by
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Mechanisms of Fetal Cardiovascular Responses to Acute Hypoxia

Thesis submitted for
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by

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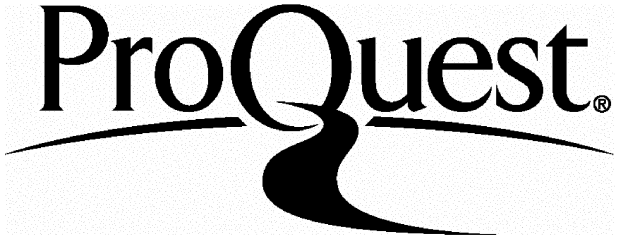

I dedicate this thesis with love to Mum and Ant
for their constant strength, lust for life, optimism
and unlimited faith.

ABSTRACT

During acute hypoxia the term fetal sheep redistributes its combined ventricular output favouring the heart, brain and adrenal glands, at the expense of peripheral vascular beds. Regional changes in organ blood flow are likely to affect their function and growth. The redistribution is characterised by an initial bradycardia, a rapid and sustained fall in peripheral blood flow and a slower rise in mean arterial pressure.

There is a large carotid chemoreceptor (CCR) component to the rapid cardiovascular changes, with α-adrenergic and muscarinic efferent limbs to the periphery and heart, respectively. Catecholamine release contributes to these responses, but there is reason to suggest the involvement of other reflexly released humoral agents, e.g. angiotensin II (AII). In addition hypoxia may act at a local tissue level, in particular on the vascular endothelium, in the control of systemic vascular tone. Renal blood flow (RBF) and urine output (UO) fall during hypoxia however it is not known whether RBF changes determine UO changes, or indeed the role played by CCRs.

In chronically instrumented sheep model I have firstly examined the role of CCRs in blood flow distribution in relation to organ function by correlating RBF and UO during hypoxia. Secondly, I have investigated the role of AII in cardiovascular responses to acute hypoxia using an angiotensin converting enzyme inhibitor, captopril, in intact fetuses and those which had been carotid sinus denervated (CSD) to remove carotid chemoreflexes. Thirdly, I have blocked endothelin-1 (ET-1) type A receptors and specifically inhibited nitric oxide synthase (NOS) to investigate the role of ET-1 and nitric oxide (NO), respectively, in cardiovascular control during normoxia and hypoxia. Finally I have investigated the role of NO in the development of fetal cardiovascular tone using chronically infused NOS-inhibitor.

My results suggest that: 1) A component of the *initial* fall in RBF during hypoxia is a carotid chemoreflex. Changes in RBF are not a major determinant of UO; 2) AII concentration rises during hypoxia but is not chemoreflexly released, although AII may play a greater role in vasoconstriction in CSD fetuses; 3) ET-1 and NO *modulate* vascular resistance and FHR during normoxia and hypoxia, and NO *mediates* the rise in carotid blood flow in hypoxia; 4) Preliminary results implicate a role for NOS in peripheral vascular development.

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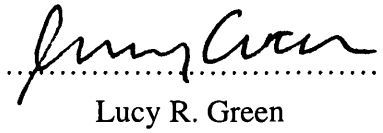
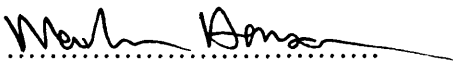
PERSONAL STATEMENT

Except as acknowledged on pages 11 and 12, the work presented in this thesis was performed solely by the candidate and is original.



Lucy R. Green

Certification by supervisor:



Professor M. A. Hanson

ABBREVIATIONS

a	arterial
AI	angiotensin I
AII	angiotensin II
AC	alternating current
ACE	angiotensin converting enzyme
ACh	acetylcholine
ACTH	adrenocorticotropic hormone
ANF	atrial natriuretic factor
$AT_{1 \text{ or } 2}$	angiotensin receptor subtype 1 or 2
ATP	adenosine triphosphate
AVP	arginine vasopressin
bpm	beats per minute
ca.	circa
Ca^{2+}	calcium ion
CBF	carotid arterial blood flow
cGMP	cyclic guanosine monophosphate
cm	centimetre
CNS	central nervous system
Co.	company
COV	coefficient of variation
CPR	cytochrome P450 reductase
CRH	corticotrophin-releasing hormone
CRL	crown-rump length
CSD	carotid sinus denervated
CSF	cerebrospinal fluid
CSN	carotid sinus nerve
CVO	combined ventricular output
CVR	carotid vascular resistance
d	day
DC	direct current
DNA	deoxyribonucleic acid
EDRF	endothelial derived relaxing factor
ECE	endothelin converting enzyme
ECG	electrocardiogram
ECoG	electrocorticogram
EMG	electromyogram
eNOS	endothelial nitric oxide synthase
ET	endothelin
ET-1	endothelin-1
$ET_{A/B/C}$	endothelin receptor subtypes A, B or C
FBF	femoral blood flow
FBMs	fetal breathing movements
FHR	fetal heart rate
$F_{i,}O_2$	inspired fraction of oxygen
FVR	femoral vascular resistance
g	gram
GFR	glomerular filtration rate
h	hour
H^+	hydrogen ion
H_2	hydrogen
Hb	haemoglobin
HbO_2	oxygenated haemoglobin
HCO_3^-	bicarbonate ion
Hct	haematocrit
^1H NMR	proton nuclear magnetic resonance

HPLC	high performance liquid chromatography
HUVEC	human umbilical vein endothelial cell
HV	high voltage
Hz	hertz
i.c.	intracisternal
i.c.v.	intracerebroventricular
I.D.	internal diameter
IGF	insulin-like growth factor
IGFBP	insulin-like growth factor binding protein
i.m.	intramuscular
iNOS	inducible nitric oxide synthase
Inc.	Incorporated
i.u.	international units
IUGR	intrauterine growth retardation
i.v.	intravenous
K^+	potassium ion
KCl	potassium chloride
L	litre
Ltd.	Limited
L-NAME	N^G-nitro-L-arginine methyl ester
L-NMMA	N^G-monomethyl-L-arginine
LV	low voltage
MAP	mean arterial pressure
MetHb	methaemoglobin
m	metre
min	minute
mg	milligram
ml	millilitre
mm	millimetre
mmHg	millimetres of mercury
msec	millisecond
MVP	mean venous pressure
MW	molecular weight
n	nano
N_2	nitrogen
Na^+	sodium ion
NADPH-d	reduced nicotinamide-adenine dinucleotide phosphate-diaphorase
NIRS	near infra-red spectroscopy
NO	nitric oxide
NOLA	N^G-nitro-L-arginine
NOS	nitric oxide synthase
nNOS	neuronal nitric oxide synthase
NPY	neuropeptide Y
NTS	nucleus tractus solitarius
O_2	oxygen
O_2ct	oxygen content
O.D.	outer diameter
p	pico
$PaCO_2$	arterial carbon dioxide partial pressure
PaO_2	arterial oxygen partial pressure
PBN	parabrachial nucleus
PBS	phosphate buffered saline
PG	prostaglandin
PGI_2	prostacyclin
Plc.	propriety limited company
PRA	plasma renin activity
RAS	renin angiotensin system
RBF	renal blood flow
RHb	reduced haemoglobin
RIA	radioimmunoassay
rpm	revolutions per minute
RSN	renal sympathetic nerve

RSNA	renal sympathetic nerve activity	UK	United Kingdom
		UO	urine output
RVR	renal vascular resistance	USA	United States of America
s	second	V	volt
S.E.M.	standard error of the mean	[ ]	concentration
		%	percent
SNP	sodium nitroprusside	°C	degree Celsius
SO_2	oxygen saturation	"	inch
U	international unit		
µ	micro		

CHAPTER 1
INTRODUCTION

1.1 General

A basic requirement by the fetus for its survival and growth is an adequate supply of oxygen (O_2) and other nutrients, for which it is reliant on a continuous supply from the mother. With this in mind, the fetal cardiovascular system needs to adapt to its changing needs, as a survival response under conditions of altered supply of nutrients or simply during the rapid period of fetal growth in late gestation. A major role for O_2 has been implicated for a long time since reduced fetal oxygenation is associated with intra-uterine growth retardation (IUGR) and postnatal neurological handicap. The mechanisms which underlie the control of the fetal cardiovascular system throughout gestation and during periods of altered oxygenation have therefore been the subject of intense investigation.

From the clinical point of view the development of techniques which allow the well-being of the fetus to be monitored *in utero* is vital. From the initial use of umbilical cord blood sampling these techniques have expanded to include measurement of intrauterine pressure, fetal-heart rate (FHR) recording, ultrasound and Doppler-ultrasound and behaviour. From this fetal surveillance, appropriate fetal size for gestational age, fetal blood gas status, fetal body and breathing movements (FBMs), FHR records and the volume of fluid compartments such as the amniotic cavity and fetal bladder can be assessed. In the event of an unusual FHR trace a biophysical profile is constructed, made up of the above parameters (see Manning, 1992). In addition, at the time of birth the collection of the first pass of urine is now sometimes used to detect fetal "birth asphyxia".

Undoubtedly these techniques provide valuable information on the fetus *in utero* but there are limitations. The first of these lies in predicting fetal outcome from these variables, for example observations of unusual FHR traces do not always relate well to fetal pH during labour or Apgar scores at birth, and do not provide a good prediction of neonatal neurological morbidity (see Spencer, 1993). In addition the physiological parameters which can be measured without (e.g. ultrasound) or with minimal invasion (ultrasound guided cordocentesis) are few, which means that information on the mechanisms of cardiovascular control is limited. Thus to date the majority of such information has been gleaned from studies using animal models where more invasive procedures are possible.

1.2 The chronic fetal sheep preparation

Early investigations in fetal research involved the use of acute experiments whereby the ewe and fetus were anaesthetised and the fetus was exteriorised and placed, with the umbilical cord still attached, on an adjacent table. In some instances a fluid-filled condom would be placed over snout of the fetus to mimic the uterine environment and prevent stimulation of the first breath (see Assali, Holm and Sehgal, 1962). However this technique had two major disadvantages: first, the physiological measurements were confounded by the effects of anaesthesia and second, the time span over which experiments could be conducted was limited.

Since the mid-1960's the chronic fetal sheep preparation has been continuously refined so that the anaesthetised fetus is instrumented under strict aseptic conditions, returned to the uterus and the ewe and fetus allowed to recover from surgery. Thus fetal cardiovascular and behavioural variables can be measured for prolonged periods of time without the influence of anaesthesia. This procedure can be carried out as early as 0.6-0.7 gestation (Iwamoto, Kaufman, Keil and Rudolph, 1989) and does not appear to increase the incidence of premature labour.

Despite the advantages over acute-anaesthetised preparations, it is still vital to ensure an adequate period of recovery from surgery (usually 4-5 days), that sterility is maintained throughout the experimental period without the prolonged use of antibiotics, and that adequate animal husbandry is carried out, such that the animal is allowed to adapt to its environment pre- and post-surgery and an adequate nutritional plan is adopted (see Mellor and Slater, 1973).

Sheep have become the primary model used in these chronic procedures. Their size means that vessels can be easily instrumented, their relatively large blood volume allows serial measurements of blood gas and hormones to be made, they tolerate surgery well and the organisation of the cardiovascular system has many similarities to that of the human. It is however vital to bear in mind the inter-species maturational differences that exist, for example, cerebral blood flow comprises a larger fraction of the cardiac output in the human than in the sheep fetus since its brain/body weight ratio is higher (see Jensen and Berger, 1993).

1.3 The use of hypoxia

A number of different methods have been developed over the years to alter fetal oxygenation. Reduction of umbilical blood flow can be achieved by either compressing the fetal abdominal aorta, occluding the umbilical cord or embolising the placental vascular bed, and results in reduced O_2 delivery in proportion to the change in flow.

Uterine blood flow reduction is produced by compression of uterine vessels, the maternal aorta or embolisation of the uterine vascular bed which interferes with materno-fetal O_2 and/or O_2 transport and produces fetal asphyxia. Fetal haemorrhage, whereby fetal blood volume is reduced by 15-20%, is another means of decreasing O_2 delivery to the fetus. Chronic reductions in fetal oxygenation have also been produced in an attempt to develop a model for fetal growth restriction (for review see Jensen abd Berger, 1993).

In this thesis maternal inspired fraction of O_2 ($F_{i,}O_2$) was manipulated, and nitrogen added to the inspirate, to reduce maternal arterial partial pressure of O_2 (PaO_2) to circa (ca.) 40mmHg and achieve a fetal PaO_2 of ca. 10-12mmHg. At the same time the fraction of maternally inspired CO_2 was increased to 3-4% to compensate for the ewe hyperventilating. The most common means of manipulating the maternal inspirate is to alter the composition of gas in a bag placed over the ewe's head, although others have administered nitrogen directly into the ewe's trachea (Block, Schlafer, Wentworth, Kreitzer and Nathanielsz, 1990). The bag method has the advantage of not requiring any additional fetal or maternal instrumentation and that a desired level of fetal PaO_2 can be maintained for an hour or more. Complicating factors may stem from the fact that as well as the fetus, the mother and placenta are exposed to reduced oxygenation and therefore the influence of raised maternal catecholamines and placental factors on the fetus cannot be dismissed.

The technique of a 50% $F_{i,}O_2$ reduction differs from other techniques such as uterine artery occlusion in that fetal arterial partial pressure of carbon dioxide ($PaCO_2$) is kept constant. Thus the effects of reduced PaO_2 are not complicated by those of a raised $PaCO_2$ (asphyxia). While asphyxia is more likely to occur in the clinical situation, it is a multifactorial insult involving changes in PaO_2, $PaCO_2$ and pH and resultant cardiovascular changes are more difficult to interpret than those resulting from a reduction in PaO_2 alone.

Reduced O_2 supply to tissues is termed *hypoxia.* However there are several types of hypoxia: *anaemic hypoxia* is due to a reduced carrying capacity of the blood perhaps due to altered haemoglobin levels; *stagnant hypoxia* occurs when cardiac output and organ blood flow is diminished so that O_2 transport is affected; *histotoxic hypoxia* is when the use of O_2 by the tissues themselves is impaired; and *hypoxic hypoxia* is characterised by a low PaO_2 (Dorland's Medical Dictionary, 1989). The manipulation of maternal inspired gases used in this thesis produces a fall in fetal PaO_2 and therefore may be termed *hypoxic hypoxia,* however since the hypoxia is induced via the maternal circulation and the fetus could be viewed in terms of being an organ being supplied by the maternal circulation the term fetal *hypoxia* may be more appropriate. Indeed this terminology

would allow for the fact that during this insult the delivery of O_2 to the different fetal vascular beds is going to vary. Throughout this thesis I have used the term *hypoxia.*

1.4 Cardiovascular and behavioural responses to hypoxia

The fetal cardiovascular responses to a reduction in O_2 delivery depend on the method of its induction, for example maternal hypoxia produces marked changes in the peripheral circulation which are absent, or occur in the opposite direction with umbilical cord compression. Furthermore certain adaptive responses have been observed when insults are prolonged (see Jensen and Berger, 1993; Thorburn and Harding, 1994). There is now quite extensive documentation of the redistribution of cardiac output which occurs during acute hypoxia. In this thesis I have used induced acute fetal hypoxia by the manipulation of maternal inspired gases. In relation to my experiments, my use of the term hypoxia refers to a fetal PaO_2 of ca. 12-13mmHg. In the following section I will outline the cardiovascular and behavioural responses under such conditions.

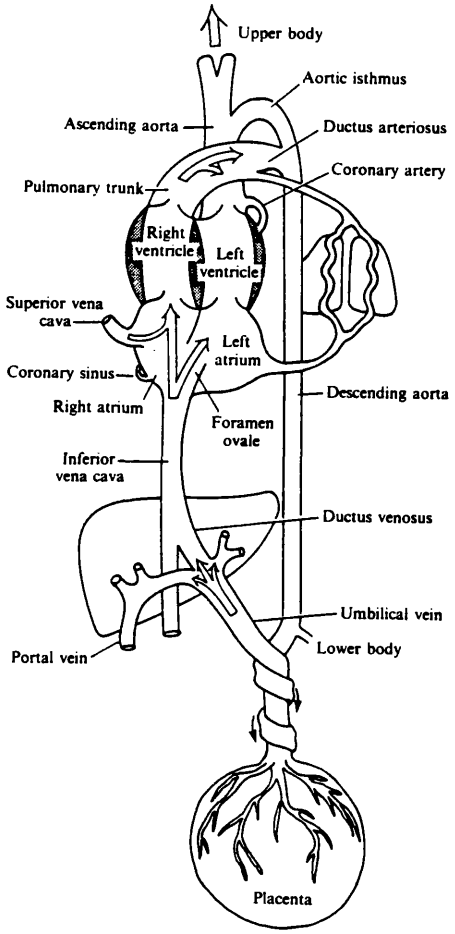


Figure 1.1 The fetal circulation. (Taken from Walker, 1993)

1.4.1 Fetal Heart Rate and Mean Arterial Pressure

In the sheep fetus there is a fall in FHR from ca. 200 to 160 beats per minute (bpm. 0.67 beats.day^{-1}) , and a rise in mean arterial pressure (MAP) from ca. 30 to ca. 50 mmHg (0.46 mmHg.day^{-1}) between the gestational ages of ca. 100 and 140 days (Boddy, Dawes, Fisher, Pinter and Robinson, 1974; Kitanaka, Alonso, Gilbert, Siu, Clemons and Longo, 1989).

In the late gestation fetus during acute episodes of hypoxia there is a, now well characterised, rapid initial fall in FHR and slower rise in MAP (Boddy *et al.*, 1974; Jones and Ritchie, 1983; Giussani, Spencer, Moore, Bennet and Hanson, 1993). The magnitude of this fall in FHR is augmented by acidaemia (Block, Schlafer, Wentworth, Kreitzer and Nathanielsz, 1990). The raised MAP is maintained for the duration of the acute insult, while FHR returns towards control levels. If hypoxia is prolonged for up to 7 days the acute FHR and MAP responses adapt so that values are no different from gestational age-matched normoxic fetuses (Kitanaka *et al.*, 1989). Earlier in gestation FHR *rises* during acute hypoxia while MAP does not change (<102 days: Boddy *et al.*, 1974; <120 days: Robillard, Weitzman, Burmeister and Smith, 1981; <99 days: Iwamoto, Kaufman, Keil and Rudolph, 1989).

1.4.2 Combined ventricular output

body weight

Oxygen consumption of the fetus is high compared to the adult, thus it is not surprising that fetal cardiac output is 3-4 times greater. The pulmonary trunk and aortic arch are connected by the *ductus arteriosus* so that right and left ventricles, respectively, contribute to the blood flow of the lower and upper fetal body, respectively. The left and right ventricles pump in parallel, so that fetal cardiac output is equivalent to the sum of the output of the two ventricles (Figure 1.1), and is therefore more commonly assigned the term combined ventricular output (CVO, see Thornburg and Morton, 1993). CVO ranges between 377 and 549 ml.min^{-1}.kg fetus^{-1} over the period of 60 days gestation until term (Rudolph and Heymann, 1970). There is a linear relationship between FHR and ventricular output such that a spontaneous or artificially-induced rise in FHR produces a rise in CVO. Furthermore, the reduction in CVO associated with a bradycardia in the fetus implies that unlike the adult, the fetus has a limited capacity to increase its stroke volume (Rudolph and Heymann, 1976). During moderate hypoxia (PaO_2 to 10-12mmHg) CVO is maintained (Perez, Espinoza, Riquelme, Parer and Llanos, 1989; Reuss, Parer, Harris and Krueger, 1982) but falls during more severe hypoxia (PaO_2 to ca. 8mmHg: Cohn, Piasecki and Jackson, 1980) and when acidaemia develops during hypoxia (Cohn, Sacks, Heymann and Rudolph, 1974; Block, Schlafer, Wentworth, Kreitzer and Nathanielsz, 1990).

While CVO is maintained during hypoxia, its distribution around the body is altered in order to maximise blood flow to the organs vital to maintaining fetal life. Thus blood flow to the brain, heart, adrenal glands and placenta is increased at the expense of flow to peripheral organs such as the lungs, kidneys, gastrointestinal tract and carcass (see Jensen and Berger, 1993).

1.4.3 Myocardial blood flow

The myocardium receives ca. 2-4% of the CVO (Rudolph and Heymann, 1970). During hypoxia (ca. 50% reduction in PaO_2) there is a large rise in myocardial blood flow ($ml.min^{-1}.100g\ tissue^{-1}$: *179±21 to 449±48* - Cohn, Sacks, Heymann and Rudolph, 1974; *218±68 to 393±178* - Reuss, Parer, Harris and Krueger, 1982; *193±20 to 718±96* - Court, Parer, Block and Llanos, 1984; *166.2±15.4 to 588.9±89.2* - Perez, Espinoza, Riquelme, Parer and Llanos, 1989) to maintain myocardial O_2 consumption (Fisher, Heymann and Rudolph, 1982). This is also observed in the more immature fetus (84-99 days gestation: Iwamoto, Kaufman, Keil and Rudolph, 1989).

1.4.4 Umbilical-placental blood flow

Placental blood flow accounts for approximately 50% of the CVO in early gestation sheep (Iwamoto *et al.*, 1989), and decreases to ca. 40% at term (Rudolph and Heymann, 1970; Cohn *et al.*, 1974) despite an increase in fetal placental blood flow throughout gestation. The increased umbilical flow <115 days gestation is due to a fall in vascular resistance, while >115 days gestation the rise in flow is arterial pressure driven (see Carter, 1993). The fetal placental circulation is characterised by its low resistance.

During hypoxia umbilical-placental flow is usually maintained (Paulick, Meyers, Rudolph and Rudolph, 1991a; Perez, Espinoza, Riquelme, Parer and Llanos, 1989; Reuss, Parer, Harris and Krueger, 1982) despite a rise in systemic blood pressure which suggests an autoregulatory vasoconstriction response. Indeed umbilical vein resistance is raised during hypoxia (Paulick *et al.*, 1991a). In contrast, <100 days gestation umbilical-placental flow falls during hypoxia (Iwamoto *et al.*, 1989).

1.4.5 Liver and ductus venosus blood flow

Umbilical venous blood flow provides >95% of the total ductus venosus blood flow (see Walker, 1993; Edelstone, Rudolph and Heymann, 1980). Thus ca. 55% of the umbilical venous blood bypasses the liver via the ductus venosus, while the remainder supplies the liver. The right lobe of the liver is supplied by umbilical and portal venous blood while the left lobe receives most of its supply from umbilical venous blood (see Jensen and Berger, 1993).

Since umbilical flow is maintained and blood flow to the liver, particularly to the right lobe, is reduced during hypoxia (Perez, Espinoza, Riquelme, Parer and Llanos, 1989; Paulick, Meyers, Rudolph and Rudolph, 1991a), a large amount of the umbilical blood flow passes through the *ductus venosus,* shunting highly oxygenated blood directly to the heart without its prior passage through the hepatic circulation (see Rudolph, 1984). However in other studies hypoxia did not alter the amount of umbilical blood flow that reached the liver, or that was shunted through the ductus venosus, nor did it affect hepatic arterial, umbilical venous or portal venous blood flows to the left and right lobes of the liver (Edelstone, Rudolph and Heymann, 1980).

1.4.6 Adrenal gland blood flow

The adrenal gland as a whole receives ca. 0.08% of the CVO. Basal adrenal blood flow is between 220 and 500 $ml.min^{-1}.100g^{-1}$ tissue, and rises 3-6 fold during hypoxia (Cohn, Sacks, Heymann and Rudolph, 1974; Court, Parer, Block and Llanos, 1984; Perez *et al.*, 1989; Paulick *et al.*, 1991a; Carter, Homan, Fraser, Richardson and Challis, 1995). This hypoxic response is also present in younger fetuses (84-99 days gestation: see Iwamoto, Kaufman, Keil and Rudolph, 1989).

In the late gestation ovine fetus blood flow to the adrenal medulla is several times greater than to the adrenal cortex, although the ratio of cortical to medullary blood flow increases during hypoxia. Differential control mechanisms have been suggested for the two regions during hypoxia: adrenocorticotropic hormone (ACTH) secretion is implicated in the rise in adrenal cortical flow, while blood flow to the medulla is more likely to be under the influence of the splanchnic nerve (Carter *et al.*, 1995).

1.4.7 Cerebral blood flow and electrocortical activity

Under basal conditions 3-4% of the CVO is distributed to the brain (Rudolph and Heymann, 1970; Cohn, Sacks, Heymann and Rudolph, 1974). Fetal sheep brain blood flow values range between 60 and 138 $ml.min^{-1}.100g\ tissue^{-1}$ (Rudolph and Heymann, 1970; Cohn *et al.*, 1974; Cohn, Piasecki and Jackson, 1980; Reuss, Parer, Harris and Kreuger, 1982; Court, Parer, Block and Llanos, 1984; Iwamoto, Kaufman, Keil and Rudolph, 1989). These measurements were obtained using the radioactive microspheres technique, for which the major disadvantage is the limited number of measurements that can be made in a given animal. More recently placement of an ultrasound flow probe around the carotid artery has provided continuous measurement of carotid arterial blood flow (CBF: Giussani, Spencer, Moore, Bennet and Hanson, 1993). This would seem to be an appropriate method of estimating cerebral blood flow since in the sheep virtually all the brain is supplied by blood from the common carotid artery, while the vertebral arteries only supply the cervical spinal cord and the caudal medulla oblongata (Baldwin and Bell, 1963). Moreover VanBel *et al.* (1994) have demonstrated a close relationship between

changes in radioactive microsphere-determined total brain blood flow and changes in CBF.

During hypoxia total brain blood flow is known to increase in young (Iwamoto, Kaufman, Keil and Rudolph, 1989) and late gestation fetuses by ca. 2-fold (Cohn, Sacks, Heymann and Rudolph, 1974; Reuss, Parer, Harris and Krueger, 1982; Perez, Espinoza, Riquelme, Parer and Llanos, 1989), and CBF rises from 95 to 120 $ml.min^{-1}$ (Giussani *et al.*, 1993). During hypoxia, the fetal cerebral circulation appears to lose its ability to autoregulate since a linear relationship develops between arterial blood pressure and cerebral blood flow indicated by a rise in CBF without a change in carotid vascular resistance (CVR: Giussani *et al.*, 1993). This is in a range of pressure that autoregulation is normally functional (see Bissonnette, Hohimer, Richardson and Machida, 1984 for discussion). However fetal cerebral autoregulation has been shown to persist under asphyxic conditions in regions such as the cerebrum and choroid plexus, thereby redirecting blood to brain stem areas where a rise in blood flow is observed (see Jensen, Hohman and Kunzel, 1987; Jensen and Lang, 1992). In contrast to the reciprocal relationship between PaO_2 and cerebral blood flow, a *linear* relationship has been observed between cerebral blood flow and $PaCO_2$ (Carter and Gu, 1988).

Electrocortical (ECoG) activity can be recorded from various sites on the brain, but prior to 112 days gestation no differentiation in state is visible, i.e. alternation between high voltage (HV-ECoG: high amplitude, low frequency) and low voltage (LV-ECoG: low amplitude, high frequency) ECoG activity. This develops gradually between 112 and 120 days gestation (Dawes, Gardner, Johnston and Walker, 1980) and by 120 days gestation there are distinct states which are most clearly differentiated in the bi-parietal ECoG recording (Clewlow, Dawes, Johnston and Walker, 1983, see Figure 2.3). Cerebral oxidative metabolism is higher during LV- than HV-ECoG activity and corresponds to an increased cerebral blood flow during LV-ECoG activity (Richardson, Patrick and Abduljabbar, 1985; Rankin, Landauer, Tian and Phernetton, 1987; Abrams, Gerhardt and Burchfield, 1991) particularly in brain stem areas (Jensen, Bamford, Dawes, Hofmeyer and Parkes, 1986). The increase in metabolism may be due to increased brain neuronal activity. During hypoxia the incidence of LV-ECoG activity decreases (Boddy, Dawes, Fisher, Pinter and Robinson, 1974), a switch to HV-ECoG activity being observed within 4 to 11 min (Clewlow, Dawes, Johnson and Walker, 1983; Koos and Sameshima, 1988). This may form part of a defence mechanism by the brain i.e., by switching to a state with lower O_2 requirements, although probably only when O_2 consumption is particularly high during LV-ECoG in the first place (Walker, Fleming, Smolich, Stunden, Horne and Maloney, 1984). In contrast other work suggests that there is no change in incidence of LV-ECoG activity during 1 h hypoxia (Sameshima and Koos, 1986; Koos, Sameshima and Power, 1987; Koos and

Sameshima, 1988). During hypercapnia the time spent in LV-ECoG activity was *increased* (Boddy, Dawes, Fisher, Pinter and Robinson, 1974) at a time at which cerebral blood flow is elevated.

Slotten *et al.* (1989) observed that a switch from LV- to HV-ECoG activity is *preceded* by changes in umbilical blood flow which suggests that the ECoG oscillations may be secondary to the processes which regulate umbilical blood flow.

1.4.8 Pulmonary blood flow

Gaseous exchange occurs in the placenta, therefore the primary function of blood flow to the lungs is to deliver nutrients for lung growth. Thus while the percent of CVO passing to the lung increases as gestation advances (Rudolph and Heymann, 1970), it still constitutes only a small proportion of the total CVO (4-8%: Rudolph and Heymann, 1970; 10-11%: Lewis, Heymann and Rudolph, 1976). Lung blood flow ranges between 87 and 126 $ml.min^{-1}.100g\ tissue^{-1}$ (Rudolph and Heymann, 1970; Reuss, Parer, Harris and Krueger, 1982; Court, Parer, Block and Llanos, 1984; Iwamoto, Kaufman, Keil and Rudolph, 1989). Fetal pulmonary vascular tone is high but falls progressively over the last half of gestation (see Heymann, 1984; Tiktinsky and Morin, 1993). On transition to the relatively well oxygenated lungs of the neonate, pulmonary blood flow rapidly increases 8-10 fold (see Fineman, Wong, Morin, Wild and Soifer, 1994; Moore, Velvis, Fineman, Soifer and Heymann, 1992) and adult levels are reached within 2-6 weeks after birth (Heymann, 1984).

Pulmonary blood flow only falls to 2% of CVO during hypoxia which suggests that pulmonary vascular changes do not contribute a great deal to the adaptation of the fetus to hypoxic stress (Cohn, Sacks, Heymann and Rudolph, 1974). Continuous measurement of pulmonary arterial blood flow by chronically implanted ultrasound flow probes has shown the fall in pulmonary blood flow and rise in pulmonary vascular resistance during hypoxia to be rapid in onset (Moore and Hanson, 1991), suggesting that they are reflexly mediated: some of the neuronal, endocrine and local hormonal mechanisms of pulmonary blood flow control will be discussed in subsequent sections [1.5].

1.4.9 Fetal breathing movements

While FBMs do not participate in gas exchange they do promote lung development and growth. By 120 days gestation FBMs, characterised by diaphragmatic EMG activity and negative tracheal pressure excursions (Figure 2.3), are almost exclusively confined to periods of LV-ECoG activity whereas prior to this point in gestation FBMs are practically continuous (Dawes, Gardner, Johnston and Walker, 1980; Clewlow, Dawes, Johnston and Walker, 1983; Johnston, 1991) .

Peculiar to the fetus is the finding that in situations of HV-ECoG activity and hypoxia FBMs are *decreased* (see Johnston, 1991). The proportion of LV-ECoG activity during which there is rapid FBMs is reduced during hypoxia (Boddy, Dawes, Fisher, Pinter and Robinson, 1974). Hypoxic inhibition of FBMs is apparent in late-gestation fetuses (>120 days: Clewlow, Dawes, Johnston and Walker, 1983; Sameshima and Koos, 1986; Koos, Sameshima and Power, 1987) and to a lesser degree (50%) in younger fetuses (<114 days: Clewlow *et al.*, 1983).

Through experimental brain stem transections (Dawes, Gardner, Johnston and Walker, 1983) and lesions at defined points within the brain stem (Gluckman and Johnston, 1987) it appears that the integrity of a particular locus lying dorsal and lateral in the rostral pons is essential for the FBM-response to hypoxia, although the specific pathways have yet to be defined. The pathways involved in co-ordinating ECoG activity and FBMs may run close to this lateral pontine area but the mechanisms are likely to be distinct from those operative in hypoxia since there is partial periodic inhibition of FBMs prior to 114 days gestation, before differentiation of ECoG activity has occurred (Clewlow *et al.*, 1983; Gluckman and Johnston, 1987). How these inhibitory mechanisms are activated is uncertain. Peripheral chemoreceptors do not influence FBMs during normoxia or hypoxia (Koos and Sameshima, 1988; Moore, Parkes, Nijhuis and Hanson, 1989; Giussani, 1992) which suggests the involvement of some other site of chemoreception, perhaps a central chemoreceptor (Hohimer, Bissonnette, Richardson and Machida, 1983). On the other hand lesioning of the lateral pons reveals a peripheral chemoreceptor-mediated tonic effect on FBMs, normally masked in the pontine-intact fetus, and a peripheral chemoreceptor mediated stimulation of FBMs in hypoxia (Johnston and Gluckman, 1993). A rise in adenosine in the fetal brain during hypoxia has also been suggested to contribute to inhibition of FBMs (Koos, Mason, Punla and Adinolfi, 1994).

The evidence for reduced O_2 consumption upon transition to HV-ECoG is tenuous [1.4.7] but appears to be consistent with the observation that FBMs, which are inhibited during HV-ECoG activity, are associated with a 30% increase in fetal O_2 consumption (Rurak and Gruber, 1983).

1.4.10 Renal blood flow

The fetal kidneys receive only 2-4% of the CVO during late gestation, in contrast to 15-18% in the newborn (see Robillard, Porter and Jose, 1994). Renal blood flow (RBF) is lower in fetal (1.6±0.2 $ml.min^{-1}.g^{-1}$ kidney) than in adult (6.4±0.3$ml.min^{-1}.g^{-1}$ kidney) sheep (Hill and Lumbers, 1988), which suggests that the fetal kidneys are under-perfused compared to the adult. However, RBF increases over the course of gestation from ca. 37 $ml.min^{-1}$ (<120 days gestation) to ca. 46 $ml.min^{-1}$ (>130 days gestation) in sheep

(Robillard, Weismann and Herin, 1981), and in human fetuses between 18 and 42 weeks gestation as evidenced from a linear fall in the renal artery pulsatility index derived from fetal Doppler flow-velocity waveforms (Vyas, Nicolaides and Campbell, 1989). There is a substantial decline in renal vascular resistance (RVR) from >130 days gestation fetuses (1.03 ± 0.08mmHg.ml^{-1}.min^{-1}) to the newborn lamb (0.51 ± 0.05mmHg.ml^{-1}.min^{-1}). There is limited information as to whether RBF is autoregulated in the immature fetal kidney as it is in the neonatal dog (9 days: Jose, Slotkoff, Montgomery, Calcagno and Eisner, 1975), although arginine vasopressin (AVP)-evoked changes in MAP do not always alter RBF which suggests that there is at least some autoregulatory capacity in the fetus (see Robillard, Porter and Jose, 1994).

Microsphere measurements have shown that RBF falls during hypoxia in the late gestation sheep fetus (247.8 ± 45.7 to 120.7 ± 25.3ml.min^{-1}.100g^{-1}: Paulick, Meyers, Rudolph and Rudolph, 1991a; 175 ± 8 to 136 ± 12ml.min^{-1}.100g^{-1}: Cohn, Sacks, Heymann and Rudolph, 1974; Nakamura, Ayres, Gomez and Robillard, 1985), although others have shown no significant change from baseline (Reuss, Parer, Harris and Krueger, 1982). Some studies show that the renal vasculature of the immature fetus (106-119 days gestation) is also able to respond to hypoxia (Robillard, Weitzman, Burmeister and Smith, 1981), however other studies using the same technique (microsphere blood flow measurement) found that the same degree of hypoxia did not elicit significant changes in kidney blood flow in even younger fetuses (84-99 days gestation: Iwamoto, Kaufman, Keil and Rudolph, 1989). Continuous measurement of RBF using a ultrasonic pulsed Doppler flow probe placed around the renal artery shows a fall in RBF which reaches its minimum after ca. 40 min hypoxia (Robillard, Nakamura and DiBona, 1986).

1.4.11 Intestinal, cutaneous and skeletal muscle blood flows

Normoxic intestinal blood flow values in late gestation fetuses range between 39 and 110ml.min^{-1}.100g^{-1} (Cohn *et al.*, 1974; Perez, Espinoza, Riquelme, Parer and Llanos, 1989; Paulick *et al.*, 1991a). Intestinal blood flow falls during hypoxia, apart from in one study in which the fall did not reach significance until acidaemia developed (Cohn *et al.*, 1974).

The carcass is defined as what is left of the fetal body once viscera, intracranial tissues and eyes have been removed; essentially skin, skeletal muscle and bones (see Peeters, Sheldon, Jones, Makowski and Meschia, 1979), and under normoxic conditions it receives 32-40% of the CVO. This percentage does not appear to change during fetal growth, but there is greater scope for mechanisms brought into action by hypoxia to effect major distributional changes in CVO via peripheral circulation than, for example, in

the pulmonary circulation (see Rudolph and Heymann, 1970). A number of studies using microsphere measurements have shown carcass blood flow to fall during hypoxia (ml.min.$^{-1}$.100g^{-1} tissue: *19.2±1.9 to 11.6±1.6* - Reuss, Parer, Harris and Krueger, 1982; *21±3 to 15±6* - Perez *et al.*, 1989; *33±3.4 to 17.5±4.8* - Paulick *et al.*, 1991a). However other studies have shown an increased carcass vascular resistance but not a fall in blood flow during hypoxia (Court, Parer, Block and Llanos, 1984), or that the fall in flow does not reach significance until acidaemia is developed (Cohn, Sacks, Heymann and Rudolph, 1974).

Femoral blood flow (FBF), commonly used as an index of skeletal muscle blood flow, rises with gestational age, and falls during hypoxia (Giussani, Spencer, Moore, Bennet and Hanson, 1993) and asphyxia (Dawes, Lewis, Milligan, Roach and Talner, 1968). Studies in exteriorised fetal sheep, and more recent observations using chronically implanted ultrasound flow probes, have revealed that the fall in FBF in response to hypoxia (Giussani *et al.*, 1993) and asphyxia (Dawes *et al.*, 1968) is rapid (ca. 2 min) in onset and then maintained for the duration of the insult. Changes in peripheral blood flow during stress correlate well with total O_2 consumption (Jensen, Hohmann and Kunzel, 1987). This suggests that the two mechanisms may be related, particularly since O_2 consumption is determined by the amount of O_2 available, which in turn is reliant on the blood flow to that organ. Indeed, if PaO_2 decreases below 1.5mM, fetal hindlimb O_2 uptake decreases towards zero. However reduction in arterial oxygenation from normal, but to above 1.5mM, produces a slight rise in blood flow and O_2 uptake is maintained by an increased extraction rate. This suggests that supply of O_2 to the hindlimb exceeds the minimum required to sustain oxidative metabolism and that reductions in O_2 delivery within this safety margin can be compensated for by increasing O_2 extraction (Boyle, Hirst, Zerbe, Meschia and Wilkening, 1990). Indeed the redistribution of blood flow depends on the degree of hypoxia, since blood flow to the musculoskeletal bed was significantly reduced by severe (8% O_2) but not by moderate (10% O_2) hypoxia (Cohn, Piasecki and Jackson, 1980).

Skin blood flow reduction during asphyxia induced by maternal aortic occlusion appears to correlate to the blood flow reductions in other peripheral organs such as the kidney and might therefore prove to be useful in fetal surveillance during a complicated labour (Jensen, Hohmann and Kunzel, 1987).

In contrast to the late gestation fetus, there was no change in musculoskeletal, cutaneous or gastrointestinal blood flow during hypoxia in younger fetuses (87-102 days gestation: Iwamoto, Kaufamn, Keil and Rudolph, 1989).

1.5 Mechanisms of cardiovascular control

During acute hypoxia adjustments in the vasculature are likely to be of paramount importance. However the function of these short-intermediate term mechanisms is limited, and therefore with chronic insults adjustment of extracellular fluid volume may become crucial. This section will focus on mechanisms involved in the control of the vascular system of the fetus during normoxia and under conditions of altered fetal oxygenation. These mechanisms appear to fall into three distinct categories: reflex neuronal, endocrine, and local mechanisms.

1.5.1 Neuronal mechanisms

The rapidity of the initial fall in heart rate and rise in peripheral and pulmonary vascular resistance at the onset of hypoxia provides the first indication of their being reflex in nature, i.e. with sensory information relayed to the brain stem via afferent pathways probably synapsing at the nucleus tractus solitarius (NTS. Felder and Mifflin, 1988 see 1.6.1) and then a command sent via efferent pathways to effector organs. Indeed early studies by Dawes *et al.* (1968) in the exteriorised anaesthetised fetus showed that if the arrival of low oxygenated blood at the hindlimb was delayed, a rapid vasoconstriction (10-20 sec) still occurred which suggested that the response was reflex rather than being due to local blood gas changes. Also, the FHR response is reminiscent of the diving response seen in adults which functions to reduce cardiac output and increase MAP in order to maintain O_2 supply to the vital organs.

The two prominent peripheral chemoreceptor locations are the carotid and aortic bodies, their afferent fibres running to the brain stem via the carotid sinus nerve (CSN: branch of the IXth nerve) and the aortic branch of the vagus (Xth) nerve, respectively. Other chemoreceptor sites exist but there is little information on them in the fetus and they will not be considered further in this thesis (see Hanson, 1988; Giussani, Spencer and Hanson, 1994c).

The carotid body is visible in early gestation, and fetal carotid (Blanco, Dawes, Hanson and McCooke, 1984) and aortic (Blanco, Dawes, Hanson and McCooke, 1982) chemoreceptors have been demonstrated to be functionally active from single fibre recordings in the last third of gestation in sheep. Basal chemoreceptor discharge, which is high compared to the adult, increases further when PaO_2 is reduced (Blanco *et al.*, 1984).

Similarly, fetal sheep carotid and aortic baroreceptor discharge was detected from ca. 85 days gestation and found to be phasic and in synchrony with the arterial pulse (see

Hanson, 1988). Arterial baroreceptors have been implicated in the regulation of arterial pressure and heart rate in the fetus (Itskovitz, LaGamma and Rudolph, 1983; Yardley, Bowes, Wilkinson, Maloney, Ritchie and Walker, 1983).

Dawes *et al.* (1968) injected cyanide into the left atrium of the fetal lamb and produced a femoral vasoconstriction which led them to suggest that, while the aortic chemoreceptors are unlikely to be the only chemosensory sites in the fetus, they probably provide the first line of defence against alterations in arterial oxygenation. Since then extensive investigations of the contribution of aortic and carotid chemo- and baroreceptors to the fetal cardiovascular responses to hypoxia have been conducted. One approach has been to sinoaortic denervate sheep fetuses by cutting not only the CSN but also the aortic and superior laryngeal nerves (Itskovitz and Rudolph, 1982; Wood, 1989; Wood, Kane and Raff, 1990). This technique has certain disadvantages in that the aortic nerve is sometimes indistinguishable from the vagus and thus the vagus could be damaged, but also that the aortic nerve itself may contain some pulmonary mechanoreceptors (for review refer to Giussani, Spencer and Hanson, 1994c). This technique did however provide further evidence for peripheral arterial chemo- and baroreceptor involvement in fetal hypoxic responses since it abolished the rapid bradycardia, the rise in MAP and the peripheral vasoconstriction during hypoxia (Itskovitz, LaGamma, Bristow and Rudolph, 1991).

In the next phase of investigations attempts were made to delineate the relative contribution of aortic and carotid chemoreceptors. Initial studies compared carotid sinus denervation (CSD), alone or in combination with vagotomy, and found that both procedures altered CVO redistribution (Jansen, Belik, Ioffe and Chernick, 1989) and attenuated the rapid fall in pulmonary blood flow (Moore and Hanson, 1991) during hypoxia. Vagotomy is not ideal since the vagus carries both afferent and efferent fibres and so any effects observed could have been due to the removal of the influence of other pathways. The work of Giussani *et al.* (1993) addressed the specific contribution of the carotid chemo- and baroreflexes by cutting the CSN alone. They found that the rapid bradycardia and femoral vasoconstriction at the onset of hypoxia were primarily carotid chemoreflexes. This was confirmed by Bartelds *et al.* (1993) who compared carotid- with aortic-denervated fetuses and found that the carotid, but not the aortic, chemoreceptors contributed to the rapid bradycardia and peripheral vasoconstriction during hypoxia. Whether these effects are carotid chemo- or baroreflexes is hard to address from these studies. However the FHR response would not appear to be a baroreflex response since it occurs before the rise in MAP. Hypoxic carotid chemoreceptor stimulation produces a fall in RBF and an anti-diuresis in adult dogs (Al-Obaidi and Karim, 1992; Karim and Al-Obaidi, 1993) and rats (Behm, Mewes, DeMuinck Keizer, Unger and Rettig, 1993), and carotid chemodenervation in adults

inhibits hypoxic renal vasoconstriction (see Honig, 1989). To date it has not been investigated whether the fall in RBF during hypoxia in the fetus is a carotid chemoreflex response. Conversely, the rise in MAP and CBF during hypoxia are not chemoreflexly mediated (Giussani, Spencer, Moore, Bennet and Hanson, 1993).

Two autonomic efferent pathways have been identified: sympathetic α- and β-adrenergic effects and vagal cholinergic effects. The sympathetic efferent pathways will be considered in section 1.5.2 (catecholamines), however it does not seem that their activity is fully developed by birth, particularly since sympathectomy of the fetus does not alter basal vascular tone, FHR or MAP (Iwamoto, Rudolph, Mirkin and Keil, 1983; Jensen and Lang, 1992) and because renal haemodynamic responses to renal nerve stimulation are not as pronounced in the fetus as in the adult (see Robillard and Nakamura, 1988). In the fetus, the *renal* sympathetic nerves do not appear to contribute to the regulation of basal renal haemodynamics or function (Smith, Sato, McWeeny, Klinkefus and Robillard, 1990) but they have been implicated in mediating the RBF responses to hypoxia (Robillard, Nakamura and DiBona, 1986), probably via α-adrenoreceptor stimulation (see Robillard, Smith, Segar, Merrill and Jose, 1992). It is interesting that following renal denervation, hypoxia produces a transient, prostaglandin (PG)-mediated initial rise followed by a progressive fall in fetal RBF (Robillard *et al.*, 1986). Adult studies have already identified these renal nerves to form the efferent limb of a carotid chemoreflex which controls RBF during hypoxia (see Honig, 1989 and DiBona, 1989), but this has yet to be shown in the fetus.

The initial rapid bradycardia during hypoxia is blocked by cholinergic receptor, but not α-adrenoceptor, antagonism which suggests a vagal cholinergic efferent limb to this reflex response (Lewis, Donovan and Platzner, 1980; Giussani, Spencer, Moore, Bennet and Hanson, 1993). Furthermore transection of the spinal cord, to remove sympathetic efferents while keeping the vagal efferents intact, shows that the MAP response to hypoxia is abolished but the rapid bradycardia remains intact (Blanco, Dawes and Walker, 1983).

1.5.2 Endocrine mechanisms

Hormones such as angiotensin II (AII), AVP, atrial natriuretic factor (ANF) and catecholamines have been implicated in a number of fetal studies of circulatory control under basal and hypoxic conditions. This section will review their varied contribution to cardiovascular control but with particular emphasis on the part played by AII. I will also review the current knowledge on the interaction between PaO_2 and these hormonal responses in particular the role of peripheral arterial chemoreceptors.

Catecholamines

Normoxia

Resting plasma [catecholamine] in the fetal circulation have been found to range between 0.5 and 1 $ng.ml^{-1}$ (Jones and Robinson, 1975; Schuijers, Walker, Browne and Thorburn, 1986). The calculated clearance rates for the fetal and maternal circulations are 840 and 4200 $ml.min^{-1}$, respectively (Jones and Robinson, 1975).

The adrenal medulla contributes no more than 30% to basal plasma [catecholamine] (Jones, Roebuck, Walker, Lagercrantz and Johnston, 1987). Sympathectomy under normoxic conditions increases plasma [adrenaline] which suggests a compensatory secretion by the adrenal medulla in the absence of sympathoneuronal function (Jensen and Lang, 1992). On the other hand, it is a rise in plasma [catecholamines] from increased activity of the sympathetic nervous system, rather than from the adrenal medulla, which is thought to account for the rise in MAP in fetal sheep between 120 and 135 days gestation (Schiujers, Walker, Browne and Thorburn, 1986).

Hypoxia

The adrenal gland is thought to be the major source of the rise in arterial [adrenaline] and [noradrenaline] during hypoxia in the fetal sheep (Cohen, Piasecki and Jackson, 1982; Cohen, Piasecki, Cohn, Young and Jackson, 1984), although plasma [noradrenaline] may be contributed to by paraganglia and sympathetic overflow (see Jones and Robinson, 1975; Jones, Roebuck, Walker, Lagercrantz and Johnston, 1987). The catecholamine response to hypoxia is functional in both early- (95-112 days) and late- (125-140 days) gestation sheep fetuses (Cohen, Piasecki and Jackson, 1982).

During hypoxia, after the initial rapid vagally-mediated bradycardia (Lewis, Donovan and Platzker, 1980), FHR returns to or above control levels. While this phenomenon could be due to increased sympathetic efferent activity, it is also associated with high circulating [catecholamines] which might exert a direct action on the heart (Jones and Robinson, 1975; Iwamoto, Rudolph, Mirkin and Keil, 1983), and is prevented by β-adrenergic blockade (Jones and Ritchie, 1983). Furthermore increased β-adrenergic activity has been implicated in limiting the vagally mediated negative chronotropic effects at the onset of hypoxia (Court, Parer, Block and Llanos, 1984).

After 1 min asphyxia [adrenaline] was higher in sympathectomised than intact fetuses, but similar to intact fetuses after 2 min asphyxia. This suggests that a massive adrenaline release from the adrenal medulla during asphyxia may have overridden any subtle difference between intact and sympathectomised fetuses (Jensen and Lang, 1992).

Furthermore hypoxia was tolerated to the same extent in sympathectomised and intact fetuses which also adds to the notion of the adrenal gland being an important source of catecholamines (Iwamoto, Rudolph, Mirkin and Keil, 1983). This is likely to be both via splanchnic nerve stimulation (Comline, Silver and Silver, 1965) and a direct action of hypoxia on the adrenal gland (Cohen, Piasecki, Cohn, Young and Jackson, 1984).

Alpha-adrenergic activity is implicated in the hypoxic vasoconstriction of hepatic, gut, spleen and carcass blood flow and the rise in blood pressure but is not responsible for renal vasoconstriction during hypoxia (Reuss, Parer, Harris and Krueger, 1982; Paulick, Meyers, Rudolph and Rudolph, 1991a; Giussani, Spencer, Moore, Bennet and Hanson, 1993). Maintenance of placental blood flow during hypoxia is due in part to increased β-adrenergic activity, either by a direct vasodilatation of placental vessels or via inotropic/chronotropic actions on the heart. Increased β-adrenergic activity may contribute to the rise in myocardial blood flow seen during hypoxia (Court, Parer, Block and Llanos, 1984). Furthermore hypoxia-induced constriction of umbilical veins is reversed by α-adrenergic blockade (Paulick *et al.*, 1991a). The variability in sensitivity of vascular beds to α-adrenergic stimulation may be due in part to differences in receptor numbers, for example the α_1-receptor density is less in intrapulmonary than in aortic smooth muscle of the late-gestation sheep fetus (Shaul, Magness, Muntz, DeBeltz and Buja, 1990).

Despite the fact that the catecholamine response is maintained in the face of chemical sympathectomy, some of the circulatory responses to hypoxia are not, for example MAP did not rise, probably due to less vasoconstriction in the periphery (Iwamoto, Rudolph, Mirkin and Keil, 1983). Thus other neuronally mediated vasoconstrictors may be involved. More recently Giussani *et al.* (1993) observed that the initial rapid cardiovascular responses to hypoxia are attenuated by section of the CSNs suggesting that carotid chemoreceptors provide the afferent limb of these reflex responses [1.5.1]. The efferent limb of the reflex, at least with regards to the vasoconstriction in the periphery (Figure 1.2), is mediated by α-adrenergic mechanisms since the rise in femoral vascular resistance (FVR) was blocked by the administration of the α_1/α_2-receptor antagonist, phentolamine. To date the effect of CSD on circulating catecholamines has not been investigated. In the study of Giussani *et al.* (1993), all fetuses in which CSD was *combined* with phentolamine treatment died. In contrast, the majority of intact fetuses treated with phentolamine survived. It was suggested that a component of the vasoconstriction during hypoxia is therefore due to carotid chemoreflexly-released vasoconstrictors that do not act via α-adrenergic mechanisms.

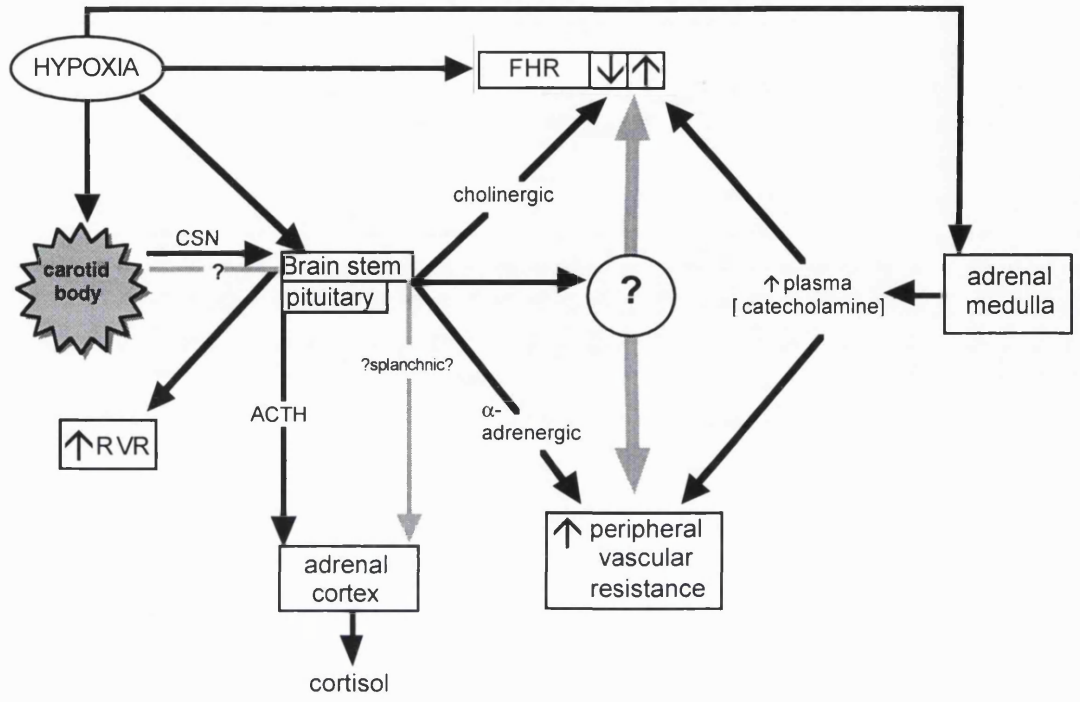


Figure 1.2 Diagram to show the carotid chemoreflexes and the question of the unidentified hormonal factor (?) as well as the question of whether the renal nerve-mediated rise in renal vascular resistance (RVR) during hypoxia is a chemoreflex response. CSN, carotid sinus nerve; FHR, fetal heart rate. The black arrows represent known pathways, whereas the grey arrows denote those that have yet to be identified (Adapted from Giussani, 1992).

Arginine vasopressin

Normoxia

Arginine vasopressin is present in fetal sheep hypothalamic extracts as early as 70 days gestation and increases between 100 and 130 days gestation (Currie and Brooks, 1992). Normal plasma [AVP] is between 0.5 and 3.5 $\mu U.ml^{-1}$. Following AVP administration, there is an initial rapid phase of clearance from the fetal circulation with a half-life of 2.8 min and clearance of $60.5\pm8.72ml.min^{-1}.kg^{-1}$. Clearance is contributed to by the fetal kidney, but not by the placenta or by fetal-maternal transport (Wiriyathian, Porter, Naden and Rosenfeld, 1983), and is reported to be three times higher than that from the maternal system (Rurak, 1978).

Two subtypes of AVP receptors have been proposed, a V_1 vascular receptor and V_2 renal receptor (Ervin, Ross, Leake and Fisher, 1992). AVP infusion produces a gradual rise in MAP, a fall in FHR, possibly via a direct action on the heart as well as a baroreflex response (Rurak, 1978; Iwamoto, Rudolph, Keil and Heymann, 1979; Wiriyathian, Porter, Naden and Rosenfeld, 1983), and redistribution of blood flow (Iwamoto *et al.*, 1979). However under normoxic conditions AVP V_1 receptor antagonism does not have any effect on fetal cardiovascular parameters (Perez, Espinoza, Riquelme, Parer and Llanos, 1989).

Hypoxia

It is now well established that plasma [AVP] rises in response to hypoxia in mid-gestation (ca. 80-100 days: Iwamoto, Kaufman, Keil and Rudolph, 1989) and late-gestation (125-141 days: Robillard, Nakamura and DiBona, 1986; 132 days: Piacquadio, Brace and Cheung, 1990; 123-144 days: Raff, Kane and Wood, 1991; 119-125 days: Giussani, McGarrigle, Spencer, Moore, Bennet and Hanson, 1994b) fetal sheep. Hypoxia and acidosis during hypoxic insults were both found to be potent stimuli for the release of AVP (Daniel, Stark, Zubrow, Fox, Husain and James, 1983), and plasma [AVP] was inversely related to arterial pH (Rurak, 1978 Wood and Chen, 1989). Hypercapnia alone however had no effect on plasma [AVP], but hypercapnic acidaemia did augment the AVP response to hypoxia (Raff, Kane and Wood, 1991; Chen and Wood, 1993).

The observation that AVP infusion caused a redistribution of blood flow similar to that seen during fetal hypoxia led investigators to suggest that AVP may be an important mediator in fetal hypoxic responses (Iwamoto, Rudolph, Keil and Heymann, 1979). Indeed later studies showed that an AVP V_1-receptor antagonist reversed the hypoxia-elevated resistance of placenta, brain, gut and liver vascular beds as well as the hypoxic bradycardia and hypertension response. Thus AVP is implicated in CVO redistribution in hypoxia (Perez, Espinoza, Riquelme, Parer and Llanos, 1989) and may play a part in the reduction of hindlimb O_2 consumption during hypoxia in the sheep fetus (Rurak, Stobbs, Kwan and Hall, 1995).

Chemical sympathectomy in fetal sheep reduces the rise in [AVP] during hypoxia (Iwamoto, Rudolph, Mirkin and Keil, 1983) and asphyxia (Jensen and Lang, 1992). This led investigators to suggest that the sympathetic nervous system is involved in stimulating AVP secretion either via peripheral chemoreceptors or central neural pathways. Indeed in adult dogs the peripheral chemoreceptors are implicated in the neurohypophysial (but not other brain region) vasodilatation during hypoxia and perhaps in the neurosecretion of AVP (Hanley, Wilson, Feldman and Traystman, 1988). However fetal sheep studies where sinoaortic-denervation (Raff, Kane and Wood, 1991), bilateral section of the CSNs and cervical vagosympathetic trunks (Chen and Wood, 1993), or bilateral CSD alone (Giussani, McGarrigle, Spencer, Moore, Bennet and Hanson, 1994b) was carried out do not implicate peripheral chemoreceptors in the AVP response to hypercapnia, normoxic hypoxia or hypercapnic hypoxia. Furthermore vagal nerve section did not block the rise in [AVP] during hypoxia which suggests that, unlike the adult, chemoreceptors other than those located in the carotid body may play a role in this response (Rurak, 1978). Thus despite the large stimulation of AVP production during hypoxia in the fetus, it does not appear to be reflexly released and therefore is not

likely to contribute to the rapid components of the fetal cardiovascular responses to hypoxia (see Giussani *et al.*, 1994b).

Atrial natriuretic factor

Normoxia

Fetal plasma [ANF] is greater than in the adult (Cheung, Gibbs and Brace, 1987). Fetal ANF clearance rates (ca 120ml.min.kg^{-1}: Brace and Cheung, 1987; Brace, Bayer and Cheung, 1989) are double than those of the maternal circulation (Ervin *et al.*, 1988), although others have reported no difference between the fetus, newborn and adult (Robillard, Nakamura, Varille, Matherne and McWeeny, 1988a). Low urine [ANF] suggest either that the fetal kidney contributes minimally to ANF clearance (Robillard *et al.*, 1988a; Brace *et al.*, 1989), or that circulating ANF is metabolised by the kidney so that little is excreted (Cheung, Gibbs and Brace, 1987). There is reported to be no fetal placental clearance of ANF (Rosenfeld, Samson, Roy, Faucher and Magness, 1992). Instead, kallikrein or other proteases may contribute to ANF degradation.

Basal ANF production from atrial muscle cells, is suggested to be primarily dependent on FHR. Basal plasma [ANF] is high in the immature fetus (110-119 days) and decreases with advancing gestation, concurrent with a fall in FHR (Cheung, 1992). Atrial natriuretic factor secretion is stimulated by endothelin-1 (ET-1), perhaps via an inotropic action on the heart and increased atrial muscle tension (Cheung, 1994), and by the infusion of AII and the α-agonist phenylephrine, which produce a concomitant rise in right atrial pressure (Rosenfeld *et al.*, 1992).

Administration of ANF antiserum, to immunoneutralise endogenous ANF, causes a delayed but sustained rise in MAP for the duration of infusion (Cheung, 1991). This is in agreement with studies in which infusion of ANF caused hypotension (Brace, Bayer and Cheung, 1989). Changes in FHR appear to be reciprocal to changes in MAP and do not suggest a direct action of ANF on the heart. In contrast, Robillard *et al.* (1988b) did not find the changes in MAP and FHR to be of significance. In some studies fetal sheep kidneys (103-128 days) are reported to be as responsive as those of adults to ANF (Shine, McDougall, Towstoless and Wintour, 1987), while others suggest that the cardiovascular, renal vasoconstriction and renal functional responses increase during maturation so that the overall response is larger in the adult (Robillard, Nakamura, Varille, Anderesen, Matherne and VanOrden, 1988).

Hypoxia

Hypoxia is a potent stimulus for elevating plasma [ANF], probably via a direct action on the fetal heart (Cheung and Brace, 1988) but there may also be contributions from the

action of AVP (Cheung, 1992), catecholamines (Ervin *et al.*, 1991) and the autonomic nervous system (Cheung, 1992). The rise in [ANF] may contribute to the reduction in blood volume (Rosenfeld, Samson, Roy, Faucher and Magness, 1992) seen during hypoxia (Cheung and Brace, 1988), probably independent of changes in urine flow (Brace, Bayer and Cheung, 1989). It also appears that the immature fetal sheep (110-119 days) displays a greater ANF response to hypoxia than the mature fetal sheep (130-135 days), probably due to the combination of a higher release and lower clearance rate of ANF (Cheung, 1992).

Inadequate ANF-mediated vasodilatation of the fetoplacental circulation may be one explanation for the raised fetoplacental vascular impedance often associated with pregnancies complicated by IUGR. However, studies have shown that umbilical vein plasma [ANF] is in fact elevated in IUGR pregnancies and so, unless there is some receptor or post-receptor mechanism defect, ANF does not appear to participate in the vascular pathology of IUGR (Kingdom, McQueen, Connell and Whittle, 1992).

ACTH and cortisol

Normoxia

The fully processed and biologically active forms of corticotrophin releasing hormone (CRH) are detected in the fetal sheep hypothalamus as early as 70 days gestation (Currie and Brooks, 1992). The rise in plasma [cortisol] over the last weeks of gestation may contribute to the increase in MAP and decrease in FHR with advancing gestation (Rose, MacDonald, Heymann and Rudolph, 1978). In the late gestation fetal sheep (127-143 days) infusion of a low dose of cortisol increased MAP, probably by a peripheral vasoconstriction, decreased FHR and decreased blood volume (Wood, Cheung and Brace, 1987). The fall in FHR could not be fully accounted for by baroreflex mechanisms. In the same study, plasma [renin] fell, perhaps by desensitising the effect of blood volume on renin secretion. Other studies show that infusion of cortisol into the immature fetal sheep (100-120 days) increases MAP but causes no further rise in, the already higher, basal MAP of the mature fetus (>130 days: Tangalakis, Lumbers, Moritz, Towstoless and Wintour, 1992). They suggested that cortisol may therefore play a greater role in basal blood pressure regulation in the immature fetus, possibly via an interaction with AII, than in the mature fetus where other cardiovascular control systems have matured.

There is evidence to suggest that stimulation of the production of cortisol by ACTH is enhanced by splanchnic nerve stimulation (Edwards and Jones, 1987) however this innervation does not appear to participate in the rise in fetal plasma [cortisol] seen after 125 days gestation (Boddy, Robinson and Ratcliffe, 1975) since splanchnic nerve section

did not alter basal plasma [cortisol] or the cortisol secretion in response to exogenous ACTH (Myers, Robertshaw and Nathanielsz, 1990).

Hypoxia

A rise in fetal plasma [cortisol] and [ACTH] is stimulated by hypoxia (Jones, Boddy, Robinson and Ratcliffe, 1975; Towell, Figueroa, Markowitz, Elias and Nathanielsz, 1987; Jackson, Morrison, Cohn and Piasecki, 1989; Brooks and Challis, 1992), but no change in circulating [CRH] occurs (Brooks and Challis, 1992). Acidaemia may be a more potent stimulant to ACTH release than hypercapnia (Chen and Wood, 1993). An intact hypothalamic-pituitary connection is required for an appropriate ACTH/cortisol response to hypoxia *in utero* (Ozolins, Young and McMillen, 1992).

Cortisol exerts a negative feedback effect on ACTH release during hypoxia in the late gestation ovine fetus, thus the elevated [cortisol] observed in older fetuses may account for their reduced ACTH response to moderate hypoxia compared to earlier in gestation (Akagi, Berdusco and Challis, 1990; Carter, Homan, Fraser, Richardson and Challis, 1995). Similarly, there is a reduced ACTH response to hypotension (131-144 days) at a time when the cortisol/ACTH ratio is increased (Rose, Meis and Morris, 1981). Furthermore, there is evidence that the rise in [ACTH] itself contributes to the increase in adrenal cortical blood flow during hypoxia (Carter *et al.*, 1995). There is an apparent dissociation in the ACTH and cortisol responses to haemorrhage in the adult dog, in that a rise in [cortisol] was detected before a rise in [ACTH] (Wood, Shinsako and Dallman, 1982). During development a rise in basal [cortisol] is detected before a rise in [ACTH] in fetal sheep (Rose, MacDonald, Heymann and Rudolph, 1978). This might be due to phasic changes in plasma [ACTH] (Wood, Shinsako and Dallman, 1982), or to changes in the adrenal sensitivity to ACTH (Wood, Shinsako, Keil, Ramsay and Dallman, 1982).

Studies in the fetal sheep suggest that the ACTH response to haemorrhage is more closely associated with the changes in pH than with the changes in MAP or central venous pressure, which suggests involvement of a chemo- rather than a mechanoreceptor mechanism (Wood, Chen and Bell, 1989; Wood and Chen, 1989). Splanchnic nerve denervation reduces the hypotension-stimulated rise in [cortisol], but probably not because of changes at the level of the hypothalamus or pituitary since there was no change in [ACTH]. Thus a splanchnic nerve-mediated pathway is implicated in the increased sensitivity of the adrenal gland to ACTH observed in response to stress in late gestation (Myers, Robertshaw and Nathanielsz, 1990). In the adult, peripheral, predominantly carotid, chemoreceptors appear to be involved in the ACTH/cortisol responses to hypoxia (Raff, Tzankoff and Fitzgerald, 1982; Raff, Shinsako and Dallman, 1984; Wood, 1989). In contrast, the ACTH response to hypercapnia is *greater* following sinoaortic denervation in the fetal sheep (Chen and Wood, 1993) but might be due to

hypotension in these fetuses. Following bilateral section of the CSNs in the sheep fetus, the rise in plasma [cortisol] during hypoxia was delayed, while the rise in [ACTH] was similar to that of intact fetuses (Giussani, McGarrigle, Moore, Bennet, Spencer and Hanson, 1994a). Thus it is possible that cortisol release in early hypoxia is mediated in part by an ACTH-independent reflex with afferents in the CSN and efferents in the splanchnic nerves, but ACTH-dependent mechanisms appear to dominate in late hypoxia. Other possible explanations for the findings of Giussani *et al.* (1994a) are that CSD altered the adrenal cortex sensitivity to ACTH during hypoxia or had some effect on the processing of the ACTH precursor, proopiomelanocortin, to ACTH during hypoxia (Castro, Valego, Zehnder and Rose, 1993).

Angiotensin II

This section will concentrate on the role of AII in fetal cardiovascular control. In addition I will review the development of the renin angiotensin system (RAS) and the types of AII receptors, as well as methods used to inhibit the RAS to provide a background to the studies contained in Chapter 4.

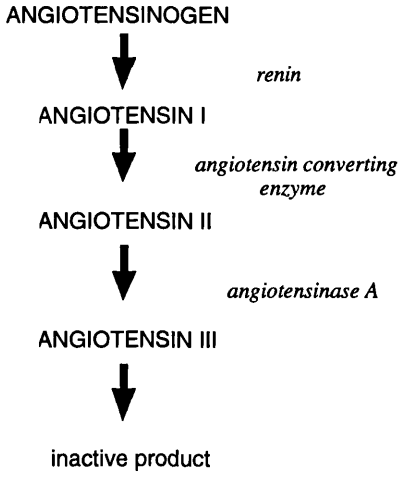


Figure 1.3 The renin angiotensin cascade. (Adapted from Guillery and Robillard, 1993).

Ontogeny of the renin angiotensin system

Angiotensinogen is the substrate for renin (Figure 1.3) and is located in the plasma and organs [see 1.5.3]. It is detected by day 11 in the rat fetus, and from 14 days gestation the majority of angiotensinogen originates from fetal liver and non-hepatic sources such as the kidney, brain and fat. Angiotensinogen gene expression is developmentally regulated and species and tissue specific (see Guillery and Robillard, 1993; Tufro-McReddie and Gomez, 1993).

The fetal kidney is thought to be the major source of the proteolytic enzyme, renin, during fetal life (see Tufro-McReddie and Gomez, 1993), although other sites of synthesis are likely [see 1.5.3]. Morphological studies of fetuses aged between 91 and 141 days gestation have revealed a well developed juxtaglomerular apparatus and renin containing cells (Smith, Lupu, Barajas, Bauer and Bashore, 1974), although a different developmental pattern has emerged in plasma renin activity (PRA). The distribution of renin containing cells shifts during development from the whole renal arterial tree to the discrete juxtaglomerular distribution characteristic of the mature animal. Below 105-106 days gestation the fetal sheep RAS cannot be stimulated by furosemide, a loop diuretic, or hypotension (Siegel and Fisher, 1980; Rawashdeh, Ray, Sundberg and Rose, 1988). After 110 days the response to furosemide increases with gestational age. In addition, PRA increases in response to hypoxia in fetuses of 131-141 days gestation but not in fetuses of between 106-119 days (Robillard, Weitzman, Burmeister and Smith, 1981), and the PRA response to haemorrhage in fetuses >120 is bigger than those <120 days gestation (Robillard, Gomez, Meernik, Kuehl and Van Orden, 1982). A greater rise in [AII] and PRA is observed in response to haemorrhage in newborn lambs than in fetal sheep (Broughton Pipkin, Kirkpatrick, Lumbers and Mott, 1974). This lack of renin response is thought to be due to a deficiency at the level of the kidney, perhaps an immaturity of β-adrenoreceptor function (Rawashdeh, Rose and Ray, 1988). Indeed *in vitro* studies on renal cortical slices taken from fetal sheep show that both renin content and basal renin secretion is lower in immature (90-112 days gestation) than mature (122 days gestation-term) kidneys (Rawashdeh, Rose and Ray, 1990), and rabbit plasma [renin] increase during the last third of gestation (Raimbach and Thomas, 1990). By 125 days gestation PRA is greater in the fetus than in the maternal circulation (Siegel and Fisher, 1980). The reason for high fetal PRA is not known but may be due to a relatively slow rate of degradation, a small volume of distribution or a high rate of secretion.

The three major mechanisms of control of renin secretion are: a) detection of decreases in tubular sodium and chloride by the macula densa. The fetal RAS like that of the adult is closely linked to renal factors that influence sodium excretion (Stevens and Lumbers, 1981); b) renal baroreceptor mechanisms, such that a fall in blood pressure (e.g. hypotension) increases, and a rise in blood pressure reduces, PRA; c) the sympathetic nervous system. Of the last mechanism, renal denervation in the fetus suppresses the rise in PRA associated with delivery (see Davis and Freeman, 1976; Smith, Smith, Guillery and Robillard, 1991). Angiotensin I (AI) infusion decreases PRA in the fetal sheep (Anderson and Binder, 1989) which supports the existence of a negative feed-back mechanism in the control of renin release. In addition AII infusion reduces PRA in adult and fetal sheep (Robillard, Gomez, VanOrden and Smith, 1982; Naden, Coultrup, Arant and Rosenfeld, 1985), and AII antagonism increases PRA (Iwamoto and Rudolph, 1979) by disinhibition of AII-inhibited renin release which may be associated with β-adrenergic receptor

receptors (Keeton, Pettinger and Campbell, 1976). The precise mechanism of this is not certain but is likely to be via a direct action of AII on the juxtaglomerular apparatus or via changes in RBF, or both.

In light of the high fetal PRA, angiotensin converting enzyme (ACE) might be the rate limiting step for AII production in the fetus. However the rate of production of AII in plasma, from endogenous renin and substrate, is higher in the fetus than in the adult (Broughton Pipkin, Lumbers and Mott, 1974). ACE appears to be functional at 120 days gestation in the sheep fetus since infusion of AI produces a rise in MAP (Anderson and Binder, 1989), although it cannot be ruled out that AI possesses some vasoconstrictor properties of its own. In every organ in which it has been studied, ACE has been located on the luminal surface of vascular endothelial cells (Caldwell, Seegal and Hsu, 1976). It is also found in epithelial cells of the renal proximal tubule. However, unlike circulating ACE, endothelial-ACE is O_2 dependent with maximal activity at a PO_2 of 100mmHg. Thus in light of the low PaO_2 of the fetus it may be that the majority of AI to AII conversion takes place in the plasma. This might explain the very high levels of plasma ACE in the fetal guinea-pig, although the characteristics and levels of ACE are species specific (Ibarra-Rubio, Pena and Pedraza-Chaverri, 1989). Fetal lungs, which are not well perfused, have low tissue ACE levels (Raimbach and Thomas, 1990) and since in adults the lungs are an important site of conversion of AI to AII this might explain the low AII/renin ratio in the fetus. Potential sources of plasma ACE are the lungs, kidneys and endothelium [1.5.3]. ACE activity is detected in rat lungs by 18 days gestation, and ACE increases during late gestation to reach, and rise above, adult levels in the first few postnatal days (Costerousse, Allegrini, Huang, Bounhik and Alhenc-Gelas, 1994; and see Guillery and Robillard, 1993; Tufro-McReddie and Gomez, 1993). It is possible that an alternative pathway for the conversion of AI to AII may exist such as via the action of neutrophil and mast cell proteinases (Reilly, Tewksbury, Schechter and Travis, 1982).

Receptor subtypes

Two AII receptor subtypes (AT_1 and AT_2) have been identified (Chiu *et al.*, 1989; see Levens, de Gasparo, Wood and Bottari, 1992). The two AII receptors are developmentally regulated, with maximal binding near term. During fetal life AT_2 receptors are 10 times more abundant than AT_1. In the rat fetus 80% of aortic AII receptors are the AT_2 type, which contrasts to the adult where 70% are AT_1 (see Tufro-McReddie and Gomez, 1993). Radioligand binding studies in the fetal rat have shown that the AT_2 (not G protein coupled) receptor is detected in early embryonic life (11 days gestation, term=21 days), reaching maximal binding on days 19-21 and decreasing 12 hours post parturition. AT_2 receptors are particularly abundant in fetal mesenchymal cells, which have the potential to differentiate into, for example, smooth and striated muscle and the connective tissue sheath surrounding muscle and blood vessels (Grady,

Sechi, Griffin, Schambeian and Kalinyak, 1991). Thus while the biological effects of AT_2 receptor-stimulation remains uncertain, they may play a role in fetal development.

AT_1mRNA expression is detected slightly later than that of AT_2 (rat day 13: Grady *et al.*, 1991; rat day 15: Tufro-McReddie, Harrison, Everett and Gomez, 1993) and whether it increases (liver) or decreases (kidney) with maturation would appear to be tissue specific (Tufro-McReddie, Harrison, Everett and Gomez, 1993). AT_1 receptor binding tends to occur in regions associated with blood pressure regulation (Grady *et al.*, 1991) but more recently AT_1 receptors (G protein coupled) have been shown to be widespread e.g. pituitary, adrenal gland, large arteries, kidney, liver, heart and mesenchymal cells (Tufro-McReddie *et al.*, 1993; Shanmugam, Monnot, Corvol and Gasc, 1994). The fetal AT_1 receptor has been further categorised into AT_{1A} and AT_{1B} types. Large blood vessels only express the AT_{1A} type, but the tissue specific developmental regulation of the two types might suggest a role in organ development as well as in cardiovascular homeostasis (Shanmugan, Corvol and Gasc, 1994; Shanmugam, Monnot, Corvol and Gasc, 1994). In the fetal sheep, renal AT_1 mRNA expression does not change during the last trimester of gestation, but decreases 1 week after birth, perhaps via the rise in cortisol rather than via changes in renal nerve stimulation (Robillard, Schutte, Page, Fedderson, Porter and Segar, 1994). In contrast, in the mouse it is the renal AT_2 receptor population which declines postnatally while the AT_1 type persists throughout adult life, and distinct developmental patterns are observed for both subtypes in the kidney (Kakuchi *et al.*, 1995); thus AII may be important in renal development (Grone, Simon and Fuchs, 1992). Cortisol may be an important modulator of the RAS components during parturition (Segar, Bedell, Page, Mazursky, Nuyt and Robillard, 1995).

The fetal circulation shows smaller MAP and heart rate responses to exogenous AII than in the maternal circulation (Robillard, Gomez, VanOrden and Smith, 1982; Yoshimura, Magness and Rosenfeld, 1990a and b). Reduced MAP and heart rate responses to exogenous AII in pregnant compared to non-pregnant women is not thought to be due to a generalised insensitivity, because post-receptor mechanisms for noradrenaline stimulation are intact, instead a high endogenous circulating [AII] is thought to down-regulate AII receptors (Siddiqi, Austin, Holroyd and Clark, 1983; Ramsay, Broughton Pipkin and Rubin, 1992). A single class of high-affinity receptor was identified in fetal aortic and placental and maternal uterine arteries. In all these vessels radioligand binding at <100 days was similar to that at >130 days. In addition, AII binding was greater in the fetus than in the maternal uterine arteries (Rosenfeld, Cox, Magness and Shaul, 1993) which would seem to rule out receptor down-regulation as an explanation for the low fetal AII responsiveness compared to adults. An alternative explanation may be the higher AII clearance rate in fetuses (121-143 days gestation: ca. 600 $ml.min.kg^{-1}$, half life=15-25 sec) than in the pregnant ewe (ranging from ca. 60 to

500ml.min.kg^{-1}, half life=49 sec) (Naden, Coultrup, Arant and Rosenfeld, 1985; Gresores, Rosenfeld, Magness and Roy, 1992; Rosenfeld, Gresores, Roy and Magness, 1995), or the degree of interaction with endothelial-derived vasodilators [1.5.4]. Intrauterine growth retardation is associated with activation of the fetal RAS but not with any change in fetoplacental vascular [AII receptor] (Kingdom, McQueen, Connell and Whittle, 1993).

Inhibition of the RAS

A number of different techniques have been employed to study the role of the RAS *in vivo*. Some studies have investigated the haemodynamic responses to exogenous AII administration, increasing plasma [AII] to that observed in pathophysiological situations, or after experimental perturbations of the system such as hypoxia or haemorrhage (e.g. Iwamoto and Rudolph, 1981b). While such experiments provide valuable information on the responsiveness to AII, they are not ideal since no account is taken of regional organ differences, particularly in light of growing evidence of local tissue RASs [1.5.3].

A more physiological approach is to inhibit some point in the renin-angiotensin cascade (Figure 1.3). Captopril, an ACE-inhibitor, has been widely used to prevent the conversion of AI to the potent vasoconstrictor AII. ACE is a dipeptidyl carboxipeptidase that removes the carboxy-terminal from AI. Alternatively AT_1-receptor antagonists, such as DUP 753 (Chiu *et al.*, 1990) and losartan, have been used to block the *action* of AII (see Timmermans, Wong, Chiu and Herbin, 1991). Both methods have their disadvantages. ACE not only converts AI to AII but is also the enzyme responsible for the breakdown of the vasodilator bradykinin [see 1.5.3- Bradykinin]. ACE-inhibitor action is therefore likely to be a combination of inhibition of AII and potentiation of the action of bradykinin. From experiments in the perfused rat hindlimb, where the effect of the circulating RAS was excluded, captopril appears to be more effective in the inhibition of local ACE than other ACE inhibitors such as enalaprilate (Nelisson-Vrancken, Leenders, Bost, Struijker-Boudier and Smits, 1992), perhaps due to greater tissue penetration. Captopril has been suggested to be teratogenic when administered maternally, being associated with fetal growth restriction (Pryde, Sedman, Nugent and Barr, 1993) and fetal death (Broughton Pipkin, Symonds and Turner, 1982; Keith, Will and Weir, 1982). Captopril is said to cross the placenta rapidly (Broughton Pipkin, Symonds and Turner, 1982), but there remains a good deal of doubt as to whether these deleterious consequences could be solely attributed to captopril or might be explained predominantly by changes in uteroplacental blood flow (see Boutroy, 1989). Thus, these observed effects might not be relevant to the use of captopril as an experimental tool administered directly to the fetus. On the other hand, AT_1-receptor antagonists will block this receptor subtype only, leaving the other receptor subtype (AT_2) available for stimulation. While the biological function of AT_2-receptors is uncertain, this may be of

particular relevance in light of the potentiation of PRA observed during AT_1 blockade (Iwamoto and Rudolph, 1979), which leads to increased AII formation and may further stimulate non-AT_1-receptor types. However it is of interest that ACE-inhibitors and AT_1 antagonists produce similar reductions in blood pressure in hypertensive adult rats (see Levens, de Gasparo, Wood and Bottari, 1992 for review).

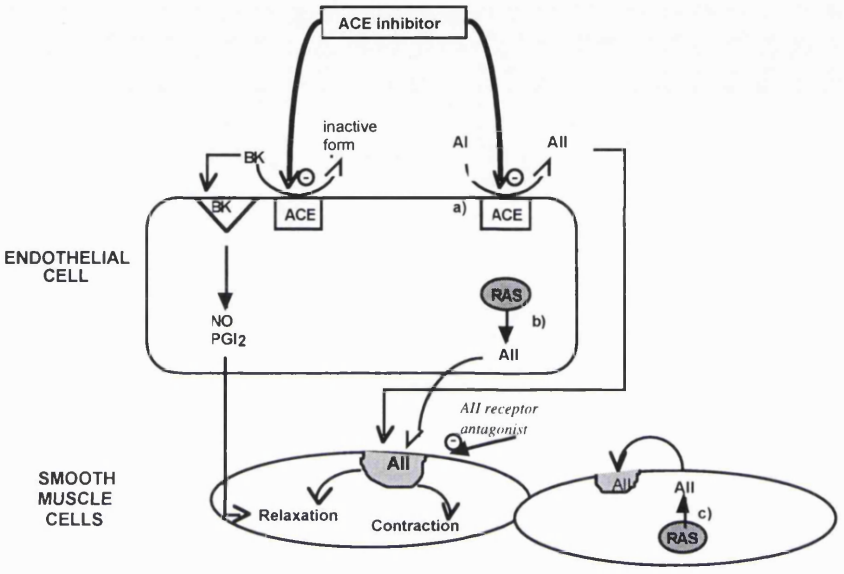


Figure 1.4 Schematic representation of the dual mechanism of action of ACE inhibitors. ACE inhibitors such as captopril inhibit the conversion of AI to AII in the circulation (a), within endothelial cells (b) and within smooth muscle cells (c). In addition ACE inhibitors prevent the breakdown of bradykinin (BK) which in turn acts in a paracrine fashion to stimulate nitric oxide (NO) and prostacyclin (PGI_2) production [refer to 1.5.3]. RAS, renin-angiotensin system. (Adapted from Rubanyi, 1993).

Normoxia

Angiotensin II infused directly into the fetus causes a vasoconstriction in the gastrointestinal, renal and thyroid vascular beds and increased blood flow to the myocardium and pulmonary vascular bed (Iwamoto and Rudolph, 1981a; Parisi and Walsh, 1989). However Iwamoto and Rudolph (1981a) saw no effect of exogenous AII on the peripheral circulation (skin, muscle and bone). Angiotensin II also results in an initial bradycardia, probably reflexly mediated, followed by a positive chronotropic response, which may be due to a direct action of AII on the heart (Iwamoto and Rudolph, 1981a), and a hypertensive response (Parisi and Walsh, 1989).

Saralasin has no effect on MAP under normoxic conditions in the fetal sheep (Mattioli, Chien, Vassenon, Crist and Lynn, 1979) in contrast to the neonatal lamb in which ACE-inhibition lowers blood pressure (4-38 days: Weismann, Herrig, McWeeny and Robillard, 1983). Earlier studies in which AII receptors were antagonised using [sar^1],[ala^8]-AII implicate endogenous AII in mediating a tonic fetal vasoconstriction, primarily in the periphery and in cerebral, thyroid and adrenal vascular beds (Iwamoto and Rudolph, 1979), but not in determining the high pulmonary vascular tone of the

normal fetus (Hyman, Heymann, Levin and Rudolph, 1975). Furthermore, there was no significant change in FHR from control with such AII receptor antagonism (Iwamoto and Rudolph, 1979). ACE-inhibition in fetal sheep causes a rise in MAP and a fall in RVR in fetuses >130 days gestation (Robillard, Weismann, Gomez, Ayers, Lawton and VanOrden, 1983), suggesting that AII may play a more important part in basal renal haemodynamics in older than in younger (<120 days gestation) fetuses (Gomez and Robillard, 1984). Thus, it appears that AII contributes to basal total peripheral resistance thereby maintaining MAP, and thus umbilical-placental flow. This would be important in ensuring adequate distribution of the CVO to the placenta and in so doing promoting gaseous exchange. AII receptors have also been located in the human full-term placenta (Wilkes, Krim and Mento, 1985).

Hypoxia

Fetal plasma [AII] and PRA rise during hypoxia (Broughton Pipkin, Lumbers and Mott, 1974; Robillard, Nakamura and DiBona, 1986). Indeed human umbilical vein [AII] increases significantly with increasing duration of the second stage of labour. This might be due to hypoxia-stimulated AII release from the fetus or from rapid changes in placental blood flow during delivery (Broughton Pipkin and Symonds, 1977). Endothelial ACE activity is reduced at PO_2<100mmHg (see Raimbach and Thomas, 1990), but this may not apply to plasma-borne ACE. It is likely that during hypoxia the O_2 tension in various vascular beds varies, providing a finely-graded mechanism for blood flow regulation (Stalcup, Lipset, Legant, Leuenberger and Mellins, 1979).

ACE inhibition alters hypoxia-induced redistribution of blood by maintaining the intestinal perfusion during hypoxia in the lamb, suggesting that AII might have a predominantly constrictive effect on intestinal vascular beds during hypoxia (Weismann, Herrig, McWeeny and Robillard, 1983). Angiotensin II is implicated in the hypertensive response to hypoxia in fetal sheep (Mattioli, Chien, Vassenon, Crist and Lynn, 1979).

The decrease in RBF during hypoxia correlated more closely to the increase in PRA than to increased plasma [AVP] in >130 day fetuses (Robillard, Weitzman, Burmeister and Smith, 1981). However, the RAS does not appear to mediate renal responses to hypoxia (Nakamura, Ayres, Gomez and Robillard, 1985), moreover AII is thought to play only a minimal role in the changes in renal haemodynamics and renal function during the transition from fetal to newborn life (Nakamura, Matherne, McWeeny, Smith and Robillard, 1987). Angiotensin II does not appear to mediate hypoxia-induced pulmonary vasoconstriction in fetal sheep (Hyman, Heymann, Levin and Rudolph, 1975).

Umbilical-placental blood flow stays constant during moderate hypoxia [1.4.4]. The vascular resistance of umbilical veins increases 2-fold during hypoxia with an increased

umbilical-placental outflow resistance. Angiotensin II receptor antagonism decreases umbilical-placental blood flow without changing calculated resistance (Iwamoto and Rudolph, 1979), and AII dramatically increases vascular resistance in large umbilical arteries (Adamson, Morrow, Bull and Langille, 1989; Yoshimura, Magness and Rosenfeld, 1990a). However, AII infusion does not alter umbilical vein resistance (Paulick, Meyers, Rudolph and Rudolph, 1991b).

The RAS is thought to play an important role in fetal cardiovascular responses to haemorrhage (Iwamoto and Rudolph, 1981b; Robillard, Gomez, Meernik, Kuehl and VanOrden, 1982; Gomez and Robillard 1984), although it may play a larger part in the neonate (Scroop, Stankewytsch-Janusch and Marker, 1992). Scroop *et al.* (1992) suggested that cardiovascular homeostasis in the fetus might not be dependent on one mechanism, rather that neuronal and hormonal mechanisms act in concert (Scroop *et al.*, 1992). The renin responses to haemorrhage are more highly correlated to changes in arterial pH than to changes in fetal MAP or central venous pressure. Thus the responses may be secondary to the acidaemia from reduced umbilical-placental perfusion during hypovolaemia and be mediated by chemo- rather than mechanoreceptors (Wood, Chen and Bell, 1989; Scroop *et al.*, 1992).

To date, the role of AII in the peripheral cardiovascular responses to hypoxia has not been fully elucidated. There is evidence to suggest that basal and stimulated PRA (and hence circulating [AII]) is under neuronal control, since sinoaortic denervation (removing both aortic and carotid body inputs) attenuates the PRA response to hypercapnic hypoxia (Wood, Kane and Raff, 1990), possibly with the efferent limb in the renal nerve (Robillard, Nakamura and DiBona, 1986). However adult studies suggest that the renin response to hypoxia is not mediated by a carotid chemoreflex (Raff, Shinsako and Dallman, 1984). In late gestation fetal sheep afferent fibres in the carotid sinus and/or aortic depressor nerves mediate the renin response to hypotension (Wood, 1989). It is possible that the rise in plasma [AII] during hypoxia (Broughton Pipkin, Lumbers and Mott, 1974) is mediated by arterial chemoreceptors, and thus contribute to the rapid peripheral vasoconstriction observed in hypoxia (Giussani, Spencer, Moore, Bennet and Hanson, 1993), and/or to the slower cardiovascular responses such as the rise in MAP and sustained peripheral vasoconstriction. The role of the carotid chemoreceptors in mediating the release of AII during hypoxia has yet to investigated.

Other hormones which may contribute to fetal cardiovascular control include neuropeptide Y (NPY) and endogenous opioids. During moderate hypoxia fetal plasma [β-endorphin], an opioid peptide, rises (Stark, Wardlaw and Daniel, 1986). β-endorphin may modulate the initial bradycardia in hypoxia, perhaps via the autonomic nervous system, contribute to maintaining CVO and placental blood flow during hypoxia

(LaGamma, Itskovitz, Rudolph, 1982), and counteract asphyxic-peripheral vasoconstriction in fetal sheep (Espinoza, Riquelme, Germain, Tevah, Parer and Llanos, 1989;). Plasma [NPY] rises during severe hypoxia in the piglet (8-20 days old: Thoresen, Dahlin, Lundberg and Lagercrantz, 1992). The interest in β-endorphin in relation to fetal cardiovascular control has been minimal over the last decade and there has been little investigation into the role played by NPY. For this reason, and because they were not studied in the experiments contained in this Thesis, I will not discuss them further.

Points to consider from this section:

1. Alpha-adrenergic mechanisms cannot fully account for the efferent limb of the chemoreflex responses to hypoxia, although it remains to be investigated whether a component of the release of catecholamines from the adrenal medulla is a carotid chemoreflex response.

2. While AVP cannot account for the, as yet, unidentified reflexly released hormonal factor other candidates such as AII cannot be ruled out (Figure 1.2).

3. Renal sympathetic nerve activity has been demonstrated in the RBF response to acute hypoxia, but it has yet to be demonstrated whether this is a chemoreflex mediated response and if so whether the afferent limb of the reflex lies in the CSN (Figure 1.2).

1.5.3 Local mechanisms

The rhythmic beating of the heart drives blood around the body in a pulsatile manner. The pulsatile blood flow exerts a mechanical stress on the vessel wall. This 'stress' has two distinct components: firstly a pulsatile pressure which acts at right angles to the long axis of the vessel cyclically stretches the vessel and secondly, a tangentially acting shear stress which, while smaller than the pressure component, is thought to be significant in regulation of vessel tone. The separation of these two components is not physiologically impossible since *in vivo* there are two likely scenarios for a rise in flow: either a rise in cardiac output and increase perfusion pressure or, on an individual vascular bed basis, from a decrease in peripheral vascular resistance in the absence of a rise in central arterial pressure. Thus there is more likely to be a pressure component in the former than in the latter case.

Myogenic response

The myogenic autoregulatory response of vessels is such that increases in intravascular pressure stimulate the vascular smooth muscle to contract, and decreases stimulate it to

relax, in an attempt to keep the volume flow through the vessel constant (Schmidt and Thews, 1987; Bevan and Henrion, 1994). This phenomenon can be experimentally induced by stretching vessels *in vitro*. The ability of smooth muscle to generate force is dependent on the position of the actomyosin cross-bridges. The myogenic response is well documented in adult small systemic resistance vessels (Hwa and Bevan, 1986; MacPherson, McLeod and Rasiah, 1991), although it may have physiological significance in larger vessels. The stretch-induced myogenic response in intra- and extrapulmonary capacitance arteries of the sheep and guinea pig is greater in the neonate and fetus than in the adult (Belik, 1994; Belik, 1995). Furthermore, the fetal myogenic response is enhanced during pulmonary hypertension. Larger pulmonary capacitance vessels increase the transmission of pressure by a stretch-induced myogenic response and stiffening of the vessel wall, ~~which would otherwise~~ resulting in a myogenic response in smaller arteries. This could have important implications for persistent pulmonary hypertension syndrome in newborns (Belik, 1995). The transduction mechanisms/receptor sites for the myogenic effect are not known but may be related to stretch-induced opening of calcium channels and subsequent smooth muscle cell-membrane depolarisation. However the myogenic response does not appear to be dependent on endothelial-derived nitric oxide (NO) or PG synthesis (MacPherson *et al.*, 1991; Belik, 1994).

Shear stress

Arteries have been found to adapt continuously the diameter of their lumen to blood flow rate. Shear stress occurs in the longitudinal axis of the vessel due to friction created by blood moving over the endothelial surface. The shear stress can be altered by both fluid viscosity and flow rate according to the equation:

$$\textit{shear stress} = 32(\textit{flow rate} \times \textit{fluid viscosity}) / \pi\ (\textit{diameter})^3$$

(Bevan and Herion, 1994; Melkumyants, Balashov and Khayutin, 1995), both components acting on arterial tone via common mechanisms. An increase in shear stress should evoke an arterial vasodilation, which will return shear stress towards its basal value. In large vessels (>100μm diameter) the shear stress is likely to be less influenced by viscosity than in smaller vessels where each blood cell occupies a large part of the vessel lumen (Giles and Trudinger, 1986).

It is hardly surprising that the vascular endothelium, the interface between blood and the underlying vessel smooth muscle, should be implicated in shear stress effects. A large number of studies have been devoted to elucidating the mechanisms that are brought into action at the endothelial level. In anaesthetised dogs *in vivo* a rise in flow was created by an arteriovenous shunt in the femoral circulation and portions of the endothelium were removed. Increased blood flow caused vasodilatation only in regions where the

endothelium was intact and this did not spread to adjacent vessel regions or to contralateral vessels (Pohl, Holtz, Busse and Bassenge, 1986 and see Smiesko and Johnson, 1993) which highlights a locally endothelial-mediated response. However there is some evidence to suggest that a component of the flow effect is via mechanisms in the sub-endothelium (see Smiesko and Johnson, 1993; Bevan and Henrion, 1994).

It is believed that the mechanisms brought into action by shear stress, that lead to changes in smooth muscle tone are mediated by deformation of the endothelial cells. Shear stress tends to push the endothelial cells in a down-stream direction and, because they are attached to the vessel wall, deforms them. Treatment of the endothelial cells with a glutaraldehyde dimer reduces their deformability and makes them insensitive to blood flow rate, while preserving their response to acetylcholine (ACh. Melkumyants, Balashov and Khayutin, 1995). An adenosine triphosphate (ATP)-dependent elevation in intracellular calcium (Ca^{2+}), perhaps by mobilisation of intracellular stores, is observed in cultured fetal bovine endothelial cells in response to shear stress (Ando, Ohtsuka, Korenaga and Kimiya, 1991). *In vitro* studies on rabbit ear resistance arteries suggest that there is a level of vascular tone at which flow-induced shear stress will cause neither relaxation or constriction of the vessel (null point), but that flow-induced relaxation is directly proportional, and constriction inversely proportional, to the initial tone of the vessel. Thus flow effects will tend to return tone to the null point (Bevan and Joyce, 1990).

In vivo and *in vitro* , elongated endothelial cells have been found in regions of high shear stress. Endothelial cell orientation is suggested to indicate the direction of flow in the immediate vicinity (Flaherty, Pierce, Ferrans, Patel, Tucker and Fry, 1972; see Nerem, Harrison, Taylor and Alexander, 1993). High shear stress is also associated with a rearrangement of actin microfilaments in the direction of flow, increased cell proliferation and migration. Structural changes have been observed in sheep common carotid arteries during development and maturation; adult endothelial cells are sheet-like in appearance and of uniform size and orientation, whereas those of the fetus are less organised and protrude into the lumen. Furthermore the transition from fetal to newborn life is associated with a reduction in endothelial cell length and width. Undoubtedly this will vary with artery type (Pearce and Longo, 1991) and its association with the effects of shear stress is not known.

There are now substantial indications from both *in vivo* and *in vitro* adult and fetal studies that endothelial-derived vasoactive substances account for a large proportion of the transduction of high shear stress into changes in vascular wall tone. The effect of shear stress on the release of ~~nitric oxide~~ (NO) and ET-1 are discussed in the following two sections. Resistance arteries are in an active state of contraction due to neural and

myogenic effects. It seems likely therefore that vasodilator mechanisms brought onto action by increased shear stress at the endothelium serve to counteract these continual constrictor factors and constitute a highly sensitive local system for maintaining adequate blood flow to the organs (see Busse, Hecker and Fleming, 1994 and see 1.5.4).

Endothelin

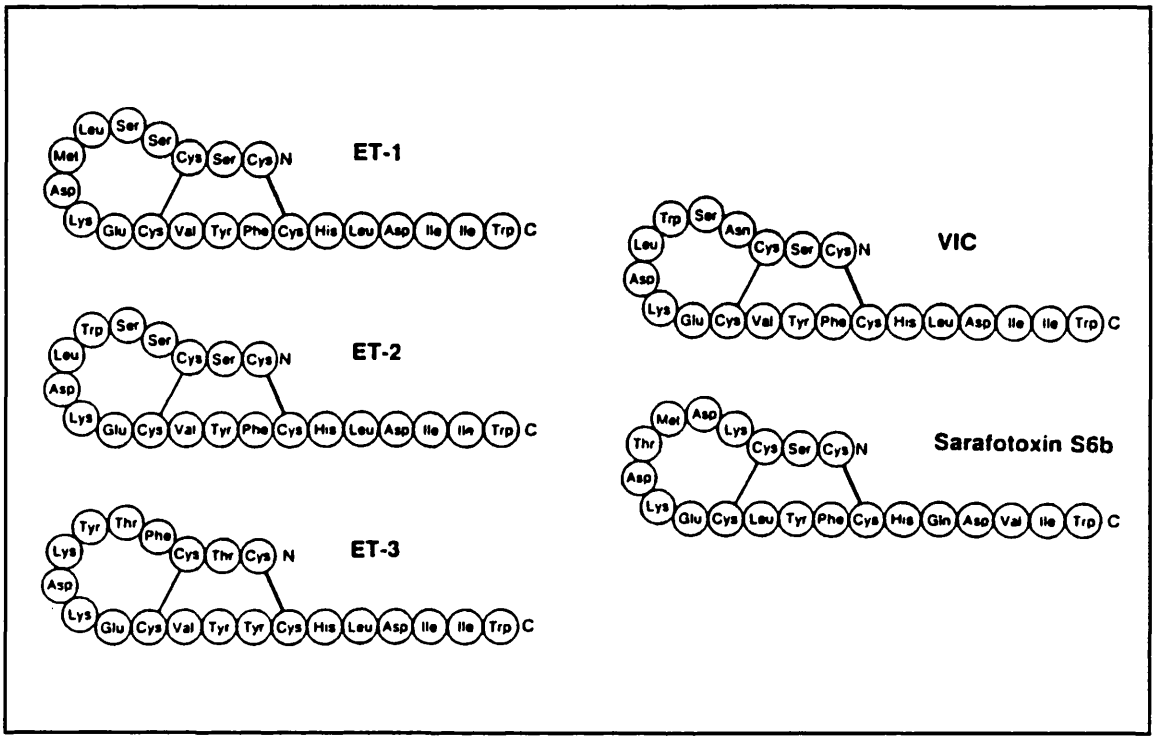
Four isoforms of endothelin (ET) have so far been identified: ET-1 (Yanagisawa *et al.*, 1988; porcine and human endothelial ET); ET-2 (2 amino acid substitutions from ET-1); ET-3 (6 amino acid substitutions from ET-1, formerly known as rat ET); and vasoactive intestinal contractor (exclusive to the mouse intestine). Each of these displays a high degree of sequence homology to safarotoxins isolated from the venom of the Israeli burrowing asp. ET-1 appears to be the major ET-subtype released by the vascular endothelium.

Endothelin synthesis and release

Endothelin-1 is derived from gene encoded preproET-1 which is cleaved by a dibasic endopeptidase to form proET-1 (or bigET-1). This is subsequently converted by a putative ET-converting enzyme (ECE) to ET-1. Immunoreactive circulating [ET-1] is low in adults (0.6 fmol.ml^{-1}: Ando, Hirata, Schichiri, Emori and Marumo, 1989; 0.3-1.7pg.ml-1: Hartter and Woloszczuk, 1989). Umbilical cord vein [ET-1], measured just after delivery in the human is greater than in the maternal peripheral circulation. This suggests that ET-1 can be actively secreted by the fetal circulation, as opposed to a passive transfer of maternal ET-1 across the placenta (Haegerstrand, Hemsen, Gillis, Larsson and Lundberg, 1989; Nakamura *et al.*, 1990). *In vivo* and cultured endothelial cells are polarised with an apical (lumen) and basolateral side. Studies on cultured human umbilical vein endothelial cells (HUVECs) have demonstrated that the secretion of ET is polarised, under basal and thromboxane-stimulated conditions, such that the majority of ET is secreted at the basolateral side of the cell (Yoshimoto, Ishizaki, Mori, Sasaki, Takakura and Murota, 1991; Wagner *et al.*, 1992). It is worth noting that this is where the smooth muscle cells would be located *in vivo*. On the basis of this, and other similar findings, the role of ET-1 as a paracrine rather than an endocrine factor has been suggested. Low plasma [ET-1] may simply reflect an over-spill into the circulation. The physiological significance of numerous studies in which exogenous ET-1 has been administered into the circulation is uncertain since this is unlikely to reflect *endogenous* ET-1 action. Indeed, intraparenchymal (i.e. abluminal) injection of ET-1 mediates constriction of the cerebral microvasculature in adult rats, while intraluminal injection causes a vasodilatation (Willette and Sauermelch, 1990). In other studies, big-ET-1 has been used in preference to ET-1 as an exogenous stimulus since its conversion to ET-1 occurs on the abluminal endothelial cell surface (Ivy, Kinsella and Abman, 1994).

Moreover in trying to estimate the ET-1 secretory response to any given stimulus, circulating levels are unlikely to be indicative of the true amount released.

A



B

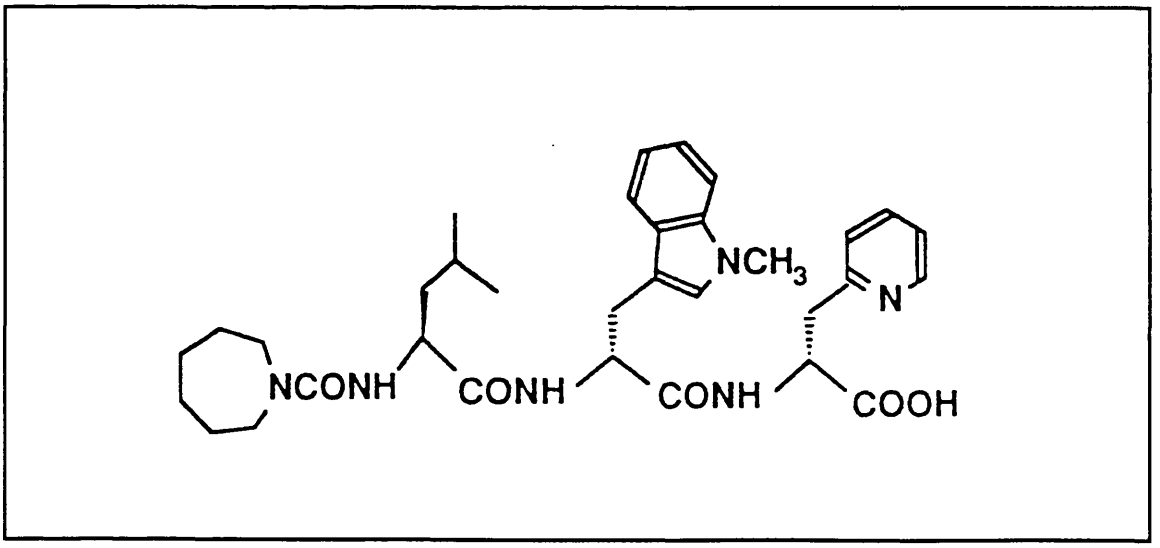


Figure 1.5 A). The structure of ET 1, 2 and 3, vasointestinal contractor (VIC) and Sarafotoxin S6b. B). The structure of the selective ET_A receptor antagonist FR139317 (Sogabe *et al.*, 1993).

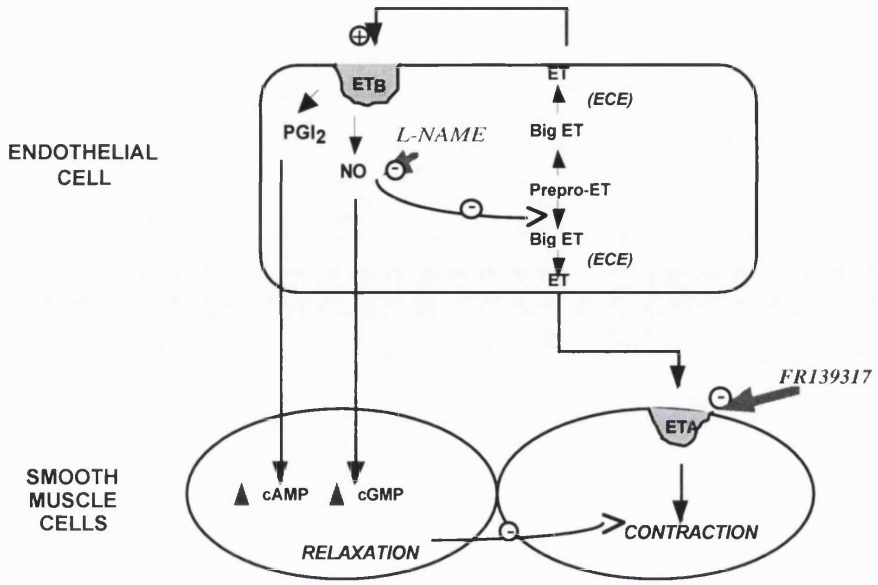


Figure 1.6 Schematic representation of ET-1 synthesis and the interaction with NO. ET, endothelin; NO, nitric oxide; ECE, endothelin converting enzyme; ET_A and ET_B, endothelin receptor subtypes.

Endothelin has also been shown to be synthesised by cultured smooth muscle cells in response to stimulation. Glucocorticoids generate ET-1 from smooth muscle cells but at a rate of ca. 1% of endothelial cells (Kanse, Takahashi, Warren, Ghatei and Bloom, 1991). Growth factors (e.g. transforming growth factor β) and vasoactive hormones, such as AII and AVP, stimulate normally quiescent cultures of vascular smooth muscle cells to express the ET-1 peptide. Endothelin-1 administration increases preproET-1 mRNA expression in smooth muscle but not endothelial cells (Hahn, Resink, Scott-Burden, Powell, Dohi and Buhler, 1990). This alternative muscle cell source of ET-1 points towards an autocrine regulatory function for ET-1 in the vasculature.

Circulating ET-1 is stable but is rapidly cleared from the circulation (half life ca. 7 min in adult rats, Shiba *et al.*, 1989), however exogenous ET-1 pressor effects can persist for a long time which suggests a high affinity for its smooth muscle receptor (Masaki, Kimura, Yanagisawa and Goto, 1991). Indeed there is little internalisation of the receptor once bound to ET-1. Endothelin-1 is cleared from the circulation via the lungs and kidneys (Masaki *et al.*, 1991) although studies in dogs suggest that the lungs do not play an important role in the removal and/or inactivation of ET-1, since concentrations in the arterial and venous pulmonary circulation are similar (Emmeluth and Bie, 1992). The removal of ET-1 may reflect the relative receptor density of different organs. To date clearance has not been examined in the fetus, although one might speculate that it would be lower since RBF is lower than in the adult [1.4.10]. One candidate for the hydrolytic inactivation of ET-1 is the enzyme neutral endopeptidase 24.11 (Vijayraghavan, Scicli, Carretero, Slaughter, Moomaw and Hersh, 1990).

Endothelin release

Increased, but low levels of shear stress (5 dynes/cm^2) are suggested to increase ET-1 release from cultured endothelial cells (Yoshizumi *et al.*, 1989), although chronic fluid-mechanical shear stress down regulates the expression of the preproET gene in cultured porcine endothelial cells (Yanagisawa *et al.*, 1988) and tends to reduce ET-1mRNA and ET-1 production from HUVECs (Noris *et al.*, 1995) probably via the release of NO (Miller and Burnett, 1992). Various hormonal factors such as adrenaline, AII and AVP induce the synthesis of preproET and bigET (Masaki, 1991).

An inverse relationship between ET-1 release and arterial oxygenation has been found by a number of investigators, that is, low PaO_2 increases plasma [ET-1]. Hypoxia initially decreases ET-1 production from primary cultures of adult cerebral microvessel endothelial cells, however this is thought more likely to be due to reduced metabolism than hypoxia itself. Indeed if the hypoxia is maintained, ET-1 production is eventually *induced* (Yoshimoto, Ishizaki, Moti, Sasaki, Takakura and Murota, 1991). Endothelin-1 production from isolated Krebs-Ringer-perfused rat mesenteric vessels is stimulated by hypoxia (Rakugi *et al.*, 1990), and in adult anaesthetised rats *in vivo* there is a negative correlation between PaO_2 and plasma [ET-1] (Horio *et al.*, 1991). Possible mechanisms for hypoxia induced ET-1 release include injury of endothelial cells. Alternatively, altered renal haemodynamics (i.e. decreased RBF) may contribute to elevated [ET-1] during hypoxia, since kidneys are implicated in the clearance of ET-1 from the circulation (Masaki, Kimura, Yanagisawa and Goto, 1991).

Immunoreactive [ET-1] in human umbilical venous blood samples is greater at a pH <7.30 than >7.30 (Hashiguchi *et al.*, 1991). Adult cerebral microvessel endothelial cells produce ET-1 in response to reduced PCO_2 (CO_2 from 5 to 2.5%. Yoshimoto *et al.*, 1991). Vaginally delivered infants often have experienced more stress, such as asphyxia, at birth than caesarean-delivered infants. Those delivered vaginally have a lower pH and higher base deficit than caesarean delivered fetuses, and those with associated asphyxia have elevated plasma [ET-1] (Isozaki-Fukuda *et al.*, 1991). In fetal sheep 3 h of hypoxia with reduced pH elevates circulating [ET-1], although 1 h of a similar insult has no effect (Jones, Abman and Wilkening, 1994).

Endothelin receptors and antagonists

Rather than measuring plasma [ET-1], a more appropriate means of studying the role of ET-1 *in vivo* would be to disrupt some stage in its synthetic pathway or block its action at its site of reception. Phosphoramidon inhibits the conversion of proET to ET (Figure 1.6) and has been shown to block the pressor activity of proET in the sheep fetus (Jones and Abman, 1994), although ECE will have to be characterised fully before specific ECE-

inhibitors can be developed (Masaki, Kimura, Yanagisawa and Goto, 1991; Yanagisawa *et al.*, 1988).

Two distinct ET receptors, ET_A and ET_B, have been cloned which display ca. 60% homology and have been classified largely on the basis of their relative affinity for the ET subtypes (see Table 1.1). A third ET receptor, ET_C, may exist but has not yet been cloned. It is suggested to be highly selective for ET-3 (for further discussion see Masaki, 1991; Masaki *et al.*, 1991; Sakurai, Yanagisawa and Masaki, 1992; Barnes, 1994). The ET_A receptor recognises the NH_2-terminal end of the ET molecule, whereas the ET_B receptor recognises the COOH-terminal end. The different types of ET have similar COOH-terminal ends which might account for the non-selective nature of the ET_B receptor (Riddihough, 1994; Barnes, 1994). ET_A receptors are associated with smooth muscle and may mediate vasoconstrictory responses to ET-1, whereas ET_B receptors are associated with endothelial cells and may mediate ET-induced production of NO (see Sakurai *et al.*, 1992 for review). However there is some evidence of a small number of ET_B receptors on porcine aortic smooth muscle cells which mediate vasoconstriction (Ihara *et al.*, 1992; Shetty, Okada, Webb, DelGrande and Lappe, 1993).

Class	*Affinity to agonists*	*Location*	*Pharmacological action*	*selective antagonists*
ET_A	ET-1>ET-2>>ET-3	smooth muscle	vasoconstriction	BQ123 FR139317
ET_B	ET-1=ET-2=ET-3	endothelial cell smooth muscle	NO & eicosanoid release vasoconstriction	IRL1038
ET_C?	ET-1<ET-3	pituitary cell	inhibition of prolactin release	

Table 1.1 Classification of ET receptor subtypes, their locations and actions mediated. (Table adapted from Masaki, 1991; Barnes, 1994)

The role played by ET-1 *in vivo* is commonly investigated using ET receptor antagonists. FR139317 (Sogabe *et al.*, 1992; Sogabe *et al.*, 1993) and BQ123 (Ihara *et al.*, 1992) are specific ET_A antagonists (Table 1.1), although other antagonists exist which target both ET_A and ET_B subtypes (Ro 46-2005: Clozel *et al.*, 1993). In adult humans and rats a high density of ET-1 binding sites have been found in blood vessels and non-vascular tissues such as the heart, lungs, intestine, kidneys and brain (Hoyer, Waeber, Palacois, 1989; Koseki, Imai, Hirata, Yanagisawa and Masaki, 1989). A recent approach has been to disrupt the endothelin gene to produce mice deficient in ($ET\text{-}1^{-/-}$ homozygous), or with reduced ($ET\text{-}1^{+/-}$ heterozygous), ET-1 (Kurihara *et al.*, 1994).

Normoxia

Endothelin-1 administration is commonly associated with a complex pattern of response, with vasodilatation and vasoconstriction components. In the fetus the majority of work on ET-1 has focused on the pulmonary vasculature. Pulmonary vascular tone is high in the fetus compared to the adult. Exogenous ET-1 causes a pulmonary vasodilatation in the fetal sheep (Chatfield, McMurtry, Hall and Abman, 1991) but a vasoconstriction in newly-ventilated lungs (Cassin, Kristova, Davis, Kadowitz and Gause, 1991) which suggests a possible relationship of response to vessel tone. Indeed in lambs, ET-1 produces pulmonary vasodilatation during pulmonary hypertension, probably mediated by NO-release and potassium (K^+) channel activation, but has no effect on basal pulmonary tone (Wong, Vanderford, Fineman, Chang and Soifer, 1993). The pulmonary vasoconstrictor effects of ET-1 are augmented, and the vasodilator effects of ET-1 attenuated, with increasing post-natal age in the lamb (Wong, Vanderford, Fineman and Soifer, 1994). Endothelin-1, -2 and -3 dilate the adult anaesthetised cat pulmonary vasculature *in vivo*, partly mediated by K^+-channel activation (Lippton, Cohen, McMurtry and Hyman, 1991). However big-ET-1 causes pulmonary vasoconstriction in the fetus (Ivy, Kinsella and Abman, 1994; Jones and Abman, 1994). The fact that big-ET-1 produces an effect suggests that the putative ECE is functional in the late-gestation sheep fetus. This response may prove to be more indicative of *endogenous* ET-1 action than the administration of ET-1 itself, since it is at the *abluminal* surface (closer to the smooth muscle) of the endothelial cell that big-ET-1 is converted to ET-1, whereas the initial site of contact for injected ET-1 will be the luminal surface of the endothelial cell. Constriction (and thus closure) of the fetal ductus arteriosus has been observed in response to ET-1 *in vivo* (Coceani, Kelsey and Seidlitz 1992) and *in vitro* (Coceani, Armstrong and Kelsey, 1989), and may contribute to pulmonary hypertension observed following systemic big-ET-1 infusion. Furthermore fetal pulmonary vessels *in vitro* exhibit a vasoconstrictor response to ET-1, perhaps due to the absence of the release of a confounding vasodilator agent from the parenchyma in the intact model (Wang and Coceani, 1992).

The contribution of ET_A receptor stimulation to high fetal pulmonary vascular tone is not clear: some studies show a sustained pulmonary vasodilatation following ET_A receptor antagonism by BQ123 (Ivy *et al.*, 1994), while others show no change in vascular tone (Wong, Fineman and Heymann, 1994). ET_B receptor stimulation causes a significant fall in pulmonary vascular tone (Wong *et al.*, 1994) which is mediated by NO release (Tod and Cassin, 1992; Ivy *et al.*, 1994). In the neonatal pig NOS-inhibition not only inhibits the vasodilator effect of ET-1, but potentiates its vasoconstrictor response *in vitro* (Perreault and De Marte, 1991). This capacity to stimulate NO release may suggest a role for ET-1 in the transitional circulation of the newborn (see nitric oxide section).

ATP-dependent K^+-channels are also implicated in ET_B receptor-mediated effects (Wong, Fineman and Heymann, 1994).

In adult sheep injection of human synthetic ET produces a marked increase in MAP with an associated increased total peripheral resistance, and fall in cardiac output and heart rate (Scoggin, Spence, Parkes, McDonald, Wade and Coghlan, 1989). Other studies have demonstrated both vasodilator and vasoconstrictor ET-1 action in the systemic vasculature (Minkes *et al.*, 1990). In the neonatal pig low doses of ET-1 (i.v.) caused an increased MAP (Bradley, Czaja and Goldstein, 1990). ET-1 and 4 Ala ET-1 (ET_B receptor agonist) injection into the left pulmonary artery does not affect systemic blood pressure (Wong, Fineman and Heymann, 1994), whereas systemic (vena caval) infusion of ET-1 produces a sustained systemic hypertension in the sheep fetus (Chatfield, McMurtry, Hall and Abman, 1991; Cheung, 1994; big-ET-1: Jones and Abman, 1994). In the newborn lamb, intrapulmonary ET-1 injection produced a systemic vasodilatation (Wong, Vanderford, Fineman, Chang and Soifer, 1993). Endothelin-1 infusion reduces mesenteric blood flow in the anaesthetised adult cat (Blank, Fuortes, Nyren and Jaffe, 1991). Endothelin-1 induced vasoconstriction in the adult human forearm is mediated by the activation of L-type calcium channels, although this effect is likely to be indirect since ET-1 does not bind to calcium binding sites on smooth muscle (Van Renterghem, Vigne, Barhanin, Schmid-Alliana, Frelin and Lazdunski, 1989; Andrawis, Gilligan and Abernethy, 1992).

In the anaesthetised adult rat ET_A receptor antagonism produces a fall in MAP and a subsequent fall in RVR which suggests a role for ET-1 in the maintenance of basal blood pressure and perhaps RVR. Binding sites for ET-1 are more numerous in vessels and interstitium of the human fetal than the adult kidney (Grone, Laue and Fuchs, 1990). It seems likely that the renal vasoconstrictor effects of ET-1 are mediated by a receptor other than the ET_A subtype, perhaps ~~the~~ a vasoconstrictory ET_B receptor (Ihara *et al.*, 1992; Shetty, Okada, Webb, DelGrande and Lappe, 1993). Studies in the conscious adult rat using a range of receptor agonists and antagonists show that low doses of ET-1 ($7.5 pmol.kg^{-1}$) cause hindlimb vasodilatation (probably mediated by ET_B receptors) and mesenteric and renal vasoconstriction (probably mediated by ET_B and ET_A receptor, respectively). Higher doses ($0.5 nmol.kg^{-1}$), after an initial depressor and hindquarter vasodilatation (probably mediated by ET_B receptors), raise MAP (dependent on $ET_A > ET_B$ receptors), and cause a vasoconstriction in renal ($ET_B > ET_A$ receptors), mesenteric ($ET_B >> ET_A$) and hindquarter ($ET_A \geq ET_B$) vascular beds (Gardiner, Kemp, March, Bennett, Davenport and Edvinsson, 1994).

Blood-borne humoral stimuli are thought to be of importance in the regulation of large cerebral arteries, and in regions lacking a blood-brain barrier such as the choroid plexus.

The cerebral endothelium will modulate these humoral influences but also directly influence vascular tone (Faraci and Heistad, 1991). Intravenous administration of ET-1 reduces total cerebral blood flow, and causes marked reductions in flow in brain regions without a blood-brain barrier such as the choroid plexus, the pineal gland and posterior lobe of the pituitary (Granstam, Wang and Bill, 1993).

Thus the diversity of responses to ET-1 between different vessels might be due to a number of factors, including a diversity in calcium channel type, the multiple ET receptor types and the two transduction mechanisms, i.e. the calcium channel and activation of phospholipase C (VanRentergham, Vigne, Barhanin, Schmid-Alliana, Frelin and Lazdunski, 1989; Masaki, 1991). Different responses within a given vascular bed might be due to route of administration, dose, experimental model and species differences.

While the bradycardic effects of ET-1 have been suggested to be a baroreflex response to a rise in blood pressure, it is possible that ET-1 could exert a direct on the heart. A two-fold increase in circulating [ET-1] in anaesthetised adult dogs produces systemic and renal vasoconstriction, decreased heart rate and cardiac output, but no increase in MAP (Lerman, Hildebrand, Aarhus and Burnett, 1991). In *conscious* adult dogs a low dose of ET-1 increased heart rate in the absence of a change in mean aortic pressure (Donckier *et al.*, 1991). ET_A and ET_B receptors have been localised to atrial and ventricular myocardium, the AV conducting system and endocardial cells. In isolated atria and in ventricular cells ET-1 exerts a positive chronotropic effect. A possible mechanistic pathway includes ET-receptor binding, activation of phospholipase C and an increase in intracellular Ca^{2+} through phosphoinositol hydrolysis (for review see Golfman, Hata, Beamish and Dhalla, 1993).

Hypoxia

Increased ET-1 production, possibly from smooth muscle, has been suggested to be more important in hypoxic pulmonary vasoconstriction than the suppression of relaxing mechanisms, such as NO. Furthermore while ET_A receptor antagonism reverses the rise in pulmonary vascular resistance during alveolar hypoxia *in vivo*, it has no effect on systemic vascular resistance or cardiac output (Wang, Coe, Toyoda and Coceani, 1995). In the conscious adult rat plasma [ET-1] increases during alveolar hypoxia, although not as rapidly as the changes in pulmonary haemodynamics occur (Shirakami *et al.*, 1991).

In contrast, there is little documentation of a role for ET-1 in either the adult or fetal *systemic* responses to hypoxia. Circulating [ET-1] rises during hypoxia although, because this only occurs after 3 h hypoxia, it has been suggested that ET-1 does not contribute to the rapid hypoxic circulatory changes (Jones, Abman and Wilkening, 1994). However there is some evidence that ET-1 contributes to the rise in hindlimb vascular

resistance and decrease in external iliac arterial blood flow, but not the rise in MAP, produced in response to 3 h maternal common iliac artery occlusion (Jones, 1995). Also increased in circulating [ET-1], triggered by birth-stress and asphyxia, may mediate the redistribution of blood flow to vital organs which takes place at the time of birth (Isozaki-Fukuda *et al.*, 1991). However as already mentioned, circulating [ET-1] will probably not accurately reflect actual local ET-1 production.

Growth and development

From cell culture studies ET-1 has been shown to be a vascular smooth muscle mitogen. This action is thought to be mediated via the ET_A receptor since it can be blocked with FR139317 (Table 1.1. Sogabe *et al.*, 1993). Endothelin-1 is implicated in pulmonary vascular remodelling and maintenance of pulmonary hypertension during the exposure of adult rats to chronic hypoxia, since pulmonary arterial pressure, plasma [ET-1], ET-1 mRNA in lung and pulmonary arteries, and ET_A and ET_B receptor mRNA levels in the lung were increased. In addition, ET_A and ET_B receptor mRNA levels were increased in the thoracic aorta and heart chambers, although no such changes were seen in organs perfused by the systemic vasculature. The stimulus for increased ET-1 gene expression is suggested to be a direct enhancement of gene transcription by hypoxia, although the molecular mechanism for this is unknown (Li *et al.*, 1994). Accordingly, the pulmonary vessels of adult human lungs most affected by morphologic abnormalities of pulmonary hypertension show increased expression of ET-1 (Giaid *et al.*, 1993).

Endothelin-1 may play a role in the pathophysiology of IUGR since a higher umbilical vein serum [ET-1,2] is observed in growth retarded fetuses than in those whose growth was normal. It is not known whether ETs cross the placenta, although ET receptors have been located on human placental membranes (Fischli, Clozel and Guilly, 1989). While maternal plasma [ET-1] is elevated in preeclampsia (Nova, Sibai, Barton, Mercer and Mitchell, 1991), this was not considered to be the primary cause of raised fetal [ET-1,2], which occured both with and without maternal signs of preeclampsia (Schiff, Weiner, Zalel, Mashiach, Sibai and Shalev, 1994).

Disruption of the ET-1 gene in mouse embryonic stem cells creates mice deficient in (homozygous $ET^{-/-}$), or with reduced (heterozygous $ET^{+/-}$), ET-1 peptide or ET-1 mRNA. The $ET^{-/-}$ homozygotes die of anoxia from respiratory failure at birth and have malformed craniofacial tissues. Thus ET-1 may play a role in mammalian ontogeny, perhaps in epithelial-mesenchymal interactions to promote pharyngeal arch development (Kurihara *et al.*, 1994; Vanhoutte, 1994). The high maximum binding sites for ET-1 in both vessels and interstitium of human fetal (18-39 weeks) compared to adult kidneys (Grone, Laue and Fuchs, 1990), and the *in vitro* mitogenic effect of ET-1 (Sogabe *et al.*, 1993), suggests that ET-1 may contribute to the growth of fetal kidneys, especially renal

vessels. For this to be a viable hypothesis the endothelium would need to be functional, and indeed ET mRNA is detected in fetal rats of a comparable gestational age (19 days. Maccumber, Ross, Glaser and Snyder, 1989).

Nitric oxide

There is a vast, and ever expanding, literature implicating NO as a messenger in the brain and peripheral nervous system, in the immune system, in myometrial activity and in the regulation of vascular tone (Forstermann, 1994).

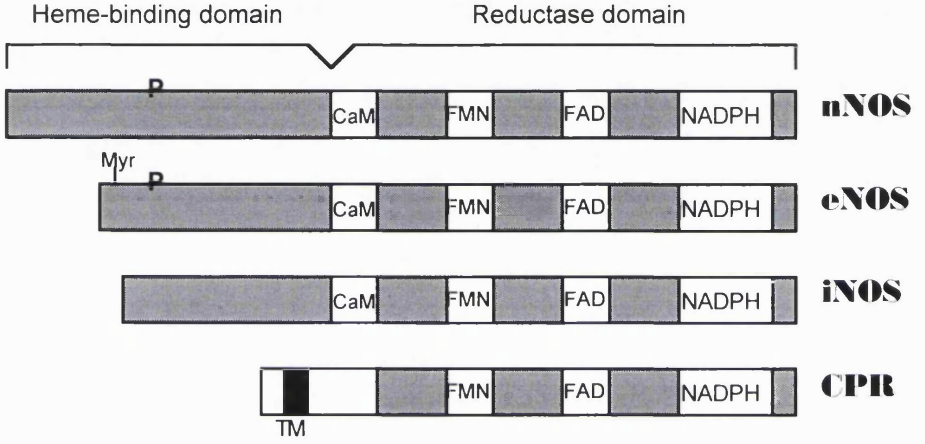


Figure 1.7 Schematic representation of the three cloned subtypes of NOS and cytochrome P450 reductase (CPR). Suggested sites for calmodulin binding (CaM), protein kinase A phosphorylation (P) and myristoylation (Myr) and recognition sites for flavin adenine dinucleotide (FAD), and flavin mononucleotide (FMN) within the enzymes are shown. TM; transmembrane domaine. (Picture adapted from Bredt and Snyder, 1994).

Nitric oxide synthesis

Early investigations showed that some vasoactive agents, such as ACh, exert a vasodilator action by acting on the endothelium to cause the release of endothelial-derived relaxing factors (EDRFs). This was paralleled by studies in which NO was found to be an active metabolite mediating the smooth muscle relaxant effect of nitroglycerin. Previously it has been suggested that EDRF may be a NO-containing compound, such as a S-nitrosothiol, more able to induce vasodilatation than NO (Myers, Minor, Guerra, Bates and Harrison, 1990). However it is now thought that NO itself can fully account for the vasodilator action of EDRF (Palmer, Ferrige and Moncada, 1987; Ignarro, Buga, Wood, Byrns and Chaudhuri, 1987; see also Marshall and Kontos, 1990; Moncada, Palmer and Higgs, 1991; Holden, 1994 for discussion), therefore in this thesis I shall consider EDRF and NO to be one and the same thing.

Nitric oxide synthase oxidises the guanidine group of L-arginine, consuming 5 electrons, to produce NO and L-citrulline. Three main types of NOS have been cloned: endothelial (eNOS), neuronal (nNOS) and inducible (iNOS), displaying ca. 50%

homology in amino acid sequence and certain similarities to CPR (Figure 1.7). These can be discriminated on the basis of their regulation by Ca^{2+} (Forstermann *et al.*, 1991; Lowenstein and Snyder, 1992; Bredt and Snyder, 1994). A rise in Ca^{2+}, either in the brain via NMDA receptor stimulation, or in blood vessels via activation of the phosphoinositide cycle, stimulates NOS. However iNOS action, from macrophages or other sources, is neither stimulated by Ca^{2+} nor inhibited by Ca^{2+}-antagonists. The tight binding of calmodulin to iNOS is thought to account for its resistance to Ca^{2+}. Further regulation of the nNOS and eNOS is possible by phosphorylation. As an enzyme, NOS is peculiar in the range of cofactors (i.e.. NADPH, FAD and FMN) it employs in its action (see Figure 1.7).

The activity and subcellular distribution of vascular eNOS is regulated by phosphorylation. Phosphorylation is associated with movement of the enzyme from the membrane to the soluble fraction of the endothelial cell. It is in the catalytically active, non-phosphorylated form that the majority of eNOS is found at the cell membrane. Although eNOS does not have a membrane-spanning domain, its myristoylation domain (Figure 1.7) ensures its membrane location. There is evidence to suggest that NO is also released from cultured smooth muscle cells (Mollace, Salvemini, Anggard and Vane, 1991), probably via iNOS (Fleming, Gray, Schott and Stoclet, 1991), and skeletal muscle cells (Balon and Nadler, 1994; for review see Schini, Busse and Vanhoutte, 1994).

Control of NO release

Nitric oxide is known to be released, perhaps incorporated into a nitrosothiol (Myers, Minor, Guerra, Bates and Harrison, 1990), from endothelial cells in both luminal and abluminal directions and to be able to penetrate the thickness of the vascular wall by diffusion and other, as yet unspecified, mechanisms (Bassenge, Busse and Pohl, 1987). Nitric oxide diffuses into the adjacent smooth muscle cells where it activates gunaylate cyclase to increase the production of guanosine 3',5'-cyclic monophosphate (cGMP) with subsequent myosin dephosphorylation and smooth muscle relaxation (see Said, 1992; Tolins, Schultz and Raij, 1991).

It is likely that endothelial NO-dependent vasodilator tone, basal or stimulated, is regulated predominantly by local mechanisms, whether they be flow-related shear stress, via the action of other endothelial-derived substances such as ET-1 via ET_B receptors or receptor-dependent stimulation (e.g. ACh, ATP and bradykinin. Shaul and Wells, 1994) on endothelial cells. Nitric oxide is therefore a good candidate for the moment-to-moment "fine-tuning" of the cardiovascular system (Moncada, Palmer and Higgs, 1991; Rongen, Smits and Thien, 1994).

Nitric oxide is released in response to shear stress. Recently a polymetric porphyrin/Naflon-coated carbon fibre microsensor with a response time of 1 millisecond has been used to show directly that there is a transient rapid NO release from cultured endothelial cells in response to shear stress (0.2-10 dynes/cm^2: Kanai, Strauss, Truskey, Crews, Grunfeld and Malinski, 1995). This is in agreement with other studies in which shear stress upregulated NO release, assessed by assay of [^{3}H]L-citrulline or measurement of nitrates and nitrites, and NOSmRNA from cultured endothelial cells (Kuchan, Jo and Frangos, 1994; 2-12 dynes/cm^2: Noris *et al.*, 1995) and isolated rabbit femoral arteries (Hecker, Mulsch, Bassenge and Busse, 1993). If shear stress is maintained, NO production appears to be sustained (Kuchan and Frangos, 1994; Kuchan, Jo and Frangos, 1994). Detailed subcellular localisation of constitutive NOS activity indicates that most is associated with the plasma membrane, which renders it more susceptible to shear stress changes (Hecker, Mulsch, Bassenge, Forstermann and Busse, 1994). The precise mechanism of stress-induced NO release is not certain but there is some indication that it is Ca^{2+}-, but not ATP-, mediated (Kuchan and Frangos, 1994; Kanai, Strauss, Truskey, Crews, Grunfeld and Malinski, 1995), and dependent on G protein activation (Kuchan *et al.*, 1994). However an ATP-dependent component has been observed by others (Fetal bovine aortic, 0.6 dyne/cm ^{2}: Korenaga *et al.*, 1994).

Nitric oxide, and to a lesser extent cyclooxygenase products, are implicated in mediating shear stress-induced pulmonary vasodilatation (induced by partial compression of the ductus arteriosus) in the sheep fetus (Cornfield, Chatfield, McQueston, McMurtry and Abman, 1992). It is possible that these shear stress mechanisms augment the stimulation of NO formation during oxygenation at the time of birth (McQueston, Cornfield, McMurtry and Abman, 1993). In adult isolated perfused rat lungs narrowing of the vessels, caused by hypoxic vasoconstriction, releases NO. This is probably via a rise in shear stress, although during chronic hypoxia other factors such as altered endothelial cell metabolism may contribute to NO release (Barer, Emery, Stewart, Bee and Howard, 1993).

Nitric oxide appears to have rather a complicated relationship with O_2, highlighted by the controversy as to the effect of hypoxia on NO-synthesis. The paradox is that while NOS requires O_2 as a substrate, its survival is enhanced during hypoxia due to reduced O_2 radical production. Indeed a popular way of inhibiting endothelium-dependent relaxation has been by the generation of O_2 radicals, for example by auto oxidation of methylene blue (Iwamoto, Yoshinaga, Yang, Krasney and Krasney, 1992). The pathway of NO inactivation, either by interacting with oxygenated haemoglobin (HbO_2) to form to nitrate and methaemoglobin (MetHb) or with the haem ring of deoxygenated haemoglobin (Hb) to form nitrosylhaemoglobin, is dependent on the proportion of HbO_2 in the blood. Since while HbO_2 acts as an O_2 donor to the NO molecule in it conversion

to nitrate ($Hb^{(Fe2+)}O_2 + NO \rightarrow Hb^{(Fe3+)} + NO_3^-$) (Wennmalm, Benthin and Petersson, 1992), other work suggests that blood-borne NO is inhibited in inverse proportion to the saturation of Hb with O_2 (Iwamoto and Morin, 1993). A number of studies of the pulmonary vasculature have suggested that decreased oxygenation attenuates NO production (Shaul, Farrar and Zellers, 1992; Kovitz, Aleskowitch, Sylvester and Flavahan, 1993; Shaul and Wells, 1994), although increased basal and agonist-stimulated NO activity has been observed during chronic hypoxic pulmonary hypertension (Isaacson, Hampl, Weir, Nelson and Archer, 1994). In the systemic vasculature, basal or stimulated (e.g. by ACh) NO production was either not altered (Shaul and Wells, 1994) or enhanced (Pohl and Busse, 1989; Tenney, 1990) by O_2 deprivation. Thus it seems possible that there is a degree of vascular bed specificity in the NO response to hypoxia, although differences may have arisen from different experimental conditions. A possible scenario for *increased* NO production seen in some studies during hypoxia centres around the importance of Ca^{2+} in NOS activity. Intracellular [Ca^{2+}] of cultured endothelial cells rises during hypoxia and may prove to be the crucial factor in stimulating rises in NO despite the low (but still in physiological range) PO_2 (see Busse, Mulsch, Fleming and Hecker, 1993).

Measurement of nitric oxide

The *in vivo* measurement of NO itself is difficult because it is not stored and has an extremely short half-life. Previously nitrite and nitrate (breakdown products of NO) have been used as an indirect measure of NO activity (Moncada, Palmer and Higgs, 1991). Direct *in vivo* measurement of NO may eventually become possible using NO electrodes (Ichimori, Ishida, Fukahori, Nakazawa and Murakami, 1994). The primary method of investigating the role of NO to date is through the inhibition of NOS. A number of NOS-inhibitors exist which are analogues of L-arginine, e.g. N^G-nitro-L-arginine methyl ester (L-NAME), N^G-iminoethyl-L-ornithine, N^G-monomethyl-L-arginine (L-NMMA) (Rees, Palmer, Hodson and Moncada, 1989), N^G-nitro-L-arginine (NOLA). The most potent inhibitor of nNOS and eNOS is NOLA (Bredt and Snyder, 1994), which along with L-NAME has more potent and longer lasting inhibitory effects than L-NMMA, perhaps due to different rates of metabolism (Gardiner, Compton, Bennett, Palmer and Moncada, 1990a; Hecker, Mitchell, Harris, Katsura, Thiemermann and Vane, 1990; Rees, Palmer, Schulz, Hodson and Moncada, 1990). Other inhibitory-compounds such as aminoguanidine (Griffiths, Messent, MacAllister and Evans, 1993; Wolff and Lubeskie, 1995) and glucocorticoids (Radomski, Palmer and Moncada, 1990; Rees, Cellek, Palmer and Moncada, 1990; Baltrons, Agullo and Garcia, 1995) target preferentially the induction of iNOS. This will eventually help delineate the NOS-subtype responsible for NO-synthesis in complex physiological and pathophysiological responses. In addition, NOS has been shown to be identical to NADPH-diaphorase (NADPH-d) which, combined with immunohistochemical localisation of NOS protein (Bredt, Hwang and

Snyder, 1990), provides a means of locating NOS in a variety of neurological preparations (Forstermann, Gorsky, Pollock, Schmidt, Heller and Murad, 1990; Klimaschewski *et al.*, 1992; Gonzalez-Hernandez, Gonzalez-Gonzalez, Mantolan-Sarmiento, Mendez-Medina, Ferres-Torres and Meyer, 1994).

Normoxia

While a large number of studies have been conducted into the role of NO-synthesis in fetal cardiovascular control, the majority of these have focused on the pulmonary vasculature. A distinctly smaller number of investigations have addressed the systemic, cerebral and renal vascular beds in the fetus.

In vitro adult studies on ovine intrapulmonary and mesenteric vessels (Bansal, Toga and Raj, 1993) and human cutaneous resistance arteries (Woolfson and Poston, 1990) have shown that while NO may contribute to venous, it does not play a role in arterial tone under basal conditions. However, it must be remembered that studies of this kind are conducted under static conditions i.e. without factors, such as shear stress from blood flow, which stimulate NO-release (Busse, Mulsch, Fleming and Hecker, 1993; Smiesko and Johnson, 1993). There is now substantial evidence from the use of NOS-inhibitors in adult animals *in vivo* to implicate endogenous NO in the modulation of basal blood pressure (Rees, Palmer and Moncada, 1989; Whittle, Lopez-Belmonte and Rees, 1989; Persson, Gustafsson, Wiklund, Moncada and Hedqvist, 1990; Tresham, Dusting, Coghlan and Whitworth, 1991) and blood flow to renal (Tolins, Palmer, Moncada and Raij, 1990; Tolins and Raij, 1991; Walder, Thiemermann and Vane, 1991; cortical: Walder *et al.*, 1991; Naess, Christensen, Kirkeboen and Kiil, 1993; Sigmon, Carretero and Beierwaltes, 1993), myocardial (Benyo, Kiss, Szabo, Csaki and Kovach, 1991), pulmonary (Persson *et al.*, 1990), internal carotid, mesenteric and hindlimb vascular beds (Gardiner, Compton, Bennett, Palmer and Moncada, 1990a and b; Gardiner, Compton, Kemp and Bennett, 1990; Richard, Gosgnach, Drieu la Rochelle, Giudicelli and Berdeaux, 1991; White, Drew, Gurden, Penny, Roach and Watts, 1993; King, Curtis, Winn, Mewburn, Cain and Chapler, 1994). The role of NO in basal pulmonary vascular tone may be species specific, since L-NAME administration increases pulmonary vascular resistance in pig, sheep and human, but not canine, isolated perfused lungs (Cremona, Wood, Hall, Bower and Higenbottam, 1994). While NOS-inhibition induced vasoconstriction may be greater in renal and mesenteric than in the hindlimb vascular bed, the effect is sustained for longer in the hindlimb (Gardiner, Compton, Bennett, Palmer and Moncada, 1990a and b; Sigmon, Carretero and Beierwaltes, 1993). Administration of L-arginine does not affect adult basal arterial blood pressure (Aisaka, Gross, Griffith and Levi, 1989) or myocardial blood flow (Benyo, Kiss, Szabo, Csaki and Kovach, 1991), or ACh-induced fetal pulmonary vasodilatation (McQueston, Cornfield, McMurtry

and Abman, 1993) which suggests that NO production of by endothelial cells is not limited by substrate availability.

In the late gestation sheep fetus, pulmonary, but not systemic (aortic), vascular smooth muscle is responsive to endothelium-independent stimuli, such as inhaled NO. The pulmonary responses are apparent as early as ca. 115 days gestation, indicating that NO-receptor mechanisms (guanylate cyclase) are functional at this age. The endothelial cells however may be *functionally* immature until later in gestation (Kinsella, McQueston, Rosenberg and Abman, 1992; Kinsella, Ivy and Abman, 1994; Skimming, DeMarco and Cassin, 1994). Acetylcholine produces an NO-mediated pulmonary vasodilatation in the late gestation ovine fetus (Tiktinsky, Cummings and Morin, 1992). Measurement of cGMP accumulation, in the presence of a phosphodiesterase inhibitor, *in vitro* demonstrates a basal NO-production in late gestation fetal pulmonary and systemic vascular beds (Shaul, Farrar and Zellers, 1992). Moreover, a marked increase in basal pulmonary artery NO production *in vitro* has been observed after 125 days gestation in the ovine fetus. However the capacity for NO production in the mesenteric vascular bed is less at this age (Shaul, Farrar and Magness, 1993), but more extensive examination of different systemic vascular beds will be required. Immunoblot analysis of NOS proteins in the fetal rat lung shows that eNOS and nNOS proteins are detectable at 16 days gestation (term=22days), but while regulation of eNOS is primarily dependent on transcription alterations or mRNA stability, that of nNOS may involve additional posttranscriptional processes (North *et al.*, 1994). Nitric oxide is implicated in the regulation of resting pulmonary arterial pressure and vascular tone in fetal lambs *in vivo* (Fineman, Heymann and Soifer, 1991). Furthermore in late-gestation ovine fetal pulmonary vessels the vasodilator action of pinacidil (Chang, Moore, Fineman, Soifer and Heymann, 1992), but not lemakalim (Cornfield, McQueston, McMurtry, Rodman and Abman, 1992), both K^+-channel activators which cause endothelial membrane hyperpolarisation and Ca^{2+} influx, is thought to be mediated in part by NO-production.

NOS-inhibition in the anaesthetised and conscious rat (Macrae, Dawson, Norrie and McCulloch, 1993; Prado, Watson, Kuluz and Dietrich, 1992; Wei, Weiss, Sinha and Chi, 1993) and goat (Dieguez, Garcia, Fernandez, Garcia-Villalon, Monge and Gomez, 1993; Fernandez, Garcia, Garcia-Villalon, Monge, Gomez and Dieguez, 1993) decreases cerebral blood flow and increases MAP and cerebral vascular resistance. However in some instances cerebral autoregulatory processes are unaffected by NOS-inhibition, since no change in cerebral blood flow occurs despite a rise in MAP (anaesthetised, ventilated rats: Buchanan and Phillis, 1993).

The cerebral blood flow response to NOS-inhibition is regionally homogenous in the adult rat (Tanaka *et al.*, 1991). However microsphere blood flow measurements in the

cat show a regionally heterogeneous response (Kovach, Szabo, Benyo, Csaki, Greenberg and Reivich, 1992), with greater reduction in flow seen in cerebellum, hypothalamus, spinal cord, pituitary and medulla oblongata, and no change in flow to the cortex and white matter (Kovach *et al.*, 1992). A widespread distribution of NOS activity has been demonstrated in the rat brain (Rodrigo *et al.*, 1994). NOS activity is high in the cerebellum, similar to the whole brain in the hypothalamus and midbrain, and low in the medulla oblongata (Forstermann, Gorsky, Pollock, Schmidt, Heller and Murad, 1990). The relative contribution of neuronal vs. endothelial sources of NO is uncertain, however use of the NOS isoform NADPH-d, as a marker of NO-synthesising neurones may prove useful in solving this problem (see Beckman, 1991). Furthermore the contribution of smooth muscle-derived NO and the direct effect of NOS-inhibitors on metabolism will need to be addressed in the context of these cerebral blood flow responses (Fernandez, Garcia, Garcia-Villalon, Monge, Gomez and Dieguez, 1993).

In the term fetus, the mechanisms by which guanylate cyclase produces vasorelaxation of cerebral arteries are fully functional; that is the fetal response to S-nitroso-N-acetyl-penicillamine is no different from the neonate and adult. However the mechanisms by which nitroglycerine activates guanylate cyclase do not appear to be fully developed at term (Pearce and Longo, 1991). Measurement of NADPH-d activity in the human fetal brain stem indicates two predominant patterns in NOS during development. The first is constant NOS activity and the second appears only transiently (19-21 weeks) in certain motor nuclei (Gonzalez-Hernandez, Gonzalez-Gonzalez, Mantolan-Sermiento, Mendez-Mendina, Ferres-Torres and Meyer, 1994). Once more the relative role of eNOS and nNOS in fetal cerebrovascular control needs to be studied.

In the fetal sheep, NOS inhibition causes a fall in RBF and rise in RVR (Bogaert, Kogan and Mevorach, 1993). Nitric oxide may play a greater role in basal renal haemodynamics in the developing piglet than in the adult pig (Solhaug, Wallace and Granger, 1993). Endothelial NOS is preferentially expressed in the glomerulus and renal vasculature of adult rat kidneys and is implicated in the regulation of RBF and glomerular filtration rate (GFR). The glomerular expression of NOSmRNA is modulated by systemic L-NAME infusion (Ujiie, Yuen, Hogarth, Danziger and Star, 1994). In addition, NO is implicated in the regulation of systemic blood pressure in the ovine fetus (Moore, Velvis, Fineman, Soifer and Heymann, 1992; Tiktinsky, Cummings and Morin, 1992).

Thus it appears that, to different extents in different vascular beds, the fetal and adult cardiovascular system is in a state of active NO-mediated vasodilatation.

In adults, inhibition of NOS *in vivo* produces a bradycardia (Aisaka, Gross, Griffith and Levi, 1989; Gardiner, Compton, Bennett, Palmer and Moncada, 1990b; Fernandez, Garcia, Garcia-Villalon, Monge, Gomez and Dieguez, 1993; Reid, Bui and Chou, 1994). This may be mediated in part by the baroreflex response to a rise in blood pressure, but endogenous NO could have direct cardiac actions. Han *et al.* (1994) have shown that NO production is important in the cholinergic inhibition of cardiac pacemaker tissue, and NO is implicated in the transduction of the positive-chronotropic effects of adrenaline on heart rate in the adult rat (Gardiner, Kemp and Bennett, 1991). Furthermore, NOS has been localised in cardiac ganglion cells and nerve fibres innervating the sinoatrial and atrioventricular nodes, the myocardium, local neurones, coronary arteries and pulmonary vessels which implicates NO in the neurogenic control of heart rate (Klimaschewski *et al.*, 1992). However studies on adult isolated atrial preparations suggest that NO does not participate in spontaneous or cholinergic/adrenergic-stimulated changes in heart rate (Kennedy, Hicks, Brian and Seifen, 1994). In contrast there have been few studies in the fetus on the role of NOS in the regulation of heart rate, and what information does exist suggests that NOS-inhibition has no effect on FHR (Moore, Velvis, Fineman, Soifer and Heymann, 1992).

Growth and development

Preeclampsia is often associated with hypertension and proteinuria, as well as fetal growth restriction secondary to an impaired placental circulation. The effect of NOS inhibitors on systemic blood pressure in adults (see previous discussion) led investigators to suggest that reduced NO-synthesis could contribute to the pathogenesis of hypertension. Subsequent studies using pregnant rats have suggested that chronic inhibition of maternal NO-synthesis provides a model of preeclampsia (Yallampalli and Garfield, 1993; Molnar, Suto, Toth and Hertelendy, 1994). Studies on resistance arteries from preeclamptic women *in vitro* reveals a dysfunctional endothelium, although it is the prostanoid, rather than the NO, component of the response to ACh stimulation which appears to be abnormal (McCarthy, Woolfson, Raju and Poston, 1993). Some rat studies however reveal minimal maternal complications with chronic L-NAME infusion, but nonetheless do observe IUGR with selective haemorrhagic disruptions of the fetal hindlimb (Diket *et al.*, 1994). More recently this study has been extended to show that these effects are likely to be due to eNOS rather than iNOS action, and that the action of L-NAME is dose and time dependent (Voelker, Miller, Zhang, Eloby-Childress, Clark and Pierce, 1995). These fetal effects are accompanied by compromised placental growth, which implies that the L-NAME-induced fetal growth restriction is due to compromised placental function. Indeed low eNOS and iNOS activity has been observed in villous samples from preeclamptic and growth restricted pregnancies (Morris *et al.*, 1995). However a direct inhibition of fetal NO production in these studies remains a possibility. Thus from these findings, and the suggestion of a role for NO in basal

placental

systemic arterial pressure in the adult and fetus, it seems reasonable to speculate that NO may play a role in fetal growth and/ or cardiovascular development.

Hypoxia

To date the majority of studies on NO function during altered oxygenation in the fetal circulation have been in the context of its role in the transition of the pulmonary circulation at birth. *Increased* oxygenation at birth is associated with a fall in pulmonary vascular resistance. A number of studies have mimicked this pulmonary vasodilatation by artificial oxygenation of the late-gestation sheep fetus *in utero*. NO-synthesis inhibition attenuates the rise in pulmonary blood flow and decrease in pulmonary vascular resistance with O_2 ventilation in the near-term fetus (Moore, Velvis, Fineman, Soifer and Heymann, 1992; Cornfield, Chatfield, McQueston, McMurtry and Abman, 1992; McQueston, Cornfield, McMurtry and Abman, 1993; Tiktinsky and Morin, 1993). Furthermore chronic NO inhibition *in utero* produces physiological derangements similar to those of persistent pulmonary hypertension in the neonate (Fineman, Wong, Morin, Wild and Soifer, 1994). Thus, NO is implicated in the rise in pulmonary blood flow at birth through stimulation of NO activity by ventilation and probably increased shear stress (Cornfield, Chatfield, McQueston, McMurtry and Abman, 1992). It is also possible that vasoconstrictor factors are inhibited at this time which would make the effect of NO more pronounced. In contrast to the fetus, the neonatal pulmonary vasodilatation in response to hyperoxia does not appear to be NO-mediated (Fineman, Wong and Soifer, 1993).

In the fetal, neonatal and adult pulmonary vascular beds there is a vasoconstrictor response to hypoxia. In adult dogs (Perella, Edell, Krowka, Cortese and Burnett, 1992) and rabbits (Persson, Gustafsson, Wiklund, Moncada and Hedqvist, 1992) , the neonatal pig (Nelin and Dawson, 1993) and fetal sheep (Fineman, Chang and Soifer, 1992) this vasoconstriction is augmented by NOS-inhibition, and in the newborn lamb it is reversed by inhalation of NO (Roberts *et al.* 1993). These results suggest a modulating role for NO in the pulmonary vascular responses to hypoxia. There is evidence to suggest that during hypoxia, NO generation declines in normally well-oxygenated portions of the adult rabbit lung, which suggests a differential regulation of lung NO-synthesis by hypoxia (Grimminger, Spriestersbach, Weismann, Walmrath and Seegar, 1995). In contrast, the adult renal vasoconstrictor response to hypoxia is not altered by NO-synthesis inhibition (Perella *et al.*, 1992).

There is some evidence that NOS-inhibition abolishes the hindlimb vasodilator response to severe hypoxia in the adult (King, Curtis, Winn, Mewburn, Cain and Chapler, 1994), although other anaesthetised dog studies suggest that NO-synthesis is not involved (Vallet, Curtis, Winn, King, Chapler and Cain, 1994). Hypoxic vasoconstriction of the human internal mammary artery may be due in part to the inhibition of NO-synthesis

(Pearson, Lin, Evora and Schaff, 1993). However, to my knowledge, there is no information to date on the role of NO in the fetal systemic vasoconstrictor responses to hypoxia.

Like the adult, fetal cerebral blood flow increases in response to hypoxia [1.4.7]. Extravascular tissue effects, such as a rise in vasodilator metabolites (e.g. hydrogen ions (H^+), see 1.5.3-The metabolic hypothesis), contribute to the cerebrovascular response to hypoxia in the adult. However the fact that hypoxia relaxes fetal cerebral vessels *in vitro* (see Longo and Pearce, 1991) implies a direct action of hypoxia on them. Nitric oxide is one candidate which may mediate such an action (Pearce, 1995). Studies in the adult sheep *in vivo* (Iwamoto, Yang, Yoshinaga, Krasney and Krasney, 1992; Iwamoto, Yoshinaga, Yang, Krasney and Krasney, 1992) suggest a role for NO in the rise in cerebral blood flow during hypoxia. However these findings are in conflict with those of others which suggest that hypoxic cerebral responses are simply *modulated* by NO (Kozniewska, Oseka and Stys, 1992; McPherson, Koehler and Traystman, 1994), or even that hypoxia inhibits cerebrovascular NO generation (Pelligrino, Koening and Albrecht, 1993). Moreover it appears that NO may act as an antagonist to sympathetic cerebro-vasoconstriction in the late-gestation fetus *in vitro* (Wagerle, Moliken and Russo, 1995). In the neonatal rat, NO-synthesis appears to play a role in the development of hypoxic-ischemic brain damage (Hamada, Hayakawa, Hattori and Mikawa, 1993). The net effect of mechanisms operated by NO, either cell damage or damage reduction by a vasodilator action, is likely to depend on the timing and intensity of the changes in NO production (Malinski, Baily, Zhang and Chopp, 1993).

Local renin-angiotensin system

The affinity for AII to bind to its receptor is reported to be in the nanomolar range while plasma [AII] is in the picomolar range. This suggests that a site of AII production, other than the renin-AII biochemical cascade already known to occur in the plasma [1.5.2-angiotensin II], is likely to exist (Dzau, 1984a). In keeping with this hypothesis is the finding that tissue extracts, such as adrenal, aorta, uterus, liver, gonads and brain, contain immunoreactive angiotensin-like material (Tufro-McReddie and Gomez, 1993; Aguilera, Schirar, Baukal and Catt, 1981). Furthermore following bilateral nephrectomy of adult rats, plasma [AII] is maintained for 24-36 h despite a fall in plasma [renin], suggesting an extra-renal renin source, probably tissue isorenin, and a tissue generation of AII. Thus while the kidneys are likely to be the principle source of renin during fetal life (Scroop, Stankewytsch-Janusch and Marker, 1992) other sources may exist.

Cultured smooth muscle and endothelial cells contain all the components (i.e. angiotensinogen, renin and AII) of the RAS (Dzau, 1984b; Kifor and Dzau, 1987). *De novo* synthesis of renin takes place in vascular endothelial (Lilly *et al.*, 1985) and smooth

muscle cells (Re, Fallon, Dzau, Quay and Haber, 1982), although the arterial wall may take up renin from the plasma (see Dzau, 1984b). Enzymes apart from renin, e.g. the tonin-α_1-macroglobulin complex, have been implicated in the cleaving of angiotensinogen thus there may not be an obligatory role for renin in local AII generation (Ikeda, Sasuguri, Maruta and Arakawa, 1988). The presence of angiotensinogen in smooth muscle cells suggests that AII synthesis takes place intracellularly (Dzau, 1984b). While the precise mechanism is uncertain it has been proposed that plasma angiotensinogen is internalised by endocytosis and fuses with a renin-containing vesicle. Angiotensin II could then be formed within this vesicle and subsequently released from the cell by exocytosis. It has not been established definitively that endothelial cells secrete AII, thus all, or part of the angiotensin could be released as AI and converted to AII at the cell surface.

Thus by virtue of a local vascular RAS, high [AII] may be produced in the immediate vicinity of its site of action, i.e. the vascular smooth muscle. This would account for the observed discrepancy between the affinity of AII for its receptor and *circulating* [AII]. At present, an appropriate means of distinguishing between theses two sources *in vivo* is not available.

Bradykinin

Kallikrein, the enzyme responsible for the conversion of kininogen to bradykinin exists in two forms: *plasma kallikrein*, which circulates, and *organ kallikrein*, which is found in exocrine glands (e.g. salivary glands and kidney) and allows local bradykinin production (see Johnston, Clappison, Anderson and Yasujima, 1982). Kininogen is synthesised predominantly in the liver, and the fetal rat liver has been shown to express kininogen genes (El-Dahr, Dipp and Chao, 1992). Bradykinin is inactivated by two sorts of kininases, kininase I and II. The most specific of these is kininase II, which is in fact identical to ACE [1.5.2- angiotensin II]. Thus the dual action of ACE/kininase II means that it can function to inactivate a dilator (bradykinin) as well as to activate a vasoconstrictor (AII). No assessment has yet been made on local vascular bradykinin production, but plasma and tissue level measurements show that ACE inhibition *in vivo* does elevate tissue [bradykinin] (see Johnston *et al.*, 1982; Holtz and Goetz, 1994).

Endogenous kinins contribute to the fall in blood pressure in the adult spontaneously hypertensive rat (Cachofeiro, Sakakibara and Nasjletti, 1992), and to the rise in renal papillary blood flow in the normal adult rat (Fenoy, Scicli, Carretero and Roman, 1991), in response to captopril administration. On the other hand ACE-inhibition increases the urinary excretion of bradykinin, but not circulating [bradykinin] in adult dogs (Clappison, Anderson and Johnston, 1981). In contrast, Robillard *et al.* (1983) observed that ACE inhibition produced a fall in fetal sheep blood pressure without any change in the

excretion of kallikrein. They suggested this to indicate that the major component of the hypotensive response to ACE-inhibition was mediated by inhibition of AII synthesis.

The role of bradykinin in the fetal circulation has been investigated predominently in the pulmonary vascular bed. Exogenous bradykinin produces pulmonary arterial vasodilatation (e.g. Konduri, Gervasio and Theodorou, 1993), but despite the fact that ventilation of fetal lungs stimulates bradykinin release, bradykinin is not crucial for oxygen-mediated pulmonary vasodilatation (Banerjee, Roman and Heymann, 1994).

The metabolic hypothesis

Local blood flow is adjusted to meet the local tissue demand of energy by the release of the products of tissue metabolism: thus low blood flow, or increased tissue energy demand, causes the build up of metabolites from a *non-vascular* element which exerts a vasodilator effect on the vasculature and restores/increases blood flow. During hypoxia, cerebral blood flow rises to match cerebral oxygen delivery to metabolic demand (see 1.4.7: Richardson, Patrick and Abduljabbar, 1985; see Bissonnette, Hohimer, Richardson and Machida, 1984). It seems likely that local metabolism will have greater influence than neurogenic mechanisms in this response (see Laudignon, Beharry, Rex and Aranda, 1990; Harper, 1990; Giussani, Spencer, Moore, Bennet and Hanson, 1993; Pearce, 1995). However fetal cerebral arteries appear to be much more sensitive to sympathetic neurotransmitters (via cerebrospinal fluid (CSF)) and sympathetic stimulation than the adult (Wagerle, Kurth and Roth, 1990), perhaps in order to protect small fetal cerebral vessels against acute fluctuations in MAP. Also in the fetal hindlimb, local metabolism may contribute to the good correlation between changes in peripheral blood flow and total O_2 consumption during stress (Jensen, Hohmann and Kunzel, 1987). Metabolic signals are likely to include H^+, K^+, adenosine, PGs and possibly NO.

To delineate the role of any metabolite in blood flow through a given vascular bed, a reliable way of measuring [metabolite] in extra-vascular sites is needed, whether it be the brain parenchyma or skeletal smooth muscle, since arterial plasma [metabolite] is unlikely to reflect local action. Increasing numbers of fetal studies report the effect of metabolites on blood flow to specific vascular beds, but techniques such as proton nuclear magnetic resonance (^{1}H NMR, Foxall, Bewley, Neild, Rodeck and Nicholson, 1995), near infra-red spectroscopy (NIRS: Wyatt, Edwards and Reynolds, 1993) and microdialysis (Koos, Mason, Punla and Adinolfi, 1994) will prove useful in addressing specifically the role of perivascular-derived metabolites on blood flow.

Prostaglandins

Nitric oxide appears to be both an endothelial-derived factor, being released by the direct action of hypoxia on the vascular endothelium, and a putative vasodilator metabolite

in the brain (see Pearce, 1995). In the same way PGs probably fall into two groups: those synthesised in the vascular endothelium and/or smooth muscle (e.g. prostacyclin (PGI_2)) which is co-released with NO in response to shear stress); and those synthesised elsewhere, for example in brain parenchyma, which then diffuse into the circulation. It is not easy to distinguish between the two sources, but PGs as a whole appear to contribute to a number of cardiovascular control mechanisms (for review see Longo and Pearce, 1991).

Prostaglandin synthesis-inhibition by indomethacin causes a rise in MAP and a baroreflex fall in FHR, followed by a sustained tachycardia. This suggests an overall peripheral vasoconstriction, probably by blocking PG-vasodilatation in specific vascular beds (Walker, Moore and Brace, 1992). In addition PGs may contribute to the rise in pulmonary blood flow seen at birth (Wang and Coceani, 1992) since PGI_2 synthesis is attenuated in low O_2 (Shaul, Campbell, Farrar and Magness, 1992), and to hypoxic cerebral vasodilatation in the neonate (see Pearce, 1995; Leffler, Busija, Fletcher, Beasley, Hessler and Green, 1985).

Administration of PGI_2 or PGE_2, or their fatty acid precursor arachidonic acid into the renal artery of anaesthetised adult rats produces a renal artery vasodilatation and attenuates the vasoconstrictor response to renal nerve stimulation (Inokuchi and Malik, 1984). Such vasodilator mechanisms appear to be functional in the fetus, since PG synthesis-inhibition by indomethacin reduces RBF (Matson, Stokes and Robillard, 1981). In addition, blockade of PG synthesis augments fetal renal vasoconstriction during hypoxia (Millard, Baig and Vatner, 1979), and a transient indomethacin-sensitive renal vasodilatation is revealed in renal denervated kidneys at the onset of hypoxia ([1.5.1] Robillard, Nakamura and DiBona, 1986). Other work does not support a modulatory role for PGs in renal hypoxic-vasoconstriction (Arnold-Aldea, Auslender and Parer, 1991). The action of PGs may be more regional, since blood is diverted from the inner to the outer cortex, where the nephrons are less mature (Stevenson and Lumbers, 1992).

H^+ and K^+

Perivascular changes in $[H^+]$ and $[K^+]$ may contribute to fetal cerebral hypoxic-vasodilatation (See Longo and Pearce, 1991). Increased *arterial* $[H^+]$ does not increase neonatal pig cerebral blood flow which indicates the presence of a functional blood-brain barrier to H^+ and reinforces the idea that changes in cerebral blood flow will only occur when H^+ is increased on the perivascular side, perhaps via increased PCO_2 (Wagerle, Kumar, Belik, Delivoria-Papadopoulos, 1988). Suffusion of the cortical surface of the neonatal pig brain with acidic cerebrospinal fluid dilates pial arterioles via an indomethacin-sensitive mechanism, perhaps by the production of arachidonic acid (Wagerle and Mishra, 1988). In the newborn lamb hydrochloric acid infusion via the

vena cava induces a metabolic acidaemia and diminishes blood flow to most vascular beds. It seems unlikely however that these responses reflect the action of H^+ as a metabolite, rather it could be partly acting as a chemoreceptor stimulant (Fisher, 1986).

Changes in extracellular [K^+] is believed to be important in linking adult brain metabolic activity with blood supply (McCarron and Halpern, 1990). Indeed in the adult rat, systemic hypoxic-vasodilatation involves the action of adenosine, mediated by the release of K^+ from skeletal muscle via ATP-sensitive K^+channels (Marshall, Thomas and Turner, 1993a). In the fetus, activation of ATP-sensitive K^+ channels causes a pulmonary vasodilatation (Cornfield, McQueston, McMurtry, Rodman and Abman, 1992), and in the adult lung a background K^+ conductance is thought to help maintain low vascular resistance (Hasunuma, Rodman and McMurtry, 1991)

ATP and adenosine

Plasma ATP is derived from red blood cells, vascular endothelium and tissues such as the heart, brain and liver. In the adult the major source of ATP is parenchymal cells but there is some suggestion that the placenta represents a significant source of adenosine throughout gestation (see Yoneyama and Power, 1992).

ATP and its metabolite adenosine are implicated in the pulmonary arterial vasodilatation during increased oxygenation in fetal sheep (Konduri, Gervasio and Theodorou, 1993) and may therefore be important in the transition of the pulmonary circulation at birth.

Adenosine receptor antagonism in fetal sheep during hypoxia suggests that adenosine modulates fetal metabolic responses, and mediates the bradycardia and raised MAP during hypoxia (Koos, Ogunyemi and Chau, 1995). ATP is found in adrenergic and cholinergic neurone synaptic terminals (see Rang and Dale, 1987), which suggests that it may be involved in the reflex neuronal responses to hypoxia. However augmented plasma [adenosine] does not alter the severity of the hypoxia-induced bradycardia or hypertension in fetal sheep (Yoneyama and Power, 1992). Marshall *et al.* (1993a) implicate adenosine in systemic hypoxic-vasodilation in adult rats, although other *in vitro* studies on adult aortic rings disagree (Yang and Metha, 1995). Furthermore adenosine administration to the fetal sheep causes a *rise* in FHR and plasma [catecholamine] which suggests adenosine-stimulated sympathetic nerve activity. Thus it is possible that during hypoxia adenosine plays a part in eliciting sympathetic peripheral *vasoconstriction* (Koos, Mason and Duscay, 1993).

In adults, blockade of adenosine receptors with theophylline reduces pial vessel diameter and cerebral blood flow, and attenuates the hypoxia-induced vasodilatation, suggesting a role for adenosine in the maintenance of cerebral vascular tone during

normoxia and hypoxia (Morii, Ngai, Ko and Winn, 1987). Microdialysis of fetal brain shows that [adenosine] is elevated at levels of hypoxia associated with the inhibition of FBMs (Koos, Mason, Punla and Adinolfi, 1994). Because adenosine dilates cerebral arterioles of fetal sheep ca. 0.7 gestation (see Pearce, 1995), the hypoxia-elevated brain [adenosine] (Koos *et al.,* 1994) may implicate adenosine in fetal cerebral vasodilatation during hypoxia.

Finally, central nervous system (CNS)-derived amino acid neurotransmitters, e.g. glutamate and aspartate, when administered topically to the neonate dilate cerebral arteries probably via NO rather than PG action (see Busija and Leffler, 1989).

1.5.4 Interaction of control mechanisms

From the previous sections it seems clear that a number of control mechanisms, i.e. neuronal, humoral and local, underlie fetal cardiovascular control. One common approach to delineating the contribution made by these different levels of control is to remove one, whether it be neuronal, humoral or local, and look at the effect on the cardiovascular system. The overriding message that has arisen from studies of this kind is that no single factor holds the complete answer to cardiovascular control. This is well illustrated by studies in the adult rat in which basal MAP is maintained as long as one of AVP, AII and α-adrenergic systems remained intact (Paller and Linas, 1984). It is interesting however that even when all these pressor systems hads been inactivated, despite a profound decrease in MAP, there is still sufficient residual blood pressure to permit survival. This is under basal conditions, but the picture is undoubtedly going to be more complicated under conditions of stress such as hypoxia where, as already outlined in previous sections, chemoreflex mechanisms are brought into action along with rises in humoral and perhaps endothelial-derived vasoactive factors. Here too the work of Giussani and colleagues (1993) showed that α-adrenergic efferent mechanisms do not fully account for the rapid reflex changes at the onset of hypoxia, and also that the rise in hormonal factors as hypoxia proceeds is likely to contribute to the slower cardiovascular changes [1.5.1 and 1.5.2]. In addition Scroop *et al.* (1992) concluded that cardiovascular homeostasis during haemorrhage in the late gestation sheep fetus is not critically dependent on one homeostatic mechanism, and that neuronal and hormonal mechanisms, in that case autonomic and AII, may act in concert.

As already mentioned pulsatile blood flow provides a continual stimulus for the release of endothelial-derived NO and PGI_2 (Busse, Hecker and Fleming, 1994. [1.5.3]). This continual release of vasodilator substances from the endothelium is likely to provide a means of counteracting neural, myogenic and hormonal vasoconstriction. For example,

NOS-inhibition augments ET-1 vasoconstriction of renal afferent arterioles *in vitro* (Ito, Juncos, Nushiro, Johnson and Carretero, 1991), and NO attenuates the vasoconstrictor response to nerve stimulation and noradrenaline in perfused segments of rat tail artery (Vo, Reid and Rand, 1991). There appear to be two possible processes which could underlie these findings: first, that increased shear stress via vasoconstriction itself promotes the release of vasodilators (see Hecker, Mulsch, Bassenge and Busse, 1993); and second, because in some instances NOS-inhibition has no effect on basal vascular tone (see Vo, Reid and Rand, 1991), it may be that vasoconstrictors can directly stimulate the production of vasodilators.

In support of the second process, numerous studies on cultured endothelial cells, isolated vessels and in the whole animal show a multitude of interactions between endothelial cells and circulating hormones, of circulating hormones and local vasoactive factors with neuronal mechanisms, and between the autocrine effects that have been demonstrated of endothelial-derived vasoactive substances. Thus a complicated picture of interactions is emerging.

Neuronal and hormonal: Some attention has already been given to the interaction between neuronal and hormonal mechanisms with regards to the extent to which the release of certain humoral factors such as ACTH/cortisol and AVP is controlled by reflex neuronal mechanisms [1.5.2]. The scope for the interaction of vasoactive substances such as AII, NO and ET-1 with the CNS in the modification of cardiovascular responses will be discussed in the following section [1.6.1]. Indeed ACE-inhibition influences sympathetic neurotransmission, probably via reduced AII formation and accumulation of bradykinin and PG (Schwieler, Kahan, Nussberger and Hjemdahl, 1993) and NO is a functional antagonist of sympathetic vasoconstriction in the premature brain (Wagerle, Moliken and Russo, 1995).

Endocrine and endothelium: The anatomical location of endothelial cells, i.e. adjacent to the blood stream, makes them an obvious target for humoral factors. The ability of endothelium to release vasoactive factors, to clear certain humoral factors from the circulation and to act as a physical or functional barrier to humoral factors makes interactions between humoral factors and the endothelium a major candidate in the control of vascular tone (see Pohl and Kaas, 1994). Alpha-adrenergic stimulation, AVP and oxytocin stimulate NO formation. On the other hand recent studies in fetal sheep show that infusion of AII does not stimulate vasodilator PG activity, which might attenuate its effects, but if anything stimulates synthesis of vasoconstrictor PGs which will augment its action (Stevenson and Lumbers, 1995), and AII does in fact stimulate ET-1 release (see Masaki, 1991; Masaki, Kimura, Yanagisawa and Goto, 1991).

There is evidence of an inverse relationship between local vascular ACE activity and endothelial NOS-expression. ACE may modulate NOS expression by altering local bradykinin production. The vasodilator action of bradykinin is mediated, at least in part, by the endothelium: bradykinin-induced relaxation of isolated strips of ductus venosus of late gestation sheep fetus is abolished by removal of the endothelium (Coceani, Kelsey and Seidlitz, 1994). Furthermore, bradykinin increases NO production by cultured fetal pulmonary endothelial cells (Shaul and Wells, 1994), and PGI_2 production by cultured HUVECs (McIntyre, Zimmerman, Satoh and Prescott, 1985), via bradykinin-receptor stimulation (Sung, Arleth, Shikano and Berkowitz, 1988). Indeed bradykinin-induced smooth muscle relaxation of intrapulmonary arterial rings *in vitro* causes an accumulation of cGMP and cAMP (Ignarro, Ryrns, Buga and Wood, 1987). A number of adult studies have shown that NOS-inhibition reduces plasma [AII] (Goyer, Biu, Chou, Evans, Keil and Reid, 1994; Reid, Bui and Chou, 1994). The interaction between the RAS and NOS will be discussed in Chapter 6.

Endothelial-derived factors also act as autocoids (Whittle, Lopez-Belmonte and Rees, 1989): for example ET-1 can, as well as acting in a paracrine fashion to vasoconstrict the underlying smooth muscle, exert a vasodilator action probably via the endothelial cell-ET_B receptor subtype to release NO and PGI_2 (Tod and Cassin, 1992). There is also evidence to suggest that the endothelial-derived PGI_2 and NO synergise in their inhibition of platelet aggregation (Radomski, Palmer and Moncada, 1987). Other interactions such as ANF-stimulated AVP release may be important under conditions of stress in the fetus (see Berry, Jaekle and Rose, 1994).

While the experimental evidence of hormonal-endothelial interactions is growing, their actual physiological/pathophysiological significance is not well understood. However, it is becoming increasingly obvious that no one control mechanism is likely to act alone in fetal haemodynamic control during normoxia or hypoxia at any given time. It is probable that local mechanisms are being recruited directly by the challenge, or their activity being co-ordinated by reflex and endocrine mechanisms, although the extent to which local vasodilator/constrictor mechanisms dominate is likely to depend on the vascular bed and the local PO_2 (see Marshall, Lloyd and Mian, 1993; Wood, 1993). While some investigations of local mechanisms have been made on the fetal pulmonary and renal circulations there is, to my knowledge, little information to date on the fetal systemic vasculature.

1.6 Integration of cardiovascular control mechanisms

1.6.1 The brain stem

There is now quite a large body of work documenting the participation of central nervous mechanisms, particularly those of the brain stem, in cardiovascular control in the adult. It is known that arterial baro- and chemoreceptor reflex afferents, along with input from the pulmonary and airway receptors, relay to the CNS mainly via the vagus and glossopharyngeal nerves and terminate in the NTS (Lipski, McAllen and Spyer, 1975) located within the medulla. This region is densely innervated by other regions of the CNS concerned with cardiorespiratory control and from electrophysiological mapping there appears to be a level of organisation within the NTS itself such that baroreceptor afferents project to different areas than chemoreceptors (Donoghue, Felder, Jordan and Spyer, 1984). However connections between the medulla, pons, midbrain and hypothalamus seem to be essential for a fully integrated response. For example, the parabrachial nucleus (PBN) has been shown to exert a powerful modulatory influence on the activity of NTS neurones which may be more potent than the influence of other descending control areas such as the hypothalamic defence area (Coote, Hilton and Perez-Gonzalez, 1979; Felder and Mifflin, 1988; see Spyer, 1981; Spyer, 1994). Similarly, activation of the cerebellar posterior vermis exerts an inhibitory action on baroreceptor input at the level of the NTS (Paton, Sila-Carvalho, Goldsmith and Spyer, 1990) via pathways involving the PBN, NTS and rostral ventrolateral medulla (see Paton and Spyer, 1992). GABA-containing neurones located within the NTS are thought not only to play a major part in the integration of the cardiorespiratory reflexes, but also to participate in modifying these reflexes (see Spyer, 1994 for recent review of this area).

Information on these brain stem integrative mechanisms in the fetus is sparse. There is some implication of descending inhibitory pathways from fetal sheep experiments, since rostral section of the brain stem (through the caudal hypothalamus) enhances the baroreflex response to phenylephrine-induced hypertension (Dawes, Gardner, Johnston and Walker, 1983). In the neonatal sheep there is a biphasic ventilatory response to hypoxia: a transient increase followed by a decrease. Cooling in the rostral pons reverses the blockade on ventilation during hypoxia which suggests a descending inhibitory influence from, or passing through this region, on ventilatory processes (Moore, Parkes, Noble and Hanson, 1991) perhaps at the level of the NTS. Similar mechanisms are implicated in the fetus (see [1.4.9]).

Angiotensin II

Modification of baroreflex control of heart rate could take place at a number of levels: by alteration of vascular distensibility, at the CNS or at the heart. Intracerebroventricular (i.c.v.) administered AII resets, but does not change the sensitivity of, the baroreflex control of heart rate, probably by acting centrally to withdraw vagal tone from the heart. This might explain the increased MAP without altered heart rate often observed in response to AII (Reid and Chou, 1990). Angiotensin II may act at the level of the area postrema, known to have projections to the NTS, to inhibit vagal tone (see Ismay, Lumbers and Stevens, 1979). A similar mechanism might explain the reduced baroreflex sensitivity seen in renin-hypertensive animals (see Moreira, Ida, Pires and Krieger, 1994). Furthermore, administration of an ACE-inhibitor prior to birth, implicates AII in the resetting of the baroreflex postnatally in the sheep model (Segar, Mazursky and Robillard, 1994), although this does not appear to be true during the perinatal period (Segar, Merrill and Robillard, 1994).

Nitric oxide

There is now quite a large body of evidence to implicate NO in the modulation of baro- and chemoreceptor activity. Nitric oxide administration to the carotid sinus region *in vitro* suppresses baroreceptor discharge. This is not related to vascular relaxation of the carotid artery or increased guanylate cyclase activity, but perhaps a direct effect on baroreceptor excitability via a K^+ channel-activated hyperpolarization (Matsuda, Bates, Lewis, Abbound and Chapleau, 1995). On the other hand, whole baroreceptor nerve recordings in the adult anaesthetised rat show that guanylate cyclase activation is involved in the NO modulation of baroreceptor resetting during hypertension (Vargas, Dias da Silva, Ballejo, Salgado and Salgado, 1994). Carotid body generated NO modulates steady-state and hypoxia-stimulated carotid chemoreceptor activity in the anaesthetised adult rat (Trzebski, Sato, Susuki and Sato, 1995; Wang, Stensaas, Dinger and Fidone, 1995). Chronic NO-inhibition enhances the bradycardic component of the baroreflex in rats (Vasquez, Cunha and Cabral, 1994). A CNS action of NO is suggested since administration of NOS-inhibitor to the ventral surface of the medulla oblongata enhances sympathetic outflow. Therefore NO may participate in blood pressure regulation by reducing sympathetic tone in peripheral vessels (Togashi *et al.*, 1992). Thus it appears that NO modulates sympathetic efferent activity by interacting with sympathetic pathways in the CNS rather than with afferent or efferent pathways in the baroreflex arc (Jimbo, Susuki, Ichikawa, Kumagai, Nishizawa and Saruta, 1994). A role for NO in the integration of autonomic cardiovascular control is further supported by the suggestion that NO is formed in NTS neurones (Tagawa *et al.*, 1994). To my knowledge the effect of NO on the integration of baro- and chemoreceptor inputs has not been addressed in the fetus.

Endothelin-I

Endothelin-1 is implicated in the central control of cardiovascular function and in cerebrovascular control. Infusion of ET-1 (i.c.v.) does not alter total or regional cerebral blood flows, but causes a marked vasoconstriction in the medulla oblongata and medulla spinalis where cardiovascular and respiratory centres are located (Granstam, Wang and Bill, 1993). It is thus of interest that i.c.v. ET-1 causes increases in heart rate, cardiac output, coronary blood flow and MAP, perhaps by increased sympathetic outflow. Ferguson and Smith (1990) injected ET-1 into the area postrema of anaesthetised rats, an area located at the dorsal surface of the medulla, with no blood brain barrier and with projections to the NTS. It is implicated as a chemoreceptor zone and may be involved in cardiovascular control. They observed that low doses of ET-1 (0.2-1.0pmol) increased, high doses of ET-1 (5.0pmol) decreased and intermediate doses (2pmol) of ET-1 cause a biphasic MAP response. Intracisternal (i.c.) ET-1 administration (directed towards the medullary cardiovascular control centres) caused a rise in cerebral blood flow in forebrain areas and a reduction in flow to areas of the brain stem (Macrae, Robinson, McAuley, Rei and McCulloch, 1991). They too observed a rise in MAP, but attributed it to brain stem ischaemia rather than a direct effect of ET-1 on cardiovascular control centres. The ET_A receptor antagonist, FR139317 (i.c.), inhibits basilar artery constriction in subarachnoid haemorrhage, but has no effect under basal conditions in dogs (Nirei, Hamada, Shoubo, Sogabe, Notsu and Ono, 1993). In the adult cat ET-1 binding sites are localised in the carotid bifurcation, as well as in the NTS where the carotid baro- and chemoreceptor afferent fibres terminate (Spyer, McQueen, Dashwood, Sykes, Daly and Muddle, 1991; Dashwood *et al.*, 1993). These findings may have important implications for the role of ET-1 in cardiovascular control.

1.6.2 The kidney

Distribution of fluid

Under normal conditions quantitative changes in extracellular fluid volume (vascular and interstitial fluid) can only be achieved by changing the balance between net fluid intake (oral fluid consumption minus fluid loss by all routes other than renal) and renal fluid output. The regulation of extracellular volume is crucial for water and electrolyte balance but also for the function of the circulatory system. Thus volume control by the fetal kidney is likely to represent an important mechanism by which long term blood pressure regulation can be achieved. In addition the kidney may provide a model in which organ function can be related to the blood flow to it.

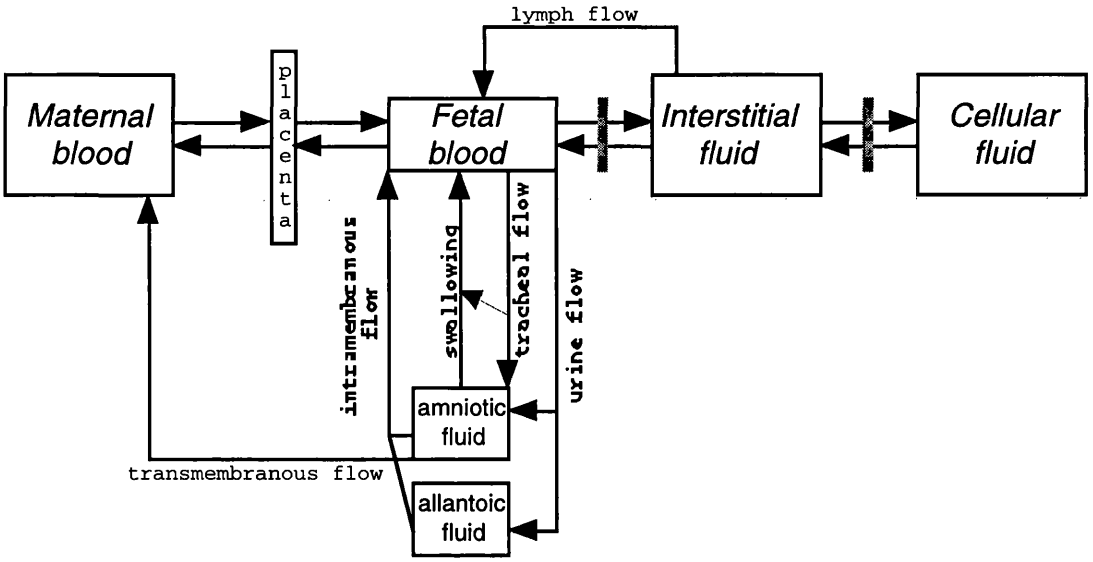


Figure 1.8 Schematic representation of fluid compartments and routes of fluid movement in the sheep fetus. Arrows indicate the direction of fluid movement and the grey bars represent membranes. Adapted from R.A. Brace 'Fetal fluid balance' In: Textbook of Fetal Physiology, 1994.

Under normal conditions the sheep fetus, in the last third of gestation, is surrounded by amniotic fluid. The allantois, not found in humans at term, forms a separate compartment stretching from the pregnant to the non-pregnant horn of the uterus. The compartment volumes remain relatively constant in relation to gestational age (Wlodek, Harding and Thorburn, 1992). The intrauterine water environment appears to be in a dynamic state. A *transmembranous* exchange of fluid and solutes occurs across the amniochorion between the amniotic cavity and the maternal blood supply of the uterine wall (Figure 1.8). An *intramembranous* exchange of fluid occurs between fetal blood and the amniotic and allantoic cavities via the fetal placental surface, fetal blood of the fetal membranes, the fetal skin and the surface of the umbilical cord (Brace, 1994). In the late gestation sheep fetus urine is the major source for amniotic and allantoic fluid, being supplied via the urethra and the urachus (passes through the umbilical cord), respectively (Ross, Ervin, Rappaport, Youssef, Leake and Fisher, 1988; Wlodek, Challis and Patrick, 1988) and appears to be essential for normal fetal development (Buddingh, Parker, Ishizaki and Tyler, 1971 Wintour and Shandley, 1993). 400-800ml of urine is voided (micturition) into each of these compartments on a daily basis by contraction of the bladder (2-4 contractions.h^{-1}: Walker, 1977; Wlodek *et al.*, 1988). This voiding appears to be of a constant frequency, but of increasing volume, with advancing gestation (Wlodek *et al.*, 1992). Micturition appears to be influenced by descending information from the brain, since bladder contractions are greater after a change to LV-ECoG than after a change to HV-ECoG activity (Wlodek, Thorburn and Harding, 1989) and usually occur in association with fetal breathing (Walker, 1977). Between 120-148 days gestation ovine fetal lung liquid is produced at a rate of 4.5±0.1ml.kg.h^{-1} (Mescher, Platzker, Ballard, kitterman, Clements and Tooley, 1975), which contributes to amniotic fluid volume.

Bladder outlet obstruction (urethra and urachus) in fetal sheep results in oligohydramnios (Peters, Docimo, Luetic, Reid, Retik and Mandell, 1991). The identification of oligohydramnios (low amniotic fluid volume) may provide some indication of an altered fetal renal or fetal membrane function. In clinical practice real-time ultrasound imaging is used routinely to make qualitative assessments of amniotic fluid volume. This measurement, combined with those of FBMs, fetal body movements and FHR, allows fetal biophysical profile scoring and recognition of a compromised (e.g. growth retarded) fetus (Lin, Sheikh and Lopata, 1990; Manning, 1992).

The determining source of water for the fetus is that derived from the mother across the placenta. There is an overall movement of fluid *into* the fetus with advancing gestation (Figure 1.8). Placental water supply is larger than actually required by the fetus which might explain why the fetus produces urine which is far more dilute than that of the adult (Hill and Lumbers, 1988). In the adult the quantity of urine produced is dependent upon GFR, proximal and distal tubular sodium and fluid absorption, and the action of AVP. In the fetal sheep GFR increases over the period between 106 and 140 days gestation. Below 130 days gestation this can be accounted for by the addition of new nephron units. However, because nephrogenesis is known to cease after ca. 130 days gestation (Robillard, Weismann and Herin, 1981), a rise in GFR after this date might be explained by increases in surface area for filtration, effective filtration pressure, and capillary filtration. That the kidney of the late-gestation ovine fetus is capable of regulating its body fluid volume is evidenced by experiments in which fetal urine output (UO) is reduced in response to a reduced water supply from the mother (Lumbers, 1983; Lumbers and Stevens, 1983) and in response to fetal haemorrhage (Gomez and Robillard, 1984), and raised in response to fetal blood volume expansion (Schroder, Gilbert and Power, 1984). The mechanisms which regulate UO in the fetus are not fully understood.

Basal regulation

The reduced ability to concentrate urine in the fetus compared to the adult might be due to an immature medulla (short loops of Henle) and high blood flow through the vasa recta which would limit the build up of an osmotic gradient by the fetal kidney, combined with the relative insensitivity of the fetal nephron to AVP (Robillard, Smith, Segar, Guillery and Jose, 1994). The fetus displays a classical antidiuretic response to infusion of AVP, but the maximum osmolarity reached (500mosmol.kg^{-1} H_2O) is lower than that in the adult (2500-3000mosmol.kg^{-1} H_2O) (Lingwood, Hardy, Horacek, McPhee, Scoggins and Wintour, 1978). The variable response to AVP in early gestation sheep fetuses (<120 days) is thought to be due to confounding AVP V_1 receptor-mediated changes in blood pressure, rather than AVP V_2 (renal) receptor immaturity (Ervin *et al.*, 1994), although it has been suggested that AVP receptors may not be fully functional in the late-

gestation fetus (Robillard and Weitzman, 1980). Aldosterone infusion to early (<115 days gestation) or late (>125 days gestation) gestation sheep fetuses does not alter UO or GFR (Robillard, Nakamura and Lawton, 1985).

Maintenance of normal renal function of the fetus is dependent on the RAS, since administration of the ACE-inhibitor captopril causes a fall in urine flow in agreement with some adult studies (Yesburg, Henderson, Wilson, Law and Cross, 1982). This is observed in conjunction with a raised RBF and reduced GFR and RVR, which suggests that AII exerts this effect via an efferent arteriolar action (Lumbers, Kingsford, Menzies and Stevens, 1992; Lumbers, Burrell, Menzies and Stevens, 1993). Similarly, saralasin infusion to the fetal sheep reduces GFR with a consequent fall in urine flow (Lumbers and Stevens, 1987). However other sheep studies suggest that this response, seen in fetuses <120 days, is absent in those >130 days gestation (Robillard, Weismann, Gomez, Ayres, Lawton and VanOrden, 1983).

Nitric oxide-synthesis is implicated in maintaining basal UO in the adult rat (Lahera, Salom, Miranda-Guardiola, Moncada and Romero, 1991; Lahera and Khraibi, 1994) and anaesthetised dog (Naess, Christensen, Kirkeben and Kiil, 1993), since L-NAME produces a fall in UO, probably via an effect on tubular reabsorption mediated by cGMP. Although NOS-inhibition in the adult rabbit causes a *diuresis* (Denton and Anderson, 1994) it has no effect on UO in the ovine fetus (Bogaert, Kogan and Mevorach, 1993). NOS is implicated in the modulation of micturition in the late-gestation fetal (Mevorach, Bogaert and Kogan, 1994) and adult (Thornbury, Hollywood and McHale, 1992) sheep. The diuretic action of exogenous ET-1 in the adult anaesthetised rat appears to be due to be a blood-pressure related phenomenon rather than a direct action on the kidney (Uzuner and Banks, 1993), although specific ET-1 binding sites, linked to PGE_2 production, have been located on adult rat renal glomeruli (Orita, Fujiwara, Ochi, Takama, Fukunaga and Yokoyama, 1989).

Sympathetic innervation of the kidney via the renal nerve does not appear to be important in the maintenance of basal UO in the fetus (Robillard, Nakamura and Dibona, 1986). In the fetus, a pressure diuresis may contribute to urine flow (Brace and Moore, 1991; Wlodek, Challis, Richardson and Patrick, 1989), indeed in fetal sheep and a 24 h rhythm has been observed in MAP and UO, with levels rising from noon to midnight (Brace and Moore, 1991).

Hypoxic regulation

In the human fetus UO correlates with the degree of fetal hypoxia (Nicolaides, Peters, Vyas, Rabinowitz, Rosen and Campbell, 1990). Indeed urine osmolarity is thought to be a good index of fetal stress (Wintour, Bell, Congui, MacIsaac and Wang, 1985). In fetal

sheep, experimental manipulation of fetal oxygenation produces either a fall (1 h asphyxia: Daniel, Yeh, Bowe, Fukunaga and James, 1975; 30 min hypoxia: Daniel, Stark, Hussain, Sanocka and James, 1984; Nakamura, Ayers, Gomez and Robillard, 1985; Walker, 1977; 1-3 h hypoxia: Brace, Wlodek, McCrabb and Harding, 1994), or no change (1 h hypoxia: Robillard, Weitzman, Burmeister and Smith, 1981; Wlodek, Challis, Richardson and Patrick, 1989) in UO. When hypoxia is prolonged UO increases above control levels (Cock, Wlodek, Hooper, McCrabb and Harding, 1994). The mechanisms of UO control during hypoxia are not understood. It may be that a component of the control of UO in hypoxia is by the direct action on the kidney of hormonal factors, whose levels are known to alter during hypoxia [1.5.2]. It is well established that plasma [AVP] rises during hypoxia (Giussani, McGarrigle, Spencer, Moore, Bennet and Hanson, 1994b; Daniel, Stark, Zubrow, Fox, Husain and James, 1983), and this is reflected by excreted levels of AVP (Daniel, Stark, Husain, Sanocka and James, 1984). Furthermore experimentally induced fetal dehydration of the fetal sheep induces AVP release, which in turn increases in urine osmolarity (Herin, Kim, Schrier, Meschia and Battaglia, 1988).

Plasma [ANF] rises during hypoxia (Cheung and Brace, 1988). The ovine kidney is known to be responsive to the natriuretic effect of ANF by the last third of gestation, and ANF infusion increases GFR without a change in MAP (Shine, McDougall, Towstoless and Wintour, 1987). In other fetal sheep studies immunosupression of ANF produces a maintained rise in MAP but a fall in UO, which suggests a diuretic role for ANF, probably via an increase in GFR (Cheung, 1991), rather than via MAP changes (Brace and Cheung, 1987). However other studies show no change in UO or GFR with ANF infusion (Robillard, Nakamura, Varille, Andressen, Matherne and VanOrden, 1988b). Volume expansion in the ovine fetus is associated with a rise in UO and rise in circulating [ANF] (Brace, Miner, Siderowf and Cheung, 1988).

Other potential hormonal modulators of UO during hypoxia include catecholamines, the RAS, ET and PGs. Intravenous infusion of adrenaline to the sheep fetus, producing a plasma [adrenaline] similar to those seen at birth, increases GFR and urine flow, independent of changes in [ANF]. The diuretic action of adrenaline is likely to be via changes in renal or extrarenal haemodynamics (Ervin, Castro, Sherman, Ross, Padbury, Leake and Fisher, 1991). While there is some evidence of a diuretic role for AII in the fetus (e.g. Lumbers, Burrell, Menzies and Stevens, 1993), other studies do not support a role for the RAS in the regulation of renal function (urine flow and GFR) under nomoxic or hypoxic conditions (Robillard, Gomez, VanOrden and Smith, 1982; Nakamura, Ayres, Gomez and Robillard, 1985). Low doses of ET-1 (1-10ng.kg^{-1}.min^{-1}) to the adult anaesthetised rat produces a diuresis, despite a systemic hypotension and low renal perfusion pressure, which suggests an suppression of renal tubular

reabsorption perhaps at a proximal site (Harris, Zhuo, Medelsohn and Skinner, 1991). In the human, management of preterm labour with PG-synthesis inhibitors, such as meclofenamate, is associated with oligohydramnios. Fetal sheep studies, infusing indomethacin or meclofenamate, suggest that this is consequent upon a reduced UO (Walker, Moore, Cheung and Brace, 1992; Walker, Moore and Brace, 1994; Wlodek, Harding and Thorburn, 1994) and lung liquid secretion (Wlodek *et al.*, 1994; Stevenson and Lumbers, 1992). Oliguria observed secondary to indomethacin treatment is associated with elevated plasma [AVP] in fetal sheep (Walker, Moore, Cheung and Brace, 1992) and has been shown to be mediated through renal AVP V_2-receptor stimulation (Walker, Moore and Brace, 1994). In other studies, a *rise* in UO after indomethacin infusion was partly attributed to a pressure diuresis (Walker, Moore and Brace, 1992).

In the adult anaesthetised rat the antidiuresis observed during hypoxia is attributed to the associated fall in MAP (Neylon, Marshall and Johns, 1995). In the fetus, because it is a rise in MAP which is usually observed during hypoxia, any pressure mediated effect is likely to be of a diuretic nature. Indeed a correlation has been demonstrated between UO and MAP in the sheep fetus (Brace and Moore, 1991; Wlodek, Challis, Richardson and Patrick, 1989).

In adults there is extensive adrenergic innervation of the afferent and efferent glomerular arterioles, proximal and distal renal tubules, ascending limb of Henle's loop and juxtaglomerular apparatus. Renal sympathetic nerve activity is implicated in the regulation of urinary water excretion, either via changes renal haemodynamics ([1.5.1 and below]. see Honig, 1989), renin release from juxtaglomerular apparatus, or by a direct action on innervated renal tubules (DiBona, 1989). There is evidence that these mechanisms operate in the sheep fetus (see Robillard, Porter and Jose, 1994) since adrenaline infusion produces a rise in UO (Ervin *et al.*, 1991), although autonomic blockade (with hexamethonium) does not alter the diuretic response to volume expansion (Miner, Cheung and Brace, 1988).

One school of thought, which would indeed provide an explanation for oligohydramnios observed in pregnancy, is that the fall in UO during hypoxia is secondary to a fall in RBF. In human fetuses a significant negative correlation has been observed between RVR (increased pulsatility index from Doppler velocity waveforms) and amniotic fluid volume (Arduini and Rizzo, 1991). $Alpha_1$-receptor stimulation produces a fall in RBF and rise in RVR which might play an important role in the concomitant reduced UO (Guillery, Segar, Merrill, Nakamura, Jose and Robillard, 1994). In addition, a significant relationship has been observed between RBF and UO in human fetuses of cocaine abusers (Mitra, Ganesh and Apuzzio, 1994). In fetal sheep

unilateral renal nerve denervation reveals a significant role for renal nerves in the fall in RBF during hypoxia but has no effect on the GFR and urinary flow rate (Robillard, Nakamura and DiBona, 1986). They suggested that a rise in circulating [catecholamines] may mask any differences in UO between intact and denervated fetuses, and indeed it may be that the denervated kidney was sensitised to the diuretic effects of catecholamines.

In light of the role of neuronal [1.5.1] and hormonal (endocrine [1.5.2] and local [1.5.3]) mechanisms in the regulation of RBF during normoxia and hypoxia, and what is known of the effect on UO by direct action of hormonal factors on the kidney, coupled with the importance of UO in the adjustment of extracellular fluid volume, the concept of the kidney being an integrator of the different levels of cardiovascular control mechanisms emerges.

1.7 Outline and Aims of Thesis

1. At the time that the work contained in this Ph.D. thesis commenced there were two initial concepts which I wanted to pursue. The first stemmed from the observed effects of hypoxia on RBF, renal sympathetic nerves (RSNs) and UO in the adult and in the fetus. Peripheral chemoreceptors had already been implicated in fetal peripheral blood flow changes during hypoxia (Giussani, Spencer, Moore, Bennet and Hanson, 1993) and there was a suggestion that they might mediate the fall in RBF in the adult during hypoxia, however it was not known whether the fetal renal vasoconstriction was chemoreflexly-mediated. Moreover up to this point continuous and direct measurements of RBF and UO had not been correlated in an animal model. Thus the aim of Chapter 3 was:

To establish a relationship between RBF and UO during normoxia and hypoxia.

I have continuously monitored RBF using ultrasound Transonic flow probes [2.3.2] with simultaneous measurement of UO during an acute hypoxic insult. In addition I have investigated the role of chemoreflex mechanisms in the control of RBF, and its relation to UO, by bilateral section of the CSNs. Furthermore I have made investigations into the vasoconstrictor- and vasodilator-hormonal mechanisms of RBF control by measuring plasma [catecholamines] in these fetuses and by blockade of NOS in a small subset of fetuses, respectively.

2. The second concept I wanted to pursue followed on from the studies of Giussani *et al.* (1993): CSD combined with α-adrenergic blockade significantly impairs fetal survival during hypoxia, whereas intact fetuses survive α-adrenergic blockade. Therefore, it is

possible that the CSNs may mediate the release of another vasoconstrictor hormone to complement the efferent α-adrenergic limb. AVP and ACTH and cortisol do not appear to fulfil this role since their rise in hypoxia is not altered by CSD [1.5.2]. However AII is another vasoconstrictor candidate. [Angiotensin II] rises during hypoxia (Broughton-Pipkin, Lumbers and Mott, 1974) and peripheral chemoreceptors have been implicated in the rise in [AII] in response to hypoxia *with* hypercapnia in the ovine fetus (Wood, Kane and Raff, 1990). Thus the aim of Chapter 4 was:

To investigate the extent to which the rise in [AII] during hypoxia is mediated by carotid chemo- and baroreflex mechanisms. *I have measured plasma [AII] during acute hypoxia in intact and CSD fetuses. In addition I have used an inhibitor of ACE, captopril, to investigate further the role of AII in the rapid and the slower (rise in MAP and sustained peripheral vasoconstriction) cardiovascular responses to hypoxia.*

3. In light of the results obtained in the AII study, and the increasing evidence of a local-vascular, as well as a circulating, RAS I went on to investigate other endothelial-derived hormonal factors which are brought into action by hypoxia at the local tissue level. Thus the aim of Chapters 5 and 6 was:

To investigate the role played by ET-1 and NO in fetal systemic cardiovascular responses to hypoxia. *To date the majority of information obtained on ET-1 and NO in the fetus has been in the pulmonary circulation. I have used an ET_A receptor blocker (FR139317) and the NOS-inhibitor, L-NAME to investigate a role for endogenous ET-1 and NO, respectively, in fetal systemic cardiovascular control.*

4. Having established the basal systemic haemodynamic effects of *short-term* NOS-inhibition, I went on to consider the role played NOS over a longer time period on systemic cardiovascular control. This preliminary study was particularly timely in view of recent evidence in which maternal administration of L-NAME has been associated with reduced fetal size and deformation of the hindlimb (Diket *et al.*, 1994). Thus the final aim of the thesis was:

To examine the effect of long-term NOS-inhibition on fetal systemic cardiovascular control.

CHAPTER 2
GENERAL METHODS

2.1 Sheep Husbandry

2.1.1 Impregnation of sheep

Pregnant Suffolk and Blue-faced Leicester Cross Mules were supplied by R. White & Co., Turville Park Farm, Turville, Oxon. The sheep has an oestrous cycle of ca. 17 days and a gestation period of ca. 147 days but a short natural breeding season (dependent on breed). This means that the period of time available for fetal research is limited. Thus it is common practice to extend the fertility period of the ewe artificially. This is done by synchronising the oestrous cycle of groups of ewes by intravaginally inserted progesterone-impregnated sponges. Pregnant mare's serum (PMS; 400 i.u. serum gonadotrophin) was injected i.m. to cause superovulation. Rams, fitted with crayon markers, were introduced and a crayon mark on the ewe's rump was taken as evidence of mating. When the ewe would not stand for mating after ca. 17 days (length of estrous cycle) pregnancy was assumed. Pregnancy was confirmed by real time ultrasound at ca. 80 days gestation.

Thus it was possible to maintain a steady supply of ewes of known gestational age for a 6-8 month period of the year.

2.1.2 Sheep maintenance

Ewes were held in straw and sawdust-lined floor pens for a minimum of 24 h following transportation from the farm to the Biological Services Department, University College London. After this period of acclimatisation ewes were put up into individual metabolic carts and transferred to an adjacent monitoring room. Ewes were accompanied at all times by at least one companion sheep.

Temperature was maintained between 16 and 18°C with ca. 40% humidity. Light-dark periods were fixed on a 12 h/12 h cycle. Ewes were given free access to hay and water and a daily bowl of concentrate pellets (Ewbol, BOCM Pauls Ltd., UK).

2.2 Materials

2.2.1 Electrodes

Electrodes were made by threading teflon-coated multi-stranded stainless steel wire (Cooner Wire, Ca. USA) through polyvinyl catheters (Portex Ltd. UK, I.D. 1.5mm, O.D. 2.1mm). Catheters were sealed, by injecting silicone sealant (RS components, UK) into the lumen for a distance of ca. 3cm. Two types of electrodes were made for each fetal instrumentation: 1) Electrocardiogram (ECG), diaphragm and earthing electrode: Vinyl tubing length=0.9m, Cooner wire length= 2 x 1.1m (Figure 2.1A). 2) ECoG electrode: Vinyl tubing length=0.9m, Cooner wire length= 3 x 1.1m. This electrode is comprised of 2 signal wires and 1 earth wire. The distal end of the earth wire was marked by a knot (Figure 2.1B).

1-2cm Cooner wire was left at the distal ends of electrodes to allow connection to head stage pins.

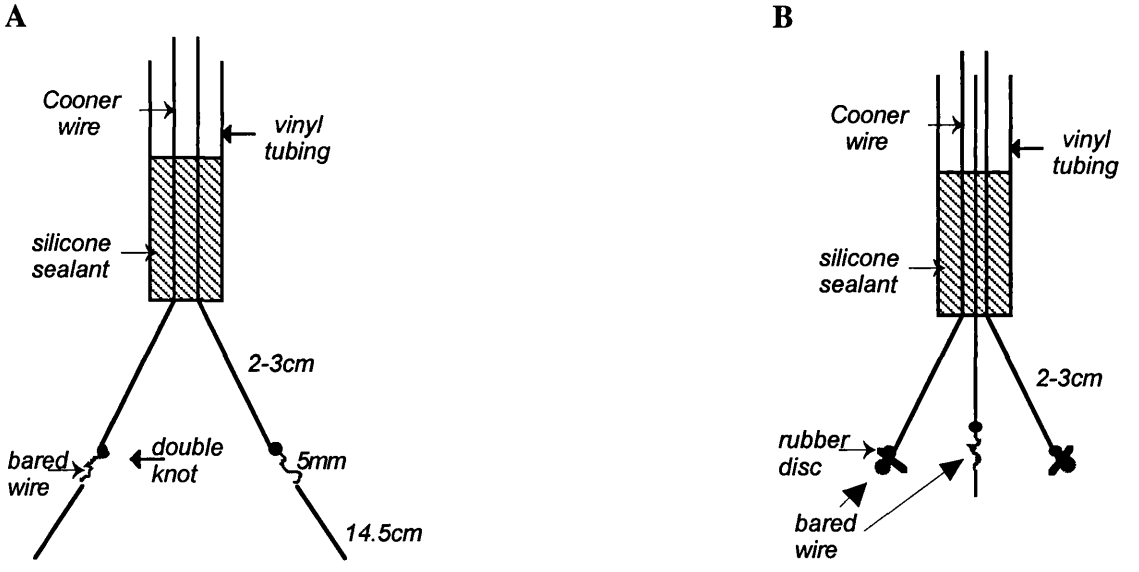


Figure 2.1 Diagrammatic representation of the proximal ends of A). ECG, diaphragm and earth electrodes, and B). the ECoG electrode.

2.2.2 Catheters

Fetal vascular, tracheal and bladder, amniotic and maternal pedal venous catheters were made of 1.5m lengths of vinyl tubing (I.D. 1.5mm, O.D. 2.0mm). An 18-gauge blunt (18G x 1", Sherwood Medical Industries Ltd., UK) was inserted in the distal end of the catheter. The proximal end of vascular catheters were cut into a bevel. Four to 5 small

holes were cut at 1cm intervals at the proximal end of the amniotic catheter. A 2cm length of vinyl tubing (I.D. 2mm, O.D. 3mm) was attached to the proximal end of the bladder catheter using silicone sealant, taking care to maintain patency of the catheter.

All catheters were colour-coded at both proximal and distal ends for easy identification.

Infusion lines were made out of 1m lengths of vinyl tubing (I.D. 1.5mm, O.D. 2.0mm). An 18-gauge blunt was inserted into each end of the catheter. A double cone-connector (Vygon (UK) Ltd.) was fitted into one of the blunts to allow connection to a vascular catheter blunt.

2.2.3 Flow probes

The ultrasound Transonic transit time flow probe, like the electromagnetic flow probe, is an integrating volume flowsensor. But unlike the electromagnetic probe, the Transonic probe is fitted loosely around the vessel, and vessel wall conductive properties are integrated in the volume flow measurement. The Transonic probe is made up of two transducers, which lie on one side of the vessel in the flow probe body, and an acoustic reflector which is positioned between the two transducers on the other side of the vessel (Figure 2.2). The 'downstream' transducer (ii) emits a plane wave of ultrasound which passes through the vessel, bounces off the reflector plate, passes through the vessel once more and is received by the upstream transducer (i). This produces electrical signals which are used to derive the time it took for the signal to pass from one transducer to the other (transit time). The same chain of events then occurs in the opposite direction with a ultrasound wave emitted from the upstream transducer (i). A second transit time is thereby derived. Both transit times are modified by a vector component of blood flow, thus the full transit time of the ultrasound beam senses the sum of the two vector components, i.e. flow itself. Misalignment of the vessel and probe has little effect on the sum of the vectors since the two vectors will show an inversely proportional relationship. The wide beam of the ultrasound integrates velocity chord elements across the full width of the flow-sensing window and thus is independent of vessel diameter.

Bench-top calibration is conducted by the manufacturer whereby the flow of a fixed temperature fluid through plastic tubing is measured by the probe itself and compared to the volume of this fluid collected over a period of time. In chronic-implant situations, fibrous tissue is likely to grow into any space between the probe and the vessel, tending to centre the vessel in the strongest part of the acoustic field. The flow probes are calibrated by the manufacturers accordingly, so that the slope of the relationship derived from the bench-top calibration equals 1.00. Each flow probe was supplied with its own specifications and all probes had a maximum error in absolute volume flow of ±10%.

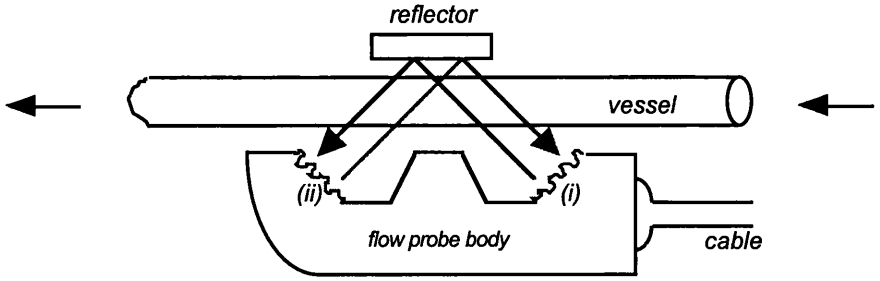


Figure 2.2 Side view of Transonic flow probe showing ultrasound waves, emitted from upstream (ii) and downstream transducers, intersecting the blood vessel, bouncing off the acoustic reflector and being received by the other transducer.

Prior to implantation the silicone cable jacket was inspected for superficial cuts. The probe was then immersed in a small container of water for 5 min and air bubbles were displaced from around the probe head. The test signal and zero-flow offset were verified and compared to the probe specification sheet.

2.3 Surgical procedures

All surgical and experimental procedures contained in this thesis were authorised by the Home Office and were conducted in accordance with the Animals (Scientific Procedures) Act, 1986.

2.3.1 Preparation and anaesthesia

Twenty-four hours prior to surgery food was withheld from the ewe, but water was allowed *ad libitum.* This precaution was taken firstly to minimise the risk of bloat (ruminal distension), which could impair ventilation of the ewe, and secondly to reduce the possibility of inhalation of the rumen contents during the regurgitation which was likely to occur during surgery. Finally reduction of rumen size provides more space for fetal manipulation during surgery. On the day of surgery a pre-operative check was carried out to ensure that the ewe was defaecating and urinating appropriately and was healthy.

In theatre, the ewe's neck was shaved and, with the head of the ewe held back to expose the jugular vein, anaesthesia (Stage III, plane 1: light with deep regular respiration and reflexes present: Haigh, 1984) was induced with 1g thiopentone sodium BP i.v. (10 ml: $0.1g.ml^{-1}$. RMB, UK). The ewe was then transferred to the operating table on its back and, with its neck extended, was intubated with a cuffed endotracheal tube (9.0mm I.D., Portex). The cuff was then inflated and the tube secured to the ewes jaw. Anaesthesia was maintained with 2% halothane (Fluothane, ICI Pharmaceuticals, UK) in O_2 ($2L.min^{-1}$) via the endotracheal tube in a closed-circuit system. Carbon

dioxide was removed by soda-lime. Ewes were allowed to breath spontaneously, although a ventilator was available in case of respiratory failure. Respiration and heart rate were monitored continuously to observe a fall in respiratory rate and heart rate and reduced corneal reflexes (Stage III, plane 2). Surgical procedures were not commenced until deep anaesthesia had been established. This was evidenced by and absence of corneal reflex and lack of response to pinching the ear (Stage III, plane 3). The ewe's ear was tagged for identification purposes.

The ewe's legs were restrained and its abdomen, flank and a hind leg shaved. A povidone-iodine solution (Pevidine: BK veterinary products Ltd., UK) was used to scrub the abdomen, flank and hind-leg three times, working within the boundaries of the previously scrubbed area. The sites of incision were given a final wash with 0.5% chlorhexidine (Depuy, UK) in 70% industrial methylated spirits.

2.3.2 Fetal instrumentation

Due to the relatively underdeveloped fetal immune system, it was vital to carry out fetal instrumentation procedures under strict aseptic conditions. Prior to surgery all surgeon-gowns, drapes, surgical instruments were sterilised by autoclave. Fetal and maternal catheters and electrodes were gamma-irradiated (Sherwood Medical Industries, UK). Transonic flow probes were cold-sterilised (Novasapa, Willows Francis Veterinary, UK). The ewe was covered with a sterile laparotomy drape and adjacent non-sterile work surfaces were covered with additional sterile drapes. Surgeons scrubbed their lower arms and hands, wore hats, overshoes, face-masks and sterile gowns and gloves (Biogel Regent, UK).

Exposure of fetus

A 15cm midline incision was made in the ewe's abdominal skin between the umbilicus and inguinal regions and sterile gauze swabs (Vernon Carus Ltd., UK) were used to retract adipose tissue and expose the underlying peritoneum. Any bleeding was stemmed by ligating vessels with 2.0 silk suture (Pearsalls Suture, UK) or the use of electrocautery (Eschmann TD311 Electrosurgical Unit, UK) on smaller vessels. A small initial scalpel-incision was made in the abdominal cavity and scissors were used to extend this along the *linea alba* (between the two rectus abdominus muscles). Care was taken not to puncture the rumen. A trocar was used to pierce the flank of the ewe and catheters, electrodes and flow probes were threaded through the cannula *into* the abdominal cavity and the cannula was removed.

The uterus was palpated to determine the fetal number and position. All the ewes in this thesis were of singleton and twin pregnancies [Appendix 1]. A 2-3cm incision was made through a portion of the uterine wall, free from cotyledons and major blood vessels,

running parallel to any smaller vessels. The edges of the wound and underlying fetal placental membranes were picked up using Babcock forceps. The hind-limb of the fetus was exteriorised first and following instrumentation was returned to the uterus. The fetus was then rotated, and the head of the fetus brought out through the same incision. Throughout these procedures uterine tearing and amniotic fluid loss were kept to a minimum. In addition care was taken not to occlude the umbilical cord and to make sure that with rotation the cord did not wrap around the fetus.

During instrumentation it was ensured that any part of the uterus or fetus exposed to the air was kept moist with sterile saline (0.9% NaCl; saline for irrigation, Baxter Healthcare Ltd., UK).

Implantation of flow probes

The femoral artery pulse was located in the femoral canal bound by the sartorius (anterior), gracilis (medial aspect) and vastus medialis (lateral aspect) muscles (Gray, 1993; Sisson and Grossman, 1938). A 3cm incision was made on the ventral aspect of, and parallel to the hindlimb. The femoral artery was exposed by blunt dissection of fascia between the muscle bands. A ca. 1cm portion of the vessel, away from any collateral vessels, was freed from fatty and connective tissue and an ultrasound flow probe (Transonic Systems Inc.: 3R with L- or U-type reflector) was implanted around the artery. Care was taken not to disturb the saphenous nerve. The probe was secured so that it could not twist on the vessel by suturing the probe's silicone flange to the surrounding muscle in four places. The wound was closed and the flow probe cable attached a two points to the fetal skin using 2.0 silk suture.

A mid-line incision was made over the trachea in the fetal neck. The carotid artery was exposed by blunt-dissection and a ca. 1cm portion of the vessel was dissected free of fatty and connective tissue. Care was taken not to disturb the vagus nerve. An ultrasound flow probe (Transonic Systems Inc.: 3R or 4R, with L- or U-type reflector) was implanted around the vessel and was positioned prior to closure of the fetal skin to minimise the risk of the probe twisting on the vessel. Once more the probe cable was secured at two points to the fetal skin.

A flow probe (2R, with U-type reflector and no silicone flange) was implanted around the renal artery as described in Chapter 3.

Catheterisation

Prior to insertion, catheters were filled with saline via stop-cocks fitted at their distal ends, and the proximal end of vascular catheters was cut into a bevel.

Jugular and brachial veins and carotid artery: The carotid artery (without flow probe) and jugular vein were located through the mid-line incision in the fetal neck. The brachial vein, or a branch thereof, is a superficial vein and was located near to the deltoid muscle through an incision made in the anterior aspect of the upper fore-limb. A 2cm length of each vessel was dissected free of connective tissue. Two 9cm lengths of 2.0 silk suture were threaded underneath the vessel and one was used to ligate the artery or vein at a point distal to the heart. A small hole was cut in the top third of the vessel and a catheter was inserted for a distance of ca. 3cm towards the heart. A 10ml saline-filled syringe was used to withdraw any air bubbles from the catheter and to ensure that blood could be easily withdrawn from, and saline flushed into, the vessel at a slow rate. The second suture was then used to tie the catheter into the vessel and its patency was reconfirmed.

Trachea: A small region of the trachea was freed from connective tissue, taking care not to puncture the trachea or the thyroid gland. A length of 1.0 silk suture was passed underneath, and tied loosely around the trachea. A curved needle was used to make a small hole in between two cartilage rings, ca. 0.5cm below the larynx, and a catheter was inserted for a distance of ca. 3cm towards the lungs. The catheter was then secured using the silk suture. Patency of the catheter was determined by very gently withdrawing a small amount of lung fluid and slowly pushing it back in. Thus tracheal pressure could be monitored while allowing the passage of fluid to and from the lungs to continue.

Bladder: see [3.2.1]

Catheters were secured to the fetal skin near to their point of insertion.

Amniotic: An amniotic catheter was sutured to the fetal skin at the time of implantation of ECG electrodes.

Implantation of electrodes

ECG: Recordings were made using the principle of Einthoven's Triangle. The bared portion of one ECG recording electrode cable was sewn subcutaneously over the fetal sternum and the other over the tip of the left ventricle. An earthing electrode was sewn onto the back of the neck. Each electrode was secured to the fetal skin in two places with suture.

Diaphragm: A 1cm incision was made in the fetal skin over the third intercostal space (counted from the bottom right side of the rib-cage) towards the midline of the fetus. The intercostal muscle was blunt dissected to expose the diaphragm and the tip of the lower

lung lobe was retracted, if necessary, with a moist cotton bud. The bared portion of the two EMG electrodes were sewed into the diaphragm, and at the free end a double knot tied down onto the surface of the muscle, thereby forming a bipolar recording configuration. A length of silk-suture was used to tie the 3rd and 4th ribs together and the skin wound was then closed.

ECoG: A ca. 6cm transverse incision was made in the fetal skin at the top of the skull and the skin was reflected and the periosteum removed to reveal the bone beneath. Bone bleeding was stemmed with bone wax (Ethicon, UK) and electrocautery was used to stop any bleeding at the edge of the wound. Bilateral 1mm holes were hand-drilled in the parietal bone at an approximate distance of 2.5cm from the Sagittal suture (formed by the junction of the two parietal bones, extending anterior to posterior) and 0.5cm anterior to the interparietal bone. Care was taken not to disturb the dura. The balls of bared electrodes were inserted into the holes so that they lay in contact with the dura of the parietal cortex beneath. The rubber discs (Figure 2.1) were glued (Cyanoacrylate adhesive, RS components UK) to the skull to secure the electrodes. The wound was then glued closed over the bone taking care not to leave excess hard glue which might irritate the uterus. The earth electrode was sewn subcutaneously to the back of the neck and all electrodes were secured to the fetal skin.

Carotid sinus denervation

An incision was made just below the angle of the jaw and the carotid body region was carefully exposed by dissection using watchmaker forceps with the aid of optical magnification x4. The CSN (arising from the carotid sinus and body and joining with the IXth or glossopharyngeal nerve) was located within the triangle formed by the common carotid artery, occipital artery and glossopharyngeal nerve and dissected free from any connective tissue. A 2-3mm portion of the CSN was cut out.

All carotid sinus denervations were carried out Professor M.A. Hanson (Department of Obstetrics and Gynaecology, University College London).

2.3.3 Closing procedures

The fetal head was returned to the uterus and the position of the umbilical cord verified. Enough catheter, electrode and flow probe cable length were looped in to the uterine cavity to allow for fetal movement. The uterus was closed, capturing fetal membranes, with a continuous 2.0 silk-suture dividing the catheter, electrode and flow probe leads into two bundles to minimise fluid loss. The wound was then over-sewn to form a water tight seal. The peritoneum (cotton umbilical tape: Ethicon, UK) and then the skin (1.0 silk-suture) were sutured closed.

At the time of closure antibiotics were administered to the ewe (4ml Streptopen i.m. Appendix 4) and amniotic cavity (via the amniotic catheter: 600mg Crystapen and 80mg Gentamicin. Appendix 4). Vascular catheters were filled with heparinised-saline ($17U.ml^{-1}$. Heparin: CP Pharmaceuticals Ltd, UK; Saline 0.9%).

An incision was made in the maternal skin parallel to the pedal vein near to the ankle joint. A trocar was inserted through the incision and tunnelled subcutaneously along the leg and out through the rump, catheters were then threaded through the cannula so that their proximal ends were at the site of incision. A 2cm length of the pedal vein was blunt dissected and freed from fatty and connective tissue. Two lengths of silk-suture were threaded under the vessel and one was used to ligate the distal portion of the vein. A small hole was then made and the heparinised polyvinyl catheter was inserted for a distance of ca. 7cm towards the heart. The catheter was then tied in and vessel patency verified by withdrawing blood slowly with a syringe.

All maternal wounds were sprayed with oxytetracycline Hydrochloride (Terramycin, Pfizer, UK) after closure.

The distal ends of the catheters were tied in a plastic bag onto the ewes back.

2.3.4 Post-operative care

After all surgical procedures had been completed the percentage of inhaled halothane in O_2 was gradually reduced to zero and respiration, heart rate and reflexes were continuously monitored. The endotracheal tube was disconnected from the oxygen supply and once regular respiratory activity and withdrawal reflexes had become firmly established the ewe was transferred from the surgery table to a metabolic cage. As soon as swallowing reflexes and chewing had returned the endotracheal cuff was deflated and the tube removed. Only then was the ewe transferred into a recording room. Food and water were withheld until sufficient muscular tone had been established for the ewe to support her own head. Ewes were encouraged to stand within 2-3 h of surgery if they had not already done so of their own volition. Ewes were checked on a daily basis for appropriate defaecation and urination.

Five days of post-operative recovery was allowed prior to experimentation during which time daily antibiotic treatment was given to the ewe (300mg Crystapen i.v.), fetus (150mg Crystapen i.v.) and amniotic cavity (150mg Crystapen). On days 1 and 2 only, Gentamicin was administered into the amniotic cavity (40mg) and to the ewe (40mg, i.v.) [Appendix 4]. Patency of the blood vessel catheters was maintained by a continuous infusion (F.T. Scientific Instruments Ltd.) of heparinised saline ($50U.ml^{-1}$ at $0.125ml.h^{-}$

[1]. Sterile 0.9% saline for infusion: Baxter Healthcare Ltd., UK) and fetal arterial blood was collected daily for analysis [2.4.3].

2.3.5 Post-mortem procedure

Ewes were killed by an overdose of barbiturate (i.v. 30-40ml: 200mg.ml^{-1} pentobarbitone sodium B.P., Rhone Merieux, UK). After maternal heart rate had ceased, the abdomen was opened and fetuses were removed and weighed. Blood flow probes were removed by dissection and catheter and electrode placement was verified. Fetal crown to rump length (CRL) was measured and fetal heart, lungs, liver and kidneys were removed and weighed and examined for any macroscopic abnormalities. Post-mortem data is recorded in Appendix 1.

2.4 Fetal monitoring

2.4.1 Biological signal processing

Transducers and amplifiers

Fetal vascular and tracheal pressures were measured using pressure transducers (PDCR 75, Druck Ltd.) connected to an Electromed preamplifier. Prior to experimentation pressures were calibrated onto chart and/or MacLab [2.4.2] using a mercury sphygmomanometer. Pressure amplifiers lay in a custom built perspex box attached to the side of the cage so that they lay at the level of the fetal heart when the ewe was standing. Ewes stood during experimental periods so that pressure recordings were kept constant. For the results presented in Chapter 4, amniotic pressure was recorded and used as a baseline to subtract from other pressure readings via a differential amplifier. Due to mechanical problems this was not carried out for the studies reported in other Chapters.

ECoG electrode signal was taken via a unity gain headstage to an AC pre-amplifier (NT114A, Neomedix Systems, Australia) with filter windows set between 0.2 and 50Hz.

Diaphragmatic and ECG signals were taken via a headstage (NL100, Neurolog System, Digitimer UK) to an AC preamplifier (NL 104), filtered (NL125) and amplified (AC DC amplifier: NL 106) with filter windows set between 50-100Hz and 300-400Hz. The diaphragmatic signal output from the AC DC amplifier was then integrated (EMG integrator: NL 703) with a time constant of ca. 100msec.

The Transonic flow meter

The Transonic flowmeter (T201 2-channel flow meter, Transonic Systems Inc., Ithaca USA) subtracts downstream transit time from upstream transit time. The flowmeter average flow output signal was interfaced to the data acquisition systems [2.4.2]. At the time of experimentation zero-flow and a maximum deflection-flow value (3R: 200ml.min^{-1}, 4R: 400ml.min^{-1}) was obtained for calibration purposes.

2.4.2 Data acquisition systems

Chart recorder

Biological variables were displayed on a video monitor (V1000, Gould, France) and oscilloscope (ISR620, Isotech UK). Polygraph recordings of all data were made via preamplifiers (SP400A, France) onto an electrostatic chart recorder (ES1000, Gould, France).

MacLab

The data presented in Chapters 5, 6 and 7 where recorded onto MacLab Chart (AD Instruments Pty Ltd.) via an 8-channel amplifier unit. Processed analogue signals (pressure and electrical) are sent to the channels (DC amplifiers), the output of which are multiplexed to an analog-digital converter. The information is then assembled by the central processing unit and sent to the MacLab Chart application on a Macintosh LCIII computer. Data was sampled at a rate of 40 samples per second.

2.4.3 Blood gas, Glucose/Lactate and Co-oximeter machines

Fetal arterial blood was sampled (0.5-0.6ml) on a daily basis after surgery to assess the well-being of the fetus after instrumentation and at intervals during experimental protocols to monitor changes in fetal oxygenation, metabolism and electrolyte balance. Blood was collected into heparinised 1ml syringes as a precaution against a clot forming in the blood gas analyser fluid pathways.

Blood gas analyser (sample volume, 240μL. IL1400 BGElectrolytes System, Instrumentation Laboratory).

The pH electrode consists of a solution of known, constant pH on one side, and the solution of unknown pH (blood) on the other side of pH sensitive glass. The potential difference which develops between the 2 solutions is proportional to the pH difference of the solutions and, the pH of one solution being constant, is representative of the solution

under investigation. A separate reference electrode, filled with saturated KCl solution, generates a constant reference potential against which the pH membrane potential can be compared. A similar principle is employed by the electrolyte electrodes (Na^+ and K^+). Their electrolyte solutions provide a fixed ion concentration and ion selective membranes ensure the specificity of each electrode.

The PCO_2 electrode combines a pH measuring and reference electrode which make contact with a solution behind a gas-permeable membrane. CO_2 can diffuse across this membrane in either direction thereby equilibrating the inner solution with the external (blood) gas pressure. Hydration of CO_2 produces hydrogen ions which are detected by the pH electrode and interpreted as a change in PCO_2.

The PO_2 electrode is polarised with a constant voltage of ca. 0.6V and generates a current by the reduction of oxygen at the cathode of the electrode ($O_2+2H_2O+4e^- \rightarrow 4OH^-$). The current is directly proportional to the partial pressure of PO_2.

Haematocrit is the percentage red blood cells of total blood volume and is determined by measuring the relative conductivity of cells and their supporting medium. This is then corrected for the sodium concentration and converted to Hct units.

Measured parameters: PCO_2 (resolution=0.1mmHg), PO_2 (resolution=1mmHg), pH (resolution=0.001pH), Na^+ (resolution=1mmol.L^{-1}), K^+ (resolution=1mmol.L^{-1}), Hct (resolution=1%).
Calculated parameters: Bicarbonate (HCO_3^-: resolution=0.1mmol.L^{-1}), Base excess (resolution 0.1mmol.L^{-1}) [Appendix 3].

Co-oximeter (sample volume, 175µL. IL482, Instrumentation Laboratory)

Anticoagulated whole blood samples are mixed with diluent, haemolysed and brought to a constant temperature. Monochromatic light is passed through the sample at four different wavelengths (535, 582.2, 594.5 and 626.6 nm) and the output is used to generate absorbances.

The analyser was specifically calibrated by the manufacturers for sheep haemoglobin.

Measured parameters: Total Hb (resolution=0.1g.dL^{-1}), %reduced Hb (%RHb: resolution=0.1%), $\%O_2Hb$ (resolution=0.1%) and %MetHb (resolution=0.1%).
Calculated parameters: oxygen content (O_2ct: resolution=0.1vol%O_2), oxygen saturation ($\%SO_2$: resolution=0.1%) [Appendix 3].

Glucose/lactate analyser (sample volume 25µl, 2300 Stat Plus, YSI Inc., USA)

Substrate (glucose or lactate) is oxidised to produce hydrogen peroxide which is in turn reduced at a platinum electrode to produce electrons. The flow of electrons is proportional to the hydrogen peroxide concentration and therefore to the concentration of substrate. The glucose/lactate reading of the whole blood sample is converted to determine that of *plasma* using the haematocrit value from the blood gas analyser.

Fetal core temperature is 39.5°C. For greater accuracy PCO_2, PO_2 and pH measured at 37°C were automatically corrected to 39.5°C by the blood gas analyser [Appendix 3].

2.4.4 Storage of plasma

Blood collected at the time of experimentation was transferred immediately to chilled 15% EDTA (K_3) tubes (Vacutainer, Becton Dickinson, UK) and spun in a cold-centrifuge at 4°C (3000 rpm. Fisons Chilspin 2, UK) for 10 min. Plasma (supernatant) was then transferred using a plastic disposable pipette to plastic storage tubes which were stored at -20°C for subsequent hormonal analysis.

Refer to Appendices for assay techniques.

2.5 Experimental protocol

2.5.1 Induction of hypoxia

In Chapters 3 to 7 the experimental protocol, or part of it, consists of a hypoxic challenge to the fetus. This was induced by manipulation of maternally inspired gases. A customised, transparent polythene bag was fitted loosely over the ewe's head with an indwelling air line. After 1 h control period ($44L.min^{-1}$) fetal hypoxia (reduction in fetal PaO_2 from ca. 22 to ca. 13mmHg) was induced for one hour by giving the ewe a 9% O_2 mixture to breathe ($12\text{-}14L.min^{-1}$ air; $0.2\text{-}0.4L.min^{-1}$ CO_2; $22L.min^{-1}$ N_2). The bag mixture was then returned to control ($44L.min^{-1}$) for a 1 h recovery period.

Fetal arterial blood was sampled 5 min after the onset of hypoxia to check that a satisfactory level of hypoxia had been induced, and again at 15, 30 and 45 min hypoxia.

2.5.2 Drug infusion

Continuous drug infusions during experimental periods was carried out using a 2-syringe digital infusion pump (Harvard Apparatus Inc., USA). Catheter dead-space was taken into account when determining time of infusion onset.

In Chapters 4, 5 and 6 fetuses were infused with a vehicle on one day and then with the experimental drug on a subsequent day. The design of these experiments approaches, but is not a true, cross-over trial. A fully randomised trial would undoubtedly have been preferable, however it was decided against in the present experiments due to the uncertainty of the half-life of the drugs used. For example, the effects of captopril have previously been reported to remain after three days of administration (Broughton Pipkin, Symonds and Turner, 1982). An obvious solution to this problem might have been to wait for a number of days following the drug-infused experimental day before conducting the vehicle-infused experiment. However the use of the fetus as an experimental model makes this an impractical solution to the problem because the results would then most likely be confounded by gestational age-related effects.

2.6 Data analysis

2.6.1 Calculation of vascular resistance

To express the Hagan-Poiseuille Law [equation 1] in terms of resistance it is usual to rearrange it by analogy with Ohm's Law [equation 2 i: current, V: voltage, R: resistance] resulting in equation 3. Thus changes in vessel radius (r) are more decisive in determining blood flow (Q) and pressure than are vessel length (l), pressure difference (ΔP) or viscosity (η). However the heterogeneous nature of blood as a whole and the elastic and branching properties of vessels means that the conditions under which this equation would be ideally satisfied are not met. It would be preferable to consider haemodynamics in the fetus in terms of impedance, since this would take into account not only resistance but vessel compliance and inertance. However in the chronic fetal preparation this is not possible since there are no means of measuring moment to moment changes in vascular compliance. Vascular resistance is therefore normally derived by the use of an equation analogous to Ohm's law [see equation 2]. In this way, volume flow is equal to the average pressure difference between arterial and venous parts of the circulation (perfusion pressure) divided by the resistance to flow in a particular region. In this thesis the predominant representation of regional haemodynamics is by blood flow changes, however an attempt has been made to address changes in vascular resistance. While it is obviously preferable to incorporate venous pressure into these calculations it was not always possible to do so. Therefore in Chapters 3, 4 and 7 MAP was used alone in the calculation of resistance. In Chapters 5 and 6, representative MVP measurements were available during normoxia and hypoxia, and this was subtracted from MAP in the calculation of vascular resistance.

Hagan-Poiseuille Law:

$$Q = \frac{\Pi \cdot r^4}{8 \cdot \eta \cdot l} \Delta P$$

.....equation [1]

Ohm's Law:

$$i = \frac{V}{R}$$

or,

$$Q = \frac{\Delta P}{R}$$

.....equation [2]

$$R = \frac{8 \cdot l \cdot \eta}{\Pi \cdot r^4}$$

.....equation [3]

2.6.2 Identification of behavioural activity

Fetal breathing movements

FBMs are known to be rapid, shallow and episodic in nature. Originally FBMs were assessed using only tracheal pressure deflections however this has since been shown to be associated with false-negative identification of FBMs due to the instability of the fetal chest wall (see Johnston, 1991). In this thesis therefore, in the instances where behaviour was investigated, FBMs were identified by positive excursions of integrated diaphragmatic EMG activity *combined* with negative pressure excursions in tracheal pressure. A further requirement was that the pressure deflections be greater than 1mmHg and at a frequency greater than 15 per min and occurred for longer than 1 min.

Electrocortical activity

It is widely recognised that there are two cyclical, episodic states of ECoG in the sheep fetus. HV-ECoG is characterised by high amplitude (40-100mV) and low frequency (3-12Hz) and LV-ECoG by low amplitude (ca. 25mV) and high frequency (10-20Hz) (Figure 2.3). However a third state, similar to LV- but with a greater amplitude, may exist (see Moore and Hanson, 1992 for review). In this thesis ECoG activity was classified qualitatively, on the basis of signal amplitude, as being either LV or HV and the points of transition between these two states were identified during the experimental periods. ECoG activity was then expressed as the percentage of time spent in LV-ECoG activity and the number of transitions made from LV- to HV-activity.

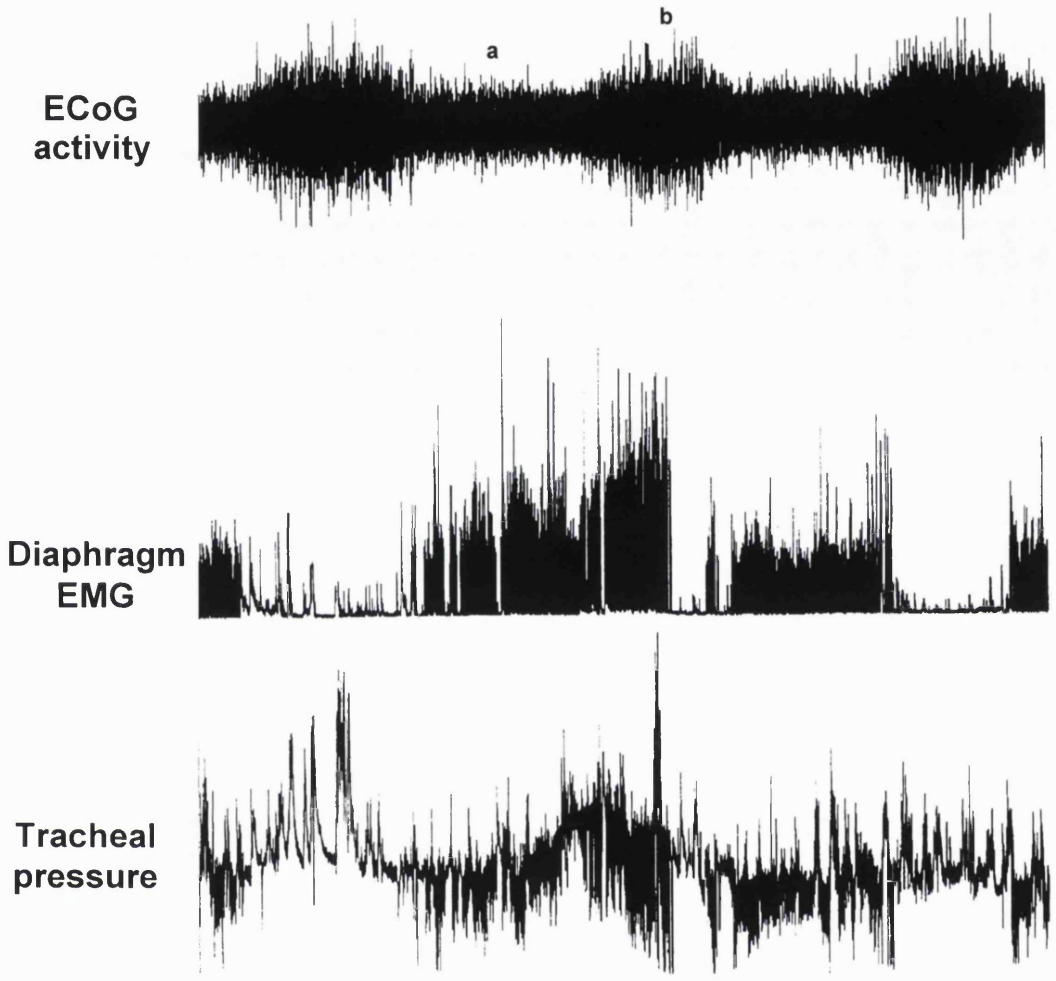


Figure 2.3 Typical trace of ECoG activity (top), integrated diaphragmatic activity (middle) and tracheal pressure (bottom). a) LV-ECoG activity and b) HV-ECoG activity. Periods of LV-ECoG activity are associated with diaphragmatic EMG activity and negative tracheal pressure excursions.

2.6.3 Statistical analysis

The statistical analysis contained in this thesis was carried after consultation with Professor S. Senn, Department of Statistical Science & Department of Epidemiology and Public Health, University College London.

The use of parametric statistics

There are two broad categories of statistical methods used in biological research: parametric and non-parametric. Parametric methods make the assumption that the observed data are from a population with a distribution that has a known theoretical form e.g. the Normal distribution, whereas non-parametric methods are 'distribution-free' being based on analysis of ranks rather than the actual data itself (Altman, 1991). In attempting to decide between these methods a test of normality is often carried out. With small sample sizes it becomes difficult to assess from a frequency histogram whether data follows a normal distribution. In addition, for the type of measurements contained within

this thesis it is in fact the *within*-animal errors that would need to be normally distributed for the use of a parametric test to be vindicated, because repeated measurements are made on the same animal (e.g. with or without antagonist drug). The problem is that to address this question correctly would itself require a parametric, i.e. assuming a normal distribution, method of analysis (Senn, 1993). The fact that non-parametric methods work by ranking data is another important consideration. Since chronic fetal sheep studies yield characteristically small sample sizes it would be a rather futile process to try to rank these data. There are only a few ways in which one can rank e.g. 5 numbers and, if this were done, it would to all intents and purposes render non-parametric methods meaningless.

On the basis of these considerations parametric methods of statistical analysis were used throughout this thesis.

Analysis of serial measurements

The correct way to approach the statistical analysis of serial or repeated measurements has been under discussion for a number of years. The present data is characterised by the fact that, while the data acquisition process is continuous, observations are made at discrete equally-spaced 15 min intervals. It is important to note that as a result there are two sources of random variation: 'between-fetus' variation and the 'within-fetus' variation which results from the repeated measurements on any given fetus. A common mistake in handling the analysis of this type of data has been to perform independent analysis, for example using a simple t-test, at each time point. This approach is flawed primarily because no account is made for the fact that the measurements at different time points are from the same individual fetuses. Clearly it is important for the chosen method of statistical analysis to focus on the way an individual fetus responds. Secondly, an important consideration is the fact that time is not an independent variable. By the very nature of the design of these experiments the measurements have a temporal sequence, i.e. this factor is non-randomisable. Thus measurements on an individual separated by a small time interval will have error components more closely correlated than those at time intervals more widely separated. Consequently it would be wrong to regard the study as a split-plot design in which time would assume the role of a variable (Finney, 1990; see Aitkin, 1981, Yates, 1982 and Wallenstein, 1982 for discussion). Thus methods such as analysis of variance, with time as a variable, are moving out of fashion and the importance of minimising the effect of sampling times, by representing the data from each animal by a smaller number of statistics that can adequately describe the time trend, has become apparent.

Summary measures analysis

One technique which is simple to use and widely applicable is analysis using summary measures (Matthews, Altman, Campbell and Royston, 1990). This method treats the individual as the basic unit and reduces the data of each subject to single numbers which summarise the aspects of utmost interest in the data. These summary measures can then be used, as if they are raw data, in an appropriate statistical analysis. In this thesis summary measures were derived from the mean of measurements taken at 15 min intervals over a given time period for an individual fetus. This can be illustrated by considering the FBF response to 1 h of hypoxia. In all fetuses there is a large fall in flow at the onset of hypoxia and this is sustained for the duration of hypoxia (Figure 2.4), thus summary measures could be created from the repeated measurements made during the 1 h prior to the onset of hypoxia (normoxia), during hypoxia and during the recovery period. When deciding on where to create the summary measures for a given variable the responses of individual fetuses are plotted. Because there was an *a priori* knowledge of rapid chemoreflex changes, for example in FHR, occurring in the first 10 min of hypoxia more frequent (5 min) measurements were displayed around this time. Otherwise data was sampled at 15 min intervals, then grouped according to summary measures. For display purposes data was generally presented in graph and table form as mean±S.E.M. However it should be borne in mind that while this is the only really feasible way of displaying large amounts of data it is not ideal since the error bars refer to between fetus error and not the within-fetus errors that should be the primary focus of attention.

The obvious advantages of using summary measures are that the method can cope with missing measurements, and that it allows comparisons to be made between serial measurements on the same subjects under different conditions e.g. vehicle vs. L-NAME infusion (Chapter 6). While at first glance it might appear that the method discards a lot of data however, as I have previously intimated, a large number of repeated measurements over time gives an impression of providing a wealth of information which is more apparent than real, since consecutive readings in an individual are likely to be highly correlated.

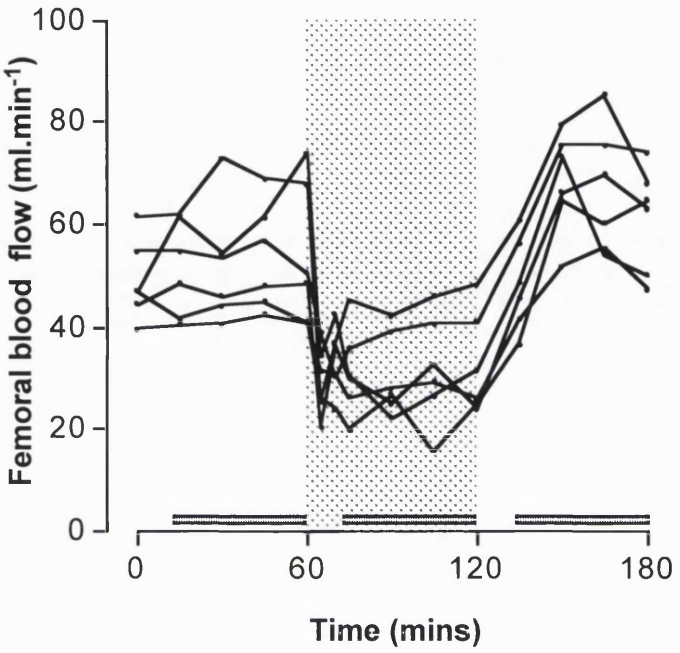


Figure 2.4 FBF responses of six individual fetuses during normoxia, hypoxia (shaded area) and recovery. The horizontal shaded bars show the time period over which data was reduced to summary measures.

Other statistical methods

Regression analysis was used to investigate the relationship between variables e.g. RBF and UO (Chapter 3). A line is fitted to the data as an estimate of the relationship between the two parameters. The slope (m) indicates the strength of the relationship between the two parameters. The R^2 statistic is the square of the correlation coefficient between the two variables and will reveal the amount of variability in the dependent variable that is explained by variation in the independent variable. The F statistic is the square of the t statistic, with a P value that describes whether the slope is significantly different from zero.

If, having selected particularly pertinent comparisons, multiple comparisons were deemed unavoidable, e.g. ACh responses in Chapter 7, the Bonferroni method of correction was used in an attempt to compensate for finding a significant difference just by chance. Prior to analysis the number of comparisons to be made was set and the P level was adjusted accordingly (i.e. 0.05 divided by the number of comparisons. Wallenstein, Zucker and Fleiss, 1980; Altman, 1991). The disadvantage of this method is that it errs on the side of non-significance. In the hypoxia protocols no method of correction was used when comparing summary measures of normoxia vs. hypoxia and normoxia vs. recovery. The primary focus for attention was the effect of hypoxia on the measured variable, and given the difficulty in defining the 'recovery' period exactly it was considered to be of secondary importance to hypoxic changes in the statistical analysis. For interest the

normoxia vs. recovery statistics have been calculated but their interpretation has been approached with caution.

Statistically significant changes are denoted on graphs and in tables. The exception to this are blood gas and electrolyte data in Chapter 4 where, for simplicity, statistics are given in the text of the results section. All description of data is of significant changes, except where otherwise indicated by the use of phrases such as 'tendency'.

CHAPTER 3
RENAL BLOOD FLOW AND URINE OUTPUT

3.1 Introduction

During hypoxia there is a redistribution of blood flow favouring the heart, brain and adrenals at the expense of peripheral vascular beds [1.4]. Previously work carried out in this laboratory has looked at the effect of CSD on this blood flow redistribution. A large component of the rapid initial peripheral blood flow changes in response to hypoxia are mediated by carotid chemoreflex mechanisms (Giussani, Spencer, Moore, Bennet and Hanson, 1993).

Given the dependence of a large number of metabolic processes on an adequate supply of oxygen (e.g. the Kreb's cycle), blood flow changes to, and thus altered oxygenation of, different vascular beds would be expected to alter their function. Indeed blood flow measurements and monitoring of blood gas and glucose/lactate composition have shown that the fall in peripheral blood flow during fetal asphyxia and hypoxia correlates well with reduced peripheral oxygen consumption and tissue metabolism (Jensen, Hohman and Kunzel, 1987; Boyle, Hirst, Zerbe, Meschia and Wilkening, 1990; Boyle, Meschia and Wilkening, 1992) and unaltered blood flow to the kidney in the face of a reduced oxygen supply has been associated with maintained renal oxygen consumption (Iwamoto and Rudolph, 1985). The ability to measure blood flow to the kidney and UO provides a potential model to relate organ function and the blood flow to it. This may add to information already available from more traditional techniques such as monitoring indicators of whole body metabolism, e.g. lactate and acid-base status, in the plasma.

The effect of hypoxia on RBF has been examined in a number of fetal sheep studies. The responses have not been consistent, with RBF reported to rise then fall (Millard, Baig and Vatner, 1979), fall (Robillard, Nakamura and DiBona, 1986) or not to change (Wlodek, Challis, Richardson and Patrick, 1989). Furthermore the mechanisms that control RBF during hypoxia are uncertain. Renal sympathetic nerve stimulation causes a renal vasoconstriction, but in the presence of α-adrenoceptor blockade it results in a renal vasodilatation, thought to be due to the action of noradrenaline released from nerve fibres acting on β_2-adrenoceptors (Robillard, Smith, Segar, Merrill and Jose, 1992). Renal denervation in the late gestation fetus attenuates the fall in RBF during hypoxia (Robillard *et al.*, 1986). In adults, stimulation of carotid chemoreceptors reduces RBF (Al-Obaidi

and Karim, 1992). Peripheral chemoreceptors have been shown to initiate the rapid vasoconstrictor responses to hypoxia in pulmonary (Moore and Hanson, 1991) and systemic (Giussani, Spencer, Moore, Bennet and Hanson, 1993) vascular beds. However there is no information to date on such afferent mechanisms in the fetal RBF responses to hypoxia. Thus the ***first aim*** of the present study was to investigate the role of peripheral chemoreceptors in the RBF responses to acute hypoxia. In addition, fetal plasma [catecholamine] is known to rise during acute hypoxia (Jones and Robinson, 1975) and has been suggested to contribute towards peripheral vasoconstriction. It is not known however what role catecholamines play in the fetal renal cardiovascular responses to hypoxia or the extent to which their release is controlled by chemoreflex mechanisms. Thus the ***second aim*** of this study was to measure plasma [catecholamine] during normoxia and hypoxia in intact and CSD fetuses.

Renal nerve section in the fetus (Robillard, Nakamura and DiBona, 1986) reveals an initial renal vasodilatation which can be blocked by PG-synthesis inhibition. Indeed there is some evidence to suggest that PG-synthesis modulates renal vasoconstriction during hypoxia (Millard, Baig and Vatner, 1979). Thus it seems possible that neuronal and hormonal renal vasoconstrictor mechanisms activated during hypoxia are operating against a background of opposing vasodilatation. However the exact nature of these vasodilator mechanisms remains to be elucidated. A prime vasodilator candidate is NO [1.5.3 and Chapter 6]. The ***third aim*** of the present study was to investigate the role played in RBF control by endogenous NO, using the NOS inhibitor L-NAME.

There is evidence to suggest an important role for amniotic fluid in fetal fluid balance and in providing an optimal environment for growth and development ([1.6.2] Wintour and Shandley, 1993). Indeed qualitative assessment of amniotic fluid volume using real-time ultrasound imaging is one variable used clinically to assess fetal well-being (Manning, 1992) The predominant input into amniotic fluid, aside from lung fluid, production (ca. 4.5ml.kg.h^{-1}: Mescher, Platzker, Ballard, Kitterman, Clements and Tooley, 1975) is fetal urine (Wlodek, Challis and Patrick, 1988; Wintour and Shandley, 1993). Obstruction of UO will lead to oligohydramnios (Peters, Docimo, Luetic, Reid, Retik and Mandell, 1991). In human fetuses fetal urine production correlates with the degree of fetal hypoxia (Nicolaides, Peters, Vyas, Rabinowitz, Rosen and Campbell, 1990). In fetal sheep studies a fall in UO has been observed in response to a range of insults including 1 h of asphyxia (Daniel, Yeh, Bowe, Fukunaga and James, 1975), 1-3 h of hypoxia (Brace, Wlodek, McCrabb and Harding, 1994) and after 30 min of hypoxia (Walker, 1977; Nakamura, Ayers, Gomez and Robillard, 1985; Daniel, Stark, Husain, Sanocka and James, 1984). However others have shown no significant change after 1 h hypoxia (Robillard, Weitzman, Burmeister and Smith, 1981; Wlodek, Challis, Richardson and Patrick, 1989). The mechanisms underlying these changes in UO in the

fetus during acute hypoxia are not understood. One school of thought is that the fall is consequent upon a fall in RBF (Daniel *et al.*, 1975; Nicolaides *et al.*, 1990). In the human fetus there is a significant correlation between raised RVR (shown by an increased pulsatility index of Doppler flow velocity waveforms) and low amniotic fluid volume (Arduini and Rizzo, 1991). However to date continuous and direct measurements of RBF and UO have not been correlated in an animal model. Thus the ***forth aim*** of the present study was to establish whether there is a relationship between RBF and UO in normoxia and hypoxia in the late gestation ovine fetus.

Some of these data have been presented in abstract form (Green, Bennet, Robson and Hanson, 1995c; Green, Robson, Bennet and Hanson, 1993) and are included in Appendices 7 and 14. Five of the 6 intact fetuses were investigated by Professor S. Robson and Dr. L. Bennet prior to the commencement of this thesis.

3.2 Methods

3.2.1 Surgical Preparation

Seventeen fetuses aged between 112 and 126 days gestation were instrumented under general anaesthesia [2.3]. The lower half of the fetus was exteriorised through the uterine incision and the left flank of the fetus was supported from the dorsal surface. A suprapubic incision was made on the left ventral surface to expose the bladder. The bladder was then catheterised via a puncture incision, and the catheter secured by a purse-string suture (4.0 prolene suture). The catheter was secured to the fetal skin and its distal end attached via a blunt needle to a 3-way stopcock. Free flow of urine via the catheter was confirmed before closing the abdominal wall and skin in separate layers using 2.0 suture. The left kidney was palpated and exposed through a second left lateral incision and intestines were retracted with cotton-gauze swabs. A small incision was made in the perirenal fat and a maximum 1cm length of the left renal artery dissected by blunt dissection. A 2R transonic flow probe was placed around the renal artery and positioned to ensure that the vessel was not distorted. The transducer cable was secured in 2 places to the fetal skin. The incision in the perirenal fat was closed using a 3.0 suture and the abdominal muscle and skin incisions were closed in separate layers using 2.0 suture. The lower portion of the fetus was returned to the uterus. The upper portion of the fetus was then exteriorised. Stainless steel electrodes were sewn onto the chest and hindlimb to monitor ECG. A catheter was sewn onto the fetal skin for drug administration into the amniotic cavity and for measuring amniotic pressure. Additional heparinised catheters were placed in a carotid artery to monitor MAP and in a fetal jugular vein and maternal pedal vein to administer antibiotics [2.3.2].

Eleven of these fetuses also underwent bilateral section of their CSNs [2.3.2].

Five days of recovery were allowed before experimentation.

3.2.2 Experimental procedure

Measurements

MAP, FHR and RBF were recorded continuously onto chart paper [2.4.2]. Fetal urine was collected by opening the 3-way stopcock and draining urine, under gravity, into sterile containers over consecutive 15 min periods. An initial 15 min draining period was always allowed prior to the onset of the protocol in an attempt minimise variability in bladder volume of individual fetuses at the onset of the protocol. During non-experimental periods the stopcock was closed so that urine passed as normal via the urachus and ureter to the allantois and amniotic cavity, respectively. The volume of urine collected over each 15 min period was recorded. Urine samples were divided into 4 groups (A. control: 15-30 with 30-45 min; B. early hypoxia: 60-75 with 75-90 min; C. late hypoxia: 90-105 with 105-120 min; D. recovery: 135-150 with 150-165 min. See Figure 3.1) and 10ml transferred to tubes and stored at -20°C for subsequent analysis of low molecular weight (MW) metabolites. Any urine collected in excess of 10ml was returned to the amniotic cavity.

2-3ml fetal arterial blood (■) was collected during control (30 min), early and late hypoxia (75 and 105 min, respectively) and recovery (150 min). Blood was transferred immediately to chilled EDTA tubes and spun at 4°C (3000 rpm) for 10 min. Plasma was then decanted into tubes and stored at -20°C for subsequent hormonal analysis. A further 0.6 ml arterial blood (●) was collected during normoxia (intact: 45 min and CSD: 30 min), hypoxia (75 and 105 min) and recovery (intact: 165 and CSD: 150 min), as well as 5 min after the onset of hypoxia (65 min) for the analysis of blood gases. In CSD fetuses involved in the L-NAME study additional plasma was taken prior to the onset of infusion for hormone (□) and blood gas (○) analysis.

Protocol

The protocol was three hours in length. An initial 1 h normoxic control period was followed by 1 h fetal isocapnic hypoxia. Ewes were then returned to normoxic conditions and measurements continued for 1 h (recovery) [2.5.1].

In three CSD fetuses this protocol was repeated on a subsequent day but with the continuous infusion of NOS inhibitor, L-NAME (initial bolus of 20mg in 2ml followed by 100mg.h^{-1} at 3ml.h^{-1} infusion, see Figure 3.1) but with no collection of urine.

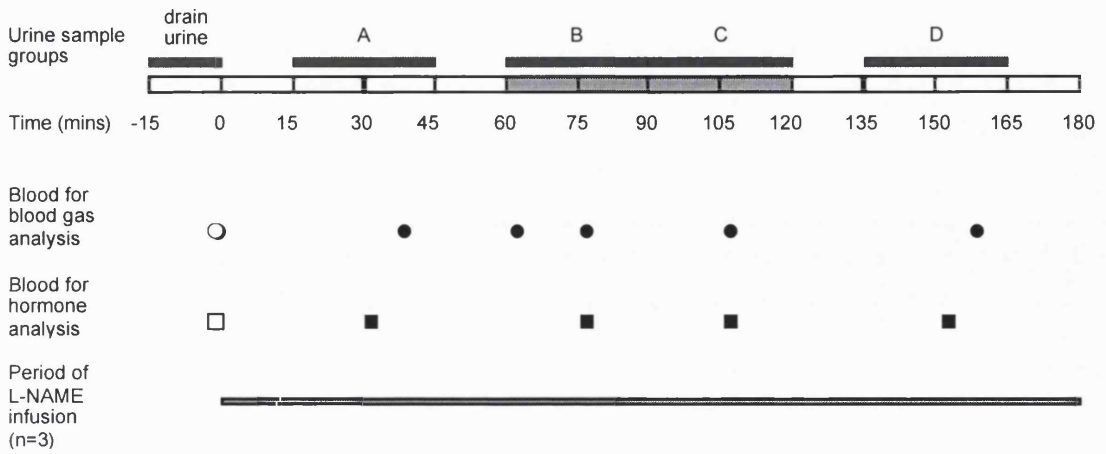


Figure 3.1 Diagram summarising the experimental procedure. The shaded region of the time scale denotes the 1 h hypoxia.

Urine and plasma analysis

Urinary inorganic acids were analysed using ^{1}H NMR by Dr P. Foxall, Department of Nephrology, Middlesex Hospital Medical School. The results of this analysis are presented in Appendix 6.

Plasma catecholamine analysis was performed by Dr C. Smith, Department of Pharmacology and Medicine, University College London. Samples from intact and CSD fetal groups were assayed in separate batches. Procedures are described in full in Appendix 5, but briefly plasma was treated with alumina and Tris buffer and catecholamines were determined by high-performance liquid chromatography (HPLC). The sensitivity of the assay was of the order of 0.1 pmol per 70µl injection onto the HPLC column.

3.3 Data analysis

Grouped data is expressed as mean ± S.E.M. Changes in blood gas and pH data from normoxia during hypoxia and recovery were tested using Student's paired t-test. An unpaired t-test was substituted in the event of uneven sample groups (see Table 3.1 e.g. PaO_2 late hypoxia). Student's paired t-test was used to test rapid transient changes in FHR and RBF. Cardiovascular measurements in normoxic, hypoxic and recovery hours for individual fetuses were reduced to summary measures [2.6.3] to describe either the whole or a portion of the hour. Summary measures were then tested using Student's paired (within intact or CSD groups) or unpaired (between intact and CSD groups) t-test.

The relationship between UO and RBF was examined using regression analysis of UO vs. RBF and UO vs. MAP data divided according to normoxia, hypoxia or recovery

periods. It is a requirement of regression analysis that only one observation be made per individual, therefore values at 30 min normoxia, hypoxia and recovery were selected for analysis. Regression analysis was performed to derive R^2, F and the probability that the slope of the line was different from zero [2.6.3].

Statistical significance was accepted when $P<0.05$.

The rise in plasma [noradrenaline] during hypoxia was tested using paired and unpaired t-test, using the Bonferroni method of correction ($P<0.025$).

No statistical analysis was used on the CSD fetuses infused with L-NAME due to the small sample size (n=3).

3.4 Results

3.4.1 Blood gases and pH

In all fetuses PaO_2 fell from ca. 24 to ca. 13mmHg during hypoxia but returned to control levels in the recovery period (Table 3.1).

In intact fetuses pH did not change from normoxic levels during hypoxia but was lower in the recovery period. In CSD fetuses pH showed a small fall during late hypoxia and recovery. $PaCO_2$ was unaltered throughout the protocol in intact fetuses and did not fall in CSD fetuses until late hypoxia and recovery (Table 3.1).

Although the sample size of the CSD fetuses treated with L-NAME was small the values appear similar to those of the other groups of fetuses.

	PaO_2 (mmHg)					$PaCO_2$ (mmHg)					pH				
	Pre-infusion	NX	early HX	late HX	REC	Pre-infusion	NX	early HX	late HX	REC	Pre-infusion	NX	early HX	late HX	REC
CONTROL															
Intact (n=6)	-	24.0 ±1.6	13.8 ±1.5 †	14.6 ±.5 *	26.2 ±1.1	-	49.7 ±0.6	51.6 ±1.4	47.9 ±1.4	44.5 ±3.2	-	7.36 ±0.01	7.30 ±0.04	7.29 ±0.03	7.27 ±0.03 ‡
CSD (n=11)	-	22.6 ±1.0	12.5 ±0.3 *	14.1 ±0.4 †	22.8 ±0.9	-	50.8 ±1.4	50.1 ±0.7	48.0 ±1.1 ‡	47.0 ±1.4 †	-	7.36 ±0.00	7.35 ±0.01	7.31 ±0.01 ‡	7.33 ±0.01 ‡
L-NAME															
CSD (n=2)	22	26.5	13.5	14.0	23.0	49.3	47.5	50.0	46.5	45.8	7.37	7.34	7.29	7.26	7.29

Table 3.1 Blood gases and pH values for intact and CSD fetuses during normoxia (NX: 45 and 30 min respectively), hypoxia (HX: early, 75 and late 105 min) and recovery (REC: 165 and 150 min respectively). Pre-infusion values are shown for those CSD fetuses infused with L-NAME. Values are shown as mean ± S.E.M. †$P<0.01$, vs. normoxia (Paired t-test, n=6), ‡$P<0.05$, vs. normoxia (Paired t-test, n=6) and *$P<0.01$, vs. normoxia (unpaired t-test, PaO_2 late HX (intact) and early hypoxia (CSD): n=5).

3.4.2 Plasma catecholamines

Plasma [noradrenaline] tended to rise during hypoxia in intact and CSD fetuses although this only reached significance in the CSD group (Figure 3.2). Plasma [adrenaline] increased in 4 out of 5 intact and all CSD fetuses during hypoxia, although this did not reach significance in either group (Figure 3.3).

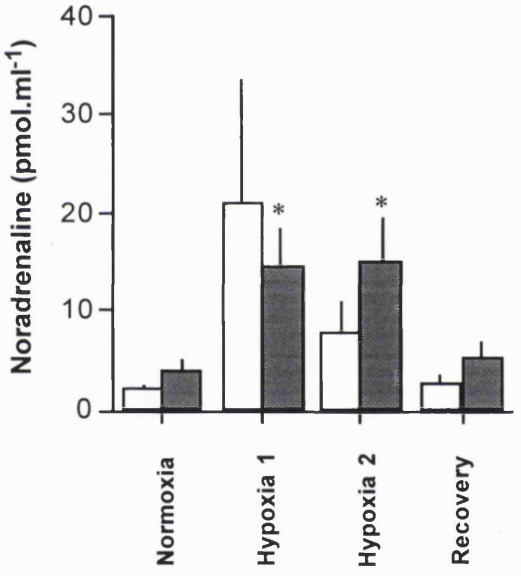


Figure 3.2 Plasma [noradrenaline] in intact (open bars, n=5) and CSD (filled bars, n=7) during normoxia, hypoxia 1 (early, 75 min), hypoxia 2 (late, 105 min) and recovery. *P<0.025, significantly different from normoxia by paired t-test.

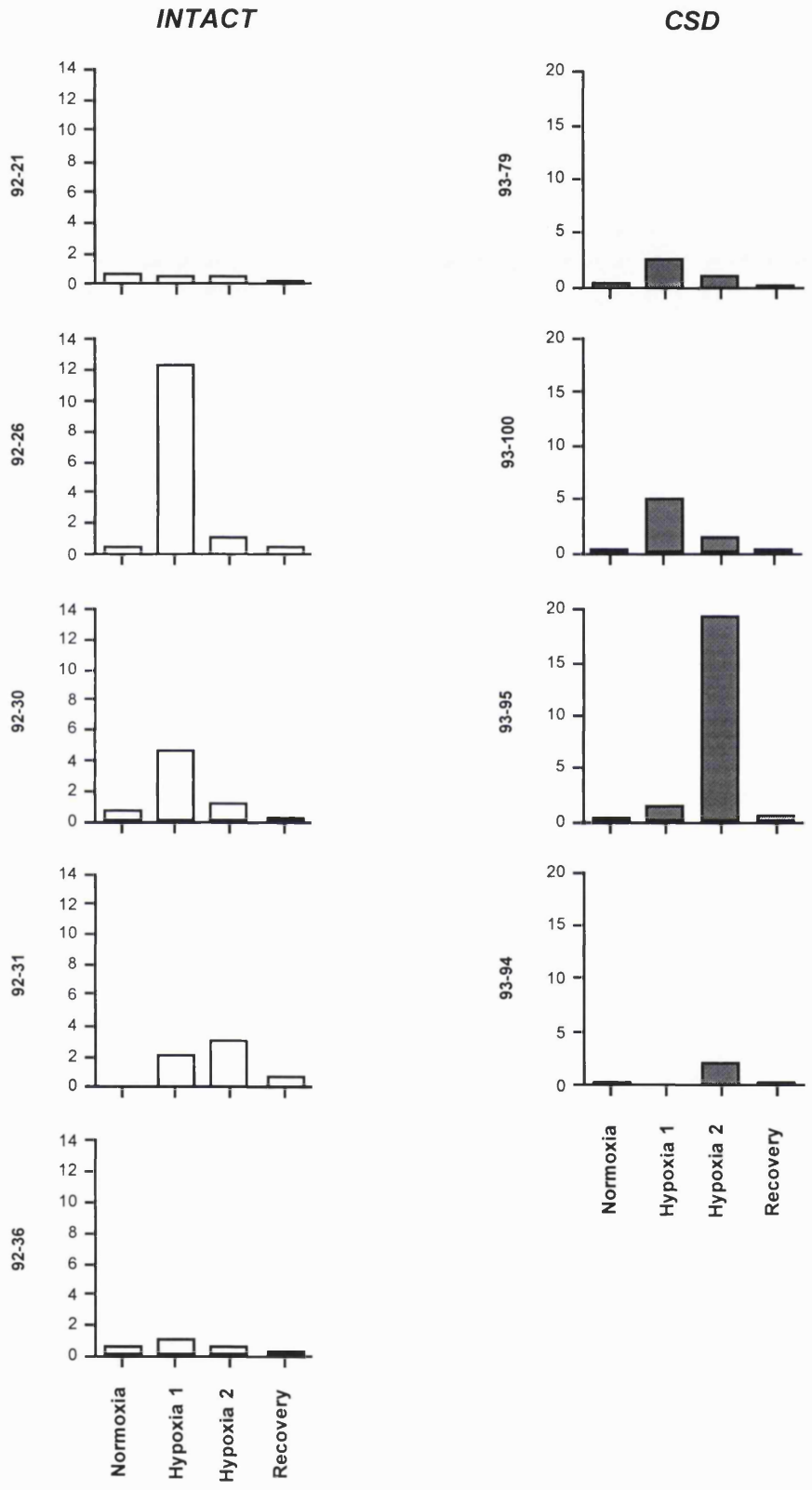


Figure 3.3 Plasma adrenaline (pmol.ml^{-1}) in intact (open bars, 5 individual fetuses) and CSD (filled bars, 4 individual fetuses) during normoxia, hypoxia 1 (early, 75 min), hypoxia 2 (late, 105 min) and recovery.

3.4.3 Fetal heart rate

FHR fell in intact fetuses 5 min after the onset of hypoxia (Figure 3.4). In CSD fetuses a significant fall did not occur. As hypoxia proceeded heart rate returned to pre-hypoxic levels. In intact, but not CSD, fetuses a tachycardia developed during recovery. Furthermore during the recovery period FHR was elevated in intact when compared to CSD fetuses.

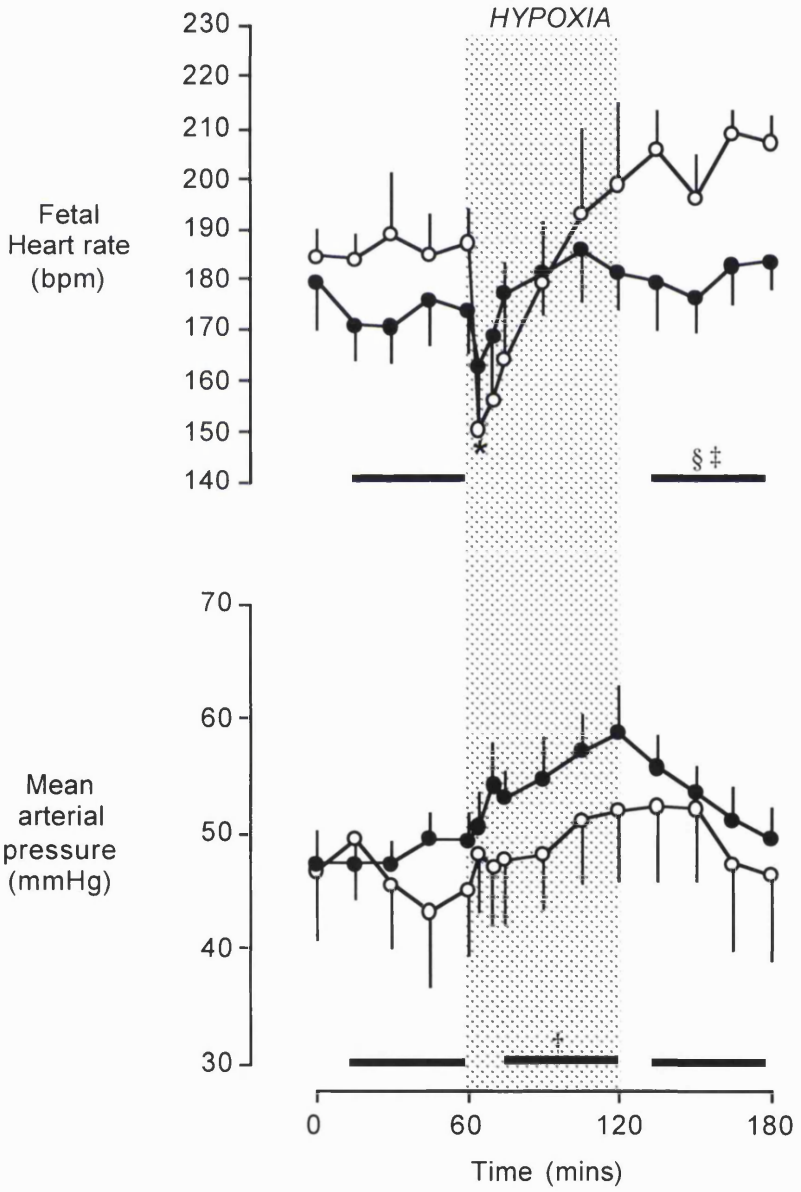


Figure 3.4 FHR and MAP during normoxia, hypoxia (shaded area) and recovery in intact (○, n=6) and CSD (●, n=10 and 11 respectively) fetuses. The horizontal shaded bars show the period over which data was reduced to summary measures. Values are shown as mean±S.E.M. *P<0.05 (n=6), intact group 60 vs. 65 min by paired t-test; †P<0.01, significantly different from normoxia in CSD group by paired t-test; ‡P<0.01, significantly different from normoxia in intact group by paired t-test and §P<0.05, CSD significantly different from intact group by unpaired t-test.

In the three CSD fetuses, during normoxia there was a large fall in FHR after 15 min of L-NAME infusion. During late-hypoxia without L-NAME infusion, the three fetuses showed a tendency for FHR to rise, however values were more variable with L-NAME infusion (Figure 3.5).

3.4.4 Mean arterial pressure

Throughout the protocol MAP was similar in both intact and CSD fetuses (Figure 3.4). During hypoxia there was a tendency for pressure to rise in both groups but this only reached significance in CSD fetuses.

The infusion of L-NAME caused a large rise in MAP during normoxia in the three fetuses studied (Figure 3.5). MAP rose during hypoxia with the infusion of L-NAME to a similar extent to that during hypoxia without L-NAME on the previous day, however pressure during hypoxia tended to be at a higher level.

3.4.5 Renal blood flow and vascular resistance

During the final 30 min of hypoxia RBF was reduced from pre-hypoxic levels in intact and CSD fetuses. CSD fetuses showed a marked initial vasodilatation (Figure 3.6) at the onset of hypoxia.

During L-NAME infusion in normoxia, RBF fell in the three CSD fetuses (Figure 3.5). The tendency for a rise in RBF during hypoxia was apparent both with and without the infusion of L-NAME.

There was large inter-animal variation in the intact group in RVR, however in the last 30 min of hypoxia RVR was elevated compared to normoxia in both intact and CSD fetuses. This was despite a tendency for an initial delay in the rise in RVR in CSD compared to the intact group (Figure 3.6).

In the three CSD fetuses, L-NAME caused a large rise in RVR during normoxia. During hypoxia with L-NAME RVR fell and then increased, in comparison to hypoxia without L-NAME in which there was a progressive rise in resistance (Figure 3.5).

3.4.6 Urine output

UO measurements were obtained in 6 intact and 8 CSD fetuses however there was a high degree of inter-animal variation in both groups. From summary statistics for individual fetuses there was no change in UO during hypoxia and recovery from normoxia in either intact or CSD fetuses (Figure 3.6). However, by examining

individual fetuses, UO appeared to be reduced from normoxia during hypoxia in 5 out of the 6 intact fetuses, and in 4 out of 8 CSD fetuses (Figure 3.7).

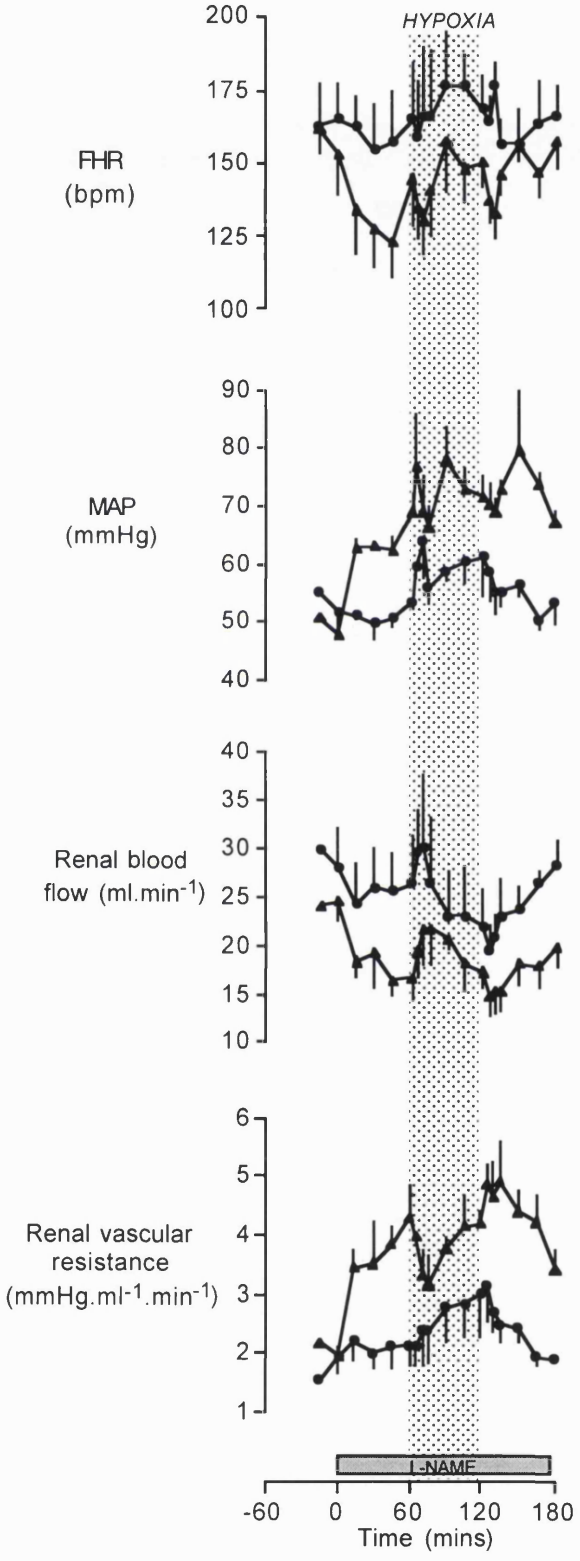


Figure 3.5 FHR, MAP, RBF and RVR during normoxia, hypoxia (shaded area) and recovery in CSD fetuses with (▲, n=3) and without (●, n=3) L-NAME infusion. Values are shown as mean±S.E.M. The horizontal shaded bar shows the duration of infusion in the L-NAME group.

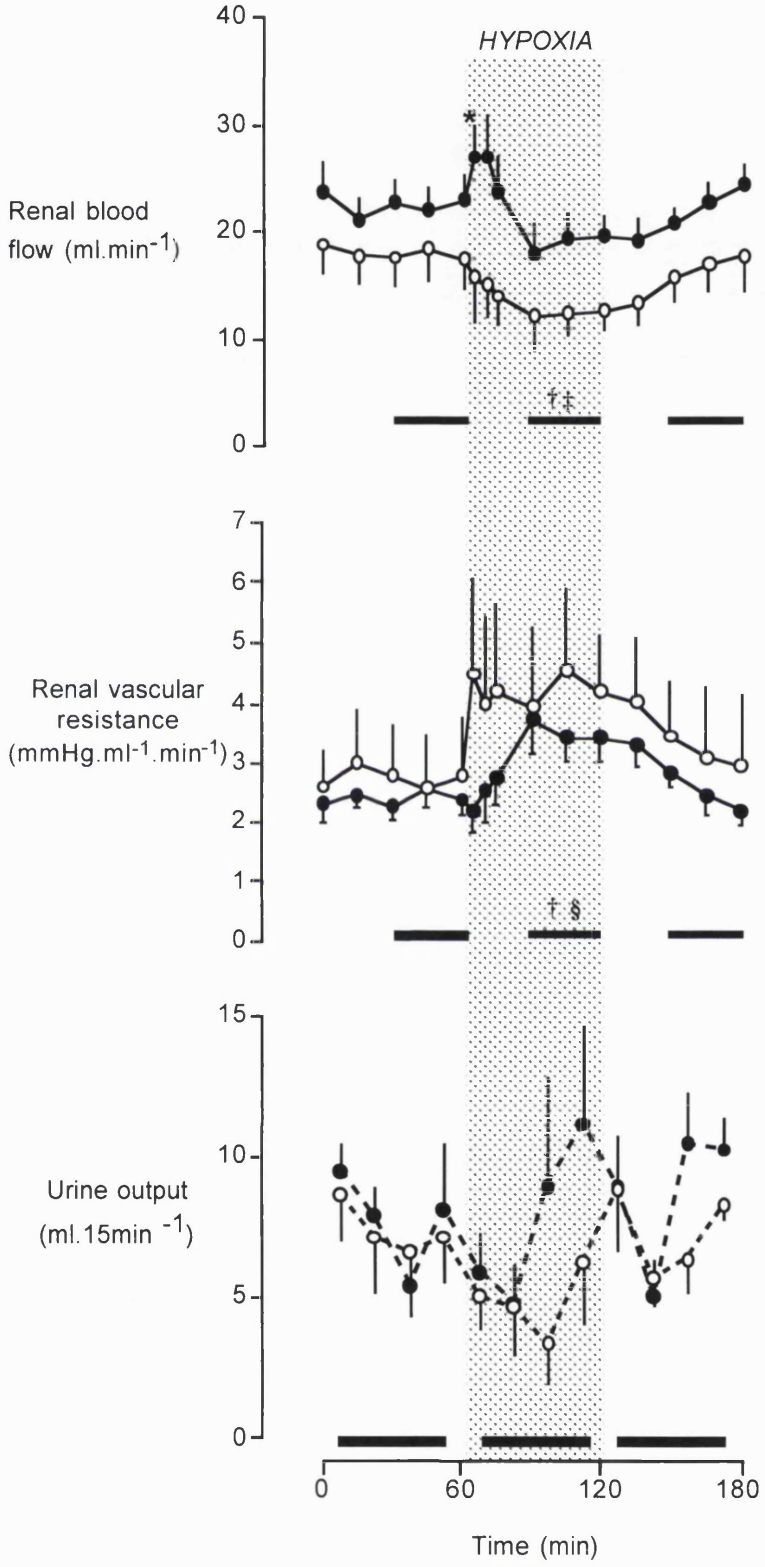


Figure 3.6 RBF, RVR and UO during normoxia, hypoxia (shaded area) and recovery in intact (❍, RBF and RVR: n=5, UO: n=6) and CSD (●, RBF and RVR: n=7, UO: n=8) fetuses. The horizontal shaded bars shows the period over which data was reduced to summary measures. Values are shown as mean±S.E.M. *P<0.01, vs. 45 min by paired t-test. †P<0.05, significantly different from normoxia in intact group by paired t-test; ‡P<0.05, significantly different from normoxia in CSD group by paired t-test and §P<0.01, significantly different from normoxia in CSD group by paired t-test.

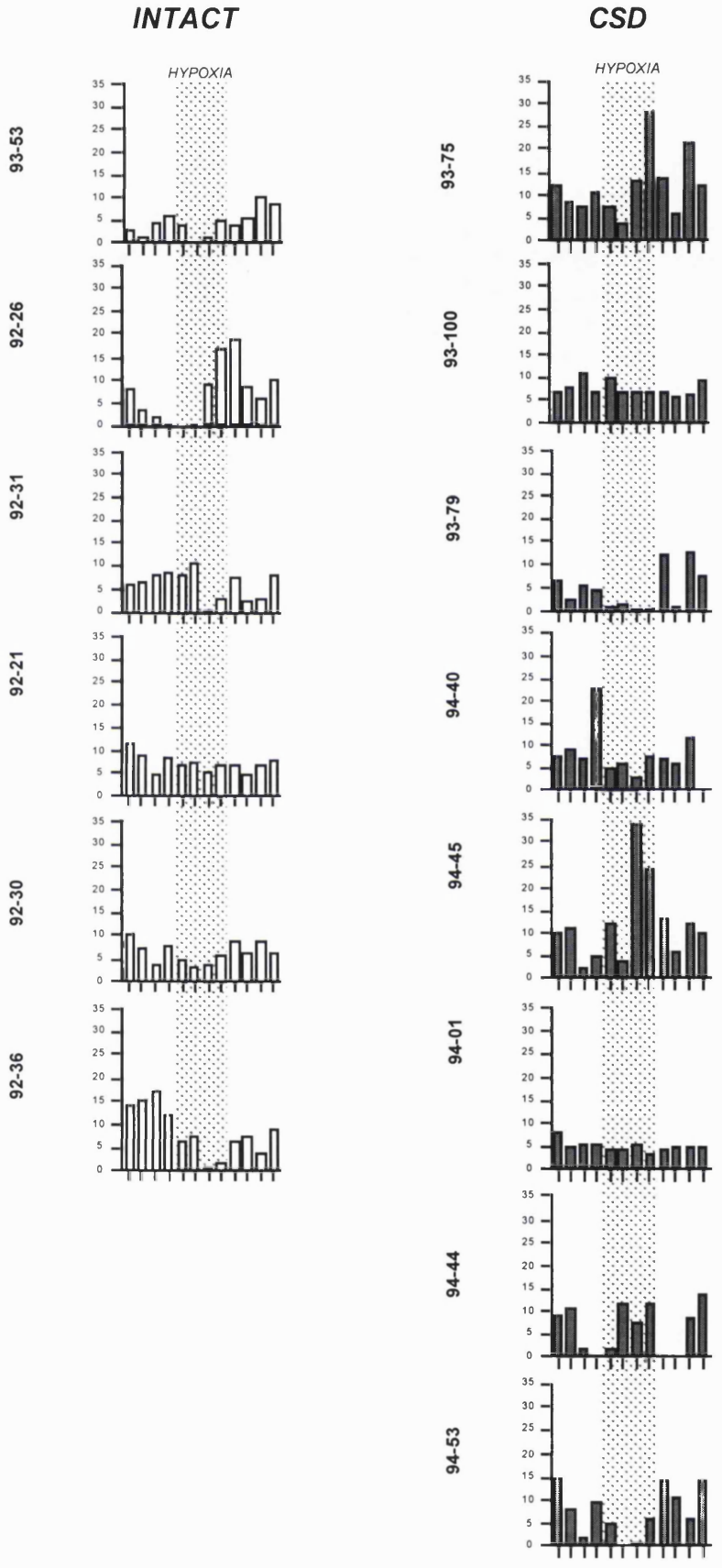


Figure 3.7 UO (Ordinate: ml.15 min^{-1}) throughout the protocol in intact (open bars) and CSD (filled bars) fetuses. The shaded region denotes the 1 h hypoxia. UO fell in 5 out of 6 intact, and 4 out of 8 CSD fetuses during hypoxia.

3.4.7 The relationship between urine output and renal blood flow

Both RBF and UO data were obtained in 5 intact and 4 CSD fetuses. There was no relationship between RBF and UO in either intact or CSD fetuses when regression analysis was carried out on UO vs. RBF, during normoxia (A), hypoxia (B) or recovery (C) periods (Figure 3.8).

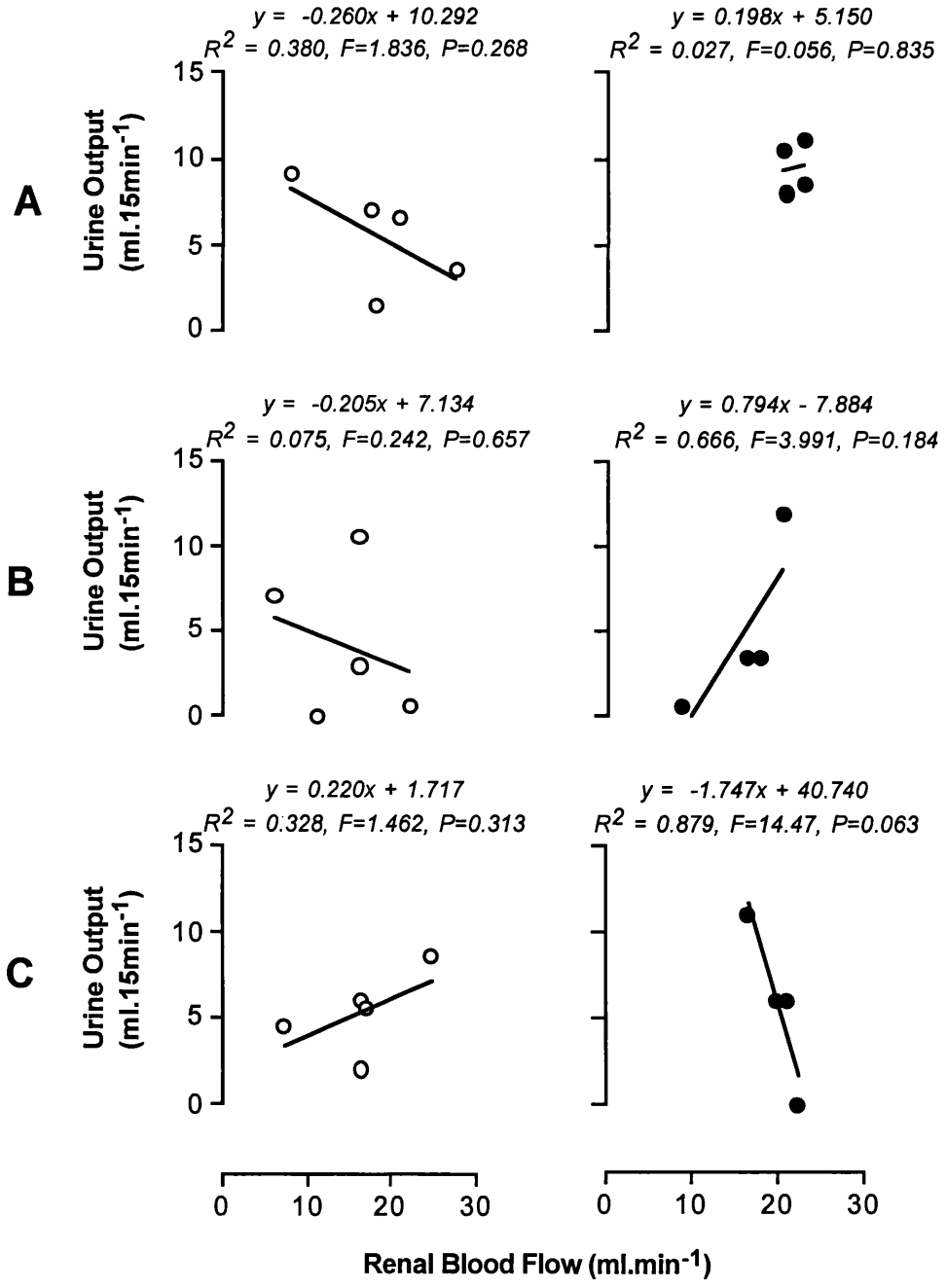


Figure 3.8 Scatter plots of UO against RBF in intact (○, n=5) and CSD (●, n=4) fetuses during A) normoxia (30 min), B) hypoxia (90 min) and C) recovery (150 min). Each plot has a fitted regression line and is represented by an equation in the form 'y=mx + c'. 'R^2' is the square of the correlation coefficient, 'F' is the square of the test statistic and 'P' is the probability that the slope (m) is significantly different from zero. These results show that there was no relation between UO and RBF in either intact or CSD fetuses in normoxia, hypoxia or recovery.

3.4.8 The relationship between urine output and mean arterial pressure

Both MAP and UO data were obtained in 6 intact and 8 CSD fetuses. In intact fetuses during normoxia (A) there was a significant relationship between MAP and UO ($P<0.05$, $n=6$) where 91% of variability in UO was explained by variation in MAP ($R^2=0.914$) (Figure 3.9). However this relationship was absent during the subsequent hypoxia (B) and recovery (C) periods. In CSD fetuses there was no relationship between MAP and UO throughout the protocol.

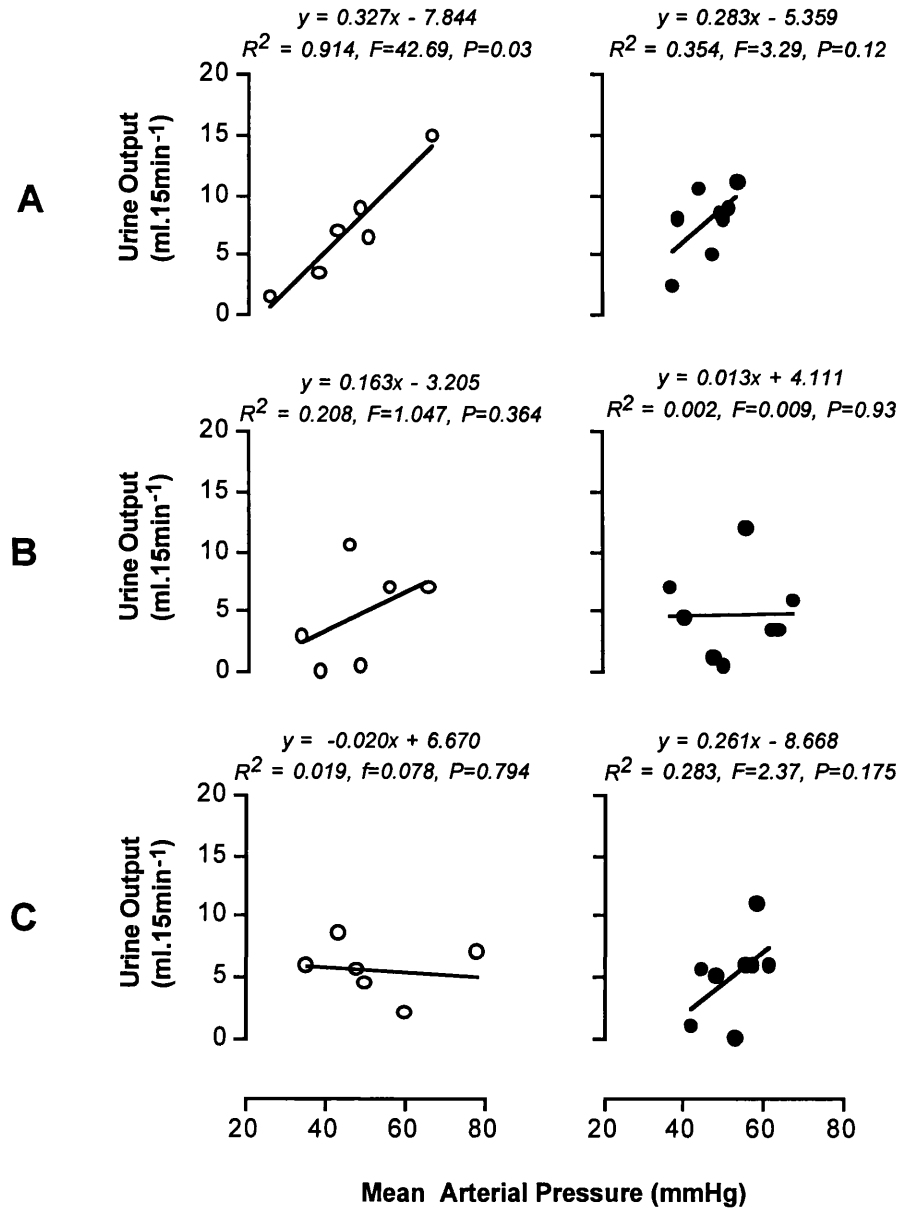


Figure 3.9 Scatter plots of UO against MAP in intact (○, n=6) and CSD (●, n=8) fetuses during A) control (30 min), B) hypoxia (90 min) and C) recovery (150 min). Each plot has a fitted regression line and is represented by an equation in the form 'y=mx + c'. 'R2' is the square of the correlation coefficient, 'F' is the square of the test statistic and 'P' is the probability that the slope (m) is significantly different from zero. There was a significant relationship between UO and MAP in intact fetuses during normoxia ($P<0.05$).

3.5 Discussion

The results presented in this chapter confirm previous findings (Giussani, Spencer, Moore, Bennet and Hanson, 1993) that a large component of the rapid initial fall in FHR at the onset of hypoxia is mediated by a carotid chemoreflex. MAP rose in the CSD group during hypoxia, but the rise did not reach significance in the intact group. Results show a reduced RBF and raised RVR in the last 30 min of hypoxia in both intact and CSD fetuses. A striking component of the CSD fetal responses was an initial rise in RBF at the onset of hypoxia. UO fell in some intact and CSD fetuses during hypoxia but from summary data there was no change in UO throughout the protocol in either group. There was no correlation between UO and RBF throughout the protocol, but there was a relationship between MAP and UO during normoxia in intact fetuses. I have also shown preliminary data in this section which demonstrate that the inhibition of NOS has marked cardiovascular effects in normoxia and continues to modify cardiovascular control in hypoxia, but does not inhibit the rise in RBF in early hypoxia.

Methodological considerations

Eleven CSD and 6 intact fetuses were initially involved in this study. MAP data was available for all fetuses, and FHR data was available for all intact and ten out of eleven CSD fetuses. RBF data was available in 5 intact and 7 CSD fetuses and UO data in all intact and 8 CSD fetuses. RBF data was unavailable usually due to total absence of measurements, but also when measurements were discarded due to abnormal readings. Difficulties in the measurement of RBF arise from the small size of the renal artery and the small space available for placement of the flow probe around the vessel. Total absence of flow readings occurred when the probe slipped off the vessel or was faulty, but may also be due to lack of sufficient fluid surrounding the vessel. Abnormal RBF was identified, at the time of experimentation, by a lack of pulsatile flow, intermittent errors in average flow meter readings, negative average flow readings and wide oscillations in average flow readings. The reason for such abnormal flow was thought to be either occlusion or twisting of the renal artery caused by positional changes of the transonic flow probe (usually confirmed at post mortem), insufficient peritoneal fluid surrounding the vessel, or a bad electrical connection in the flow probe itself.

UO values of 5-10 ml.15 min^{-1} during normoxia in this study are compatible with those of previous studies using similar collection methods (Robillard, Weitzman, Burmeister and Smith, 1981; Nakamura, Ayres, Gomez and Robillard, 1985). The data from all fetuses in which free flow of urine was observed in the 15 min draining period prior to the onset of the protocol were included in the present study. Urine flow was distinguished from amniotic fluid by its colour and viscosity. Absence of UO during experimental periods in CSD fetuses was attributed, at the time of post mortem, to the

catheter having come out of the bladder and free floating in the amniotic cavity, a kink in the catheter or the catheter tip being too close to the bladder wall. UO data from fetuses in which RBF data was missing was considered viable unless there was evidence of twisting or occlusion of the renal artery.

CSD does not alter baseline renal haemodynamics

During normoxia, CSD was not associated with a change in baseline FHR or MAP as observed in previous studies (Giussani, Spencer, Moore, Bennet and Hanson, 1993). In addition, although RBF tended to be greater, and RVR to be lower, in CSD than in intact fetuses, it was not statistically significant. Thus carotid chemoreflex mechanisms do not appear to play a role in the control of baseline renal haemodynamics. These findings are similar to those of Robillard *et al.* (1986) in which the *renal* nerve was unilaterally sectioned.

Basal RBF values reported in this study are lower than those of comparatively aged fetal sheep in previous studies (Robillard, Weitzman, Burmeister and Smith, 1981; Nakamura, Ayers, Gomez and Robillard, 1985). While I do not know the reason for this difference, I did not observe any effect of flow probe implantation on kidney size or weight, or find evidence of renal artery occlusion at post mortem. It may be due in part to methodological differences since I used implanted ultrasound flow probes whereas previous workers used the radiolabelled microsphere technique. As RBF is quite variable from minute to minute, the latter technique may give unreliable results. Moreover the use of implanted transducers provides continuous measurement of flow which allows investigation of rapid, reflexly-mediated changes in flow.

Carotid chemoreceptors play a role in rapid renal vascular responses to hypoxia

Low umbilical arterial PO_2 has been linked to a high impedance to renal arterial flow in human fetuses (Vyas, Nicolaides and Campbell, 1989) and experimentally induced acute hypoxia in fetal sheep is reported to produce a fall in RBF (Cohn, Sacks, Heymann and Rudolph, 1974; Robillard *et al.*, 1981; Nakamura *et al.*, 1985; Robillard, Nakamura and DiBona, 1986;) and in some instances a rise in RVR (Robillard *et al.*, 1981; Reuss, Parer, Harris and Kreuger, 1982; Robillard *et al.*, 1986). Furthermore, elevated fetal $PaCO_2$ causes a fall in RBF (Beguin, Dunihoo and Quilligan, 1974). Results presented in this chapter confirm a fall in RBF and a rise in RVR during hypoxia in intact fetuses. However these findings conflict with other studies in which blood flow to the kidney is not altered during hypoxia (Wlodek, Challis, Richardson and Patrick, 1989). The reason for similarities and differences between RBF responses in this study and the observations of others is not clear. There does not appear to be an obvious relationship between the

observation of a fall in RBF in response to hypoxia and the technique for blood flow measurement used whether it be by Doppler ultrasound, microsphere or transit time ultrasound methods.

Adult dog studies have shown that carotid chemoreceptor stimulation reduces RBF (Al-Obaidi and Karim, 1992). Furthermore, renal vasoconstriction during hypoxia was attenuated in fetal studies in which the *renal* nerves had been cut (Robillard *et al.*, 1986). Previous work has shown that peripheral chemoreceptors initiate the rapid phase of the vasoconstrictory responses to acute hypoxia in pulmonary (Moore and Hanson, 1991) and systemic (Giussani, Spencer, Moore, Bennet and Hanson, 1993) vascular beds. Thus we might have expected CSD to attenuate changes in RBF during hypoxia. In the present study CSD fetuses show a fall in RBF and rise in RVR during hypoxia to a similar extent as that observed in intact fetuses, but they also show a transitory initial rise in RBF at a time when MAP was increasing. Indeed RVR tended to rise more slowly in CSD than in intact fetuses. These findings indicate an important role for carotid chemoreceptors in the initial renal vasoconstriction during hypoxia since it is only once this reflex input has been removed that the initial rise in RBF is revealed.

The significant fall in RBF during late hypoxia after section of the CSNs makes it clear however that other vasoconstrictor mechanisms, in addition to the reflex neuronal pathway, must be in operation during hypoxia. It is widely established that fetal plasma [catecholamine] rise significantly during hypoxia (Jones and Robinson, 1975; Schuijers, Walker, Browne and Thorburn, 1986) and has been suggested to contribute towards peripheral vasoconstriction (Giussani, 1992). Furthermore α_1-adrenoceptor stimulation in the late gestation fetus produces a fall in RBF (Guillery, Segar, Merrill, Nakamura, Jose and Robillard, 1994). Thus it is possible that the rise in [catecholamine] in fetuses in this study was sufficient to produce a vasoconstriction by mid-hypoxia which would mask vasodilatation, even in CSD fetuses. This of course assumes that catecholamines rise to similar levels in intact and CSD fetuses. To date there has been no comparison between plasma [catecholmine] in intact and CSD fetuses. Measurements presented in this Chapter show a rise in plasma [catecholamine] in the majority of fetuses during hypoxia, and do not suggest a difference between intact and CSD groups. Other hormonal candidates which may contribute to the renal vasoconstriction during hypoxia include AII (Robillard, Weitzman, Burmeister and Smith, 1981) and ETs (Simonson and Dunn, 1991). Indeed a role for ET-I is implicated in the modulation of peripheral vascular tone at rest and during acute hypoxia (Green, Bennet and Hanson, 1994a; Green, McGarrigle, Bennet and Hanson, 1995e: Appendices 10 and 13, and Chapter 5 for discussion) and for AII in MAP and femoral vascular tone responses to acute hypoxia (Green, McGarrigle, Bennet and Hanson, 1994c and Appendix 8, and Chapter 4 for discussion).

The role of NOS in renal vascular responses to hypoxia

The marked, but transient rise in RBF at the onset of hypoxia in CSD fetuses, suggests a renal vasodilatation. This observation concurs with previous fetal studies in which the *renal* nerves had been severed. Prostaglandins have been implicated in this initial vasodilatation since treatment with indomethacin was found to block the response (Robillard, Nakamura and DiBona, 1986). Prostaglandins are released from the adult carotid body in response to hypoxia (Gomez-Nino, Almaraz and Gonzalez, 1994), and decrease action potential frequency in the CSN either by altering carotid body blood flow (McQueen and Belmonte, 1974) or by a direct action on chemoreceptor cells (Gomez-Nino *et al.*, 1994). It is possible that PGs act at the level of the carotid body to counteract chemoreflex renal vasoconstrictor mechanisms at the onset of hypoxia. Indeed a more intense fetal renal vasoconstriction in response to hypoxia has been observed after PG-synthesis blockade in some studies (Millard, Baig & Vatner, 1979), although not in others (Robillard, *et al.*, 1986; Arnold-Aldea, Auslender & Parer, 1991). Another vasodilatory candidate is NO. PGI_2 and NO are co-released from endothelial cells (Tenney, 1990). There is evidence to suggest that they may act in synergy in their platelet anti-aggregating actions (Radomski, Palmer and Moncada, 1987) and that NO can increase the production of PGI_2 (Davidge, McLaughlin and Roberts, 1994). Preliminary data presented in this chapter shows that blockade of NOS using L-NAME during normoxia in three fetuses causes a fall in FHR and rise in MAP, as subsequently observed in a larger group of fetuses (Green, Bennet and Hanson, 1995a/b, Appendices 9 and 11, see Chapter 5). NOS appears to play a role in the modulation of renal vascular tone in normoxia since RBF tended to fall and RVR to rise in normoxia, with L-NAME infusion. These findings are in accordance with adult (Walder, Thiemermann and Vane, 1991; Tolins and Raij, 1991) neonatal (Solhaug, Wallace and Granger, 1993) and fetal studies (Bogaert, Kogan and Mevorach, 1993). Furthermore there is evidence in the adult rat that NOS is more important in the maintenance of baseline blood flow in the renal than in the femoral vascular bed (Sigmon, Carretero and Beierwaltes, 1993).

During hypoxia the initial rise in RBF was still in evidence after blockade of NOS. The role for NO in the renal haemodynamic responses to hypoxia are not clear from the results of this study. The removal of a vasodilatatory influence by inhibition of NOS during hypoxia might be expected to *augment* renal vasoconstriction. Alternatively, in light of the large RVR which was established in normoxia after the infusion of L-NAME, the scope for *vasodilatation* during hypoxia might have been expected to be great. This study shows that during hypoxia the initial rise in RBF was prolonged and RVR fell with the infusion of L-NAME. Thus these results indicate that NOS does not play a role in the initial rise in RBF at the onset of hypoxia, but also that it is not a major vasodilatatory

factor in the modulation of renal vascular responses to hypoxia. It seems possible that the effect of another vasodilator substance is being unmasked after the removal of NOS during hypoxia.

From the data presented in this Chapter it may be concluded that both vasodilatatory and vasoconstrictory mechanisms are operating in hypoxia to control RBF.

UO responses to hypoxia

Previous fetal studies have shown UO to fall after 30 min - 3 h of hypoxia (Nakamura, Ayers, Gomez and Robillard, 1985; Brace, Wlodek, McCrabb and Harding, 1994), while others show no significant change after ca. 1 h of hypoxia (Robillard, Weitzman, Burmeister and Smith, 1981; Wlodek, Challis, Richardson and Patrick, 1989). In this study, while there was a tendency for UO to fall during hypoxia in the majority of intact and half of the CSD group, this was not statistically significant when summary measures were compared between normoxia and hypoxia. The reason for a clear fall in UO being absent in this study is not obvious. Variability in UO in this study was larger than in previous studies using a similar sample size. All the fetuses were involved in an identical protocol however I was unable to control for the time of day that the study was carried out. There is evidence to suggest that urine production rate is greater as the fetus switches between behavioural states (Stigter, Visser and Mulder, 1995). While we had no measure of behavioural state in these fetuses, it is possible that differences in behavioural states could have contributed to the wide variety of UO values observed. Using a nomogram to determine the power of the present study it is apparent that with a sample size of 6 intact fetuses (normoxia: mean=7.33, Standard deviation=4.29) there is only 21% probability of detecting a 50% change in UO at the 0.05 significance level [see Appendix 2]. Thus in order to determine whether there was a fall or not in UO during hypoxia it is possible that a larger group of fetuses would have been required, although from previous studies there does not appear to be an obvious link between group size and observation of a fall in UO.

A variety of regimes have been used previously to measure urine flow. Similar techniques to the one used in this study have involved collection of urine over periods of time ranging from 20 min - 1 h. More recently techniques have been developed for the continuous removal of urine by pump while keeping a constant bladder pressure (Brace and Moore, 1991), and continuous drainage of the bladder via a flow meter (Brace *et al.*, 1994). However, there does not appear to be any relationship between the method of UO measurement employed in these studies and the detection of a fall in UO during hypoxia.

RBF is not a major control of UO

That the fetus can modify its UO in order to maintain volume and composition of its body fluids has been demonstrated in the sheep by a reduced fetal UO under conditions of reduced transplacental water transfer (Lumbers and Stevens, 1983). The mechanisms that underlie control of urine production during normoxia and hypoxia are not fully understood. It has been proposed that reduced UO observed in hypoxic fetuses be secondary to changes in RBF (Nicolaides, Peters, Vyas, Rabinowitz, Rosen and Campbell, 1990; Daniel, Yeh, Bowe, Fukunaga and James, 1975). However while marked changes in RBF were observed in this study during hypoxia in both intact and CSD fetuses, these were not accompanied by changes in UO in all fetuses. Furthermore there was no significant relationship between UO and RBF during normoxia, hypoxia or recovery. Thus, these data do not support a major role for RBF in the control of UO.

In the sheep fetus, a 24 h rhythm has been observed in MAP and UO with levels rising from the noon to midnight (Brace and Moore, 1991) and UO has been shown to correlate to fetal arterial pressure (Brace and Moore, 1991; Wlodek, Challis, Richardson and Patrick, 1989). In the present study we have confirmed a strong relationship between UO and MAP during normoxia in intact fetuses. Thus a pressure diuresis may contribute to basal urine flow. It is possible that diurnal variations in MAP and the increase in MAP seen during hypoxia may have had an effect on the urine measurements obtained in this study.

It therefore seems quite possible that the primary control of UO is via the direct action of hormonal factors on the kidney. Possible anti-diuretic candidates include AVP and NO. AVP administration to the fetus decreases urine flow rate (Lingwood, Hardy, Horacek, McPhee, Scoggins and Wintour, 1978), and NOS inhibition increases capacity, and decreases voiding volume of the fetal bladder (Mevorach, Bogaert and Kogan, 1994). Diuretic candidates include ANP (Cheung, 1991), catecholamines (Ervin, Castro, Sherman, Ross, Padbury, Leake and Fisher, 1991), AII (Lumbers, Burrell, Menzies and Stevens, 1993) and ET-1 (Uzuner and Banks, 1993).

3.6 Conclusion

The results of this study confirm that a fall in RBF occurs during hypoxia and show that the initial, rapid component of this response is reflexly mediated with a CSN afferent limb. The efferent limb is probably via the renal nerves. It is likely that vasoconstrictor hormonal factors (e.g. catecholamines) contribute to the sustained response. While NOS does not appear to contribute to the rapid vasodilatation seen after CSD at the onset of hypoxia, it does seem possible that if hypoxia is prolonged, a balance exists between vasoconstrictory and vasodilatatory agents, such as PGs and NO. In addition, results

from this study do not reveal a fall in UO during hypoxia and indicate that hypoxic changes in renal vascular tone do not provide a major control of UO. While comparisons to the human situation should be made with caution, it is clear that UO is variable and depends on a range of factors: mechanisms other than hypoxia may account for diminished urine production seen in oligohydramnios in human pregnancies.

CHAPTER 4

THE ROLE OF ANGIOTENSIN II IN THE CARDIOVASCULAR RESPONSES TO HYPOXIA

4.1 Introduction

Previous studies in this laboratory have investigated the role of peripheral arterial chemoreceptors on regional blood flow distribution during acute isocapnic hypoxia. This redistribution consists of cardiovascular responses with two temporal components. *Initial* responses are characterised by a rapid fall in FHR and FBF and have been shown to be carotid (Giussani, Spencer, Moore, Bennet and Hanson, 1993; Bartelds, van Bel, Teitel and Rudolph, 1993) but not aortic chemoreflexes [1.5.1]. The efferent limbs of these reflex responses are provided by α-adrenergic (femoral vascular bed) or vagal (heart) mechanisms (Giussani *et al.*, 1993). However it is clear [1.5.2] that other, as yet unidentified, reflexly released mediators participate in these rapid responses. Arginine vasopressin (Giussani, McGarrigle, Spencer, Moore, Bennet and Hanson, 1994b) and ACTH and cortisol (Giussani, McGarrigle, Moore, Bennet, Spencer and Hanson, 1994a) are not likely to fulfil this role since their rise during hypoxia is not altered by CSD. An alternative candidate is the potent vasoconstrictor, AII. Fetal plasma [AII] is known to rise during hypoxia (Broughton-Pipkin, Lumbers and Mott, 1974). Therefore, the ***first aim*** of this study was to determine whether AII is released during hypoxia by chemoreflex mechanisms.

The *second* phase of cardiovascular responses is slower and manifested by a return of FHR to control, a maintained peripheral vasoconstriction, a rise in MAP, and a vasodilatation of cerebral, myocardial and adrenal beds. These responses are not chemoreflexes as they occur in CSD fetuses. A rise in plasma [catecholamine] (Jones and Robinson, 1975), due in part to a direct action of hypoxia on the adrenal gland, and in [AVP] (Giussani *et al.*, 1994b), and [ACTH] and [cortisol] (Giussani, *et al.*, 1994a) are likely to contribute to, but do not fully account for, the second phase of responses. The role played by AII is not certain. AII is produced from the conversion of AI by ACE in the plasma, but there is also evidence that endothelial and smooth muscle cells contain all the components of the RAS, which suggests a local RAS [1.5.3] that may play a role in the regulation of local blood flow (Nelissen-Vrancken, Leenders, Bost, Struijker-Boudier and Smits, 1992). Captopril is thought to be one of the more effective ACE inhibitors in

reaching this local ACE. While exogenous AII administration to the fetus, simulating levels seen in moderate haemorrhage, does not alter peripheral blood flow (Iwamoto and Rudolph, 1981a), a role for *endogenous* AII in maintaining a high basal vascular resistance in the periphery and in cerebral, thyroid and adrenal vascular beds has been implicated by the administration of the angiotensin receptor blocker, Saralasin (Iwamoto and Rudolph, 1979). Thus the ***second aim*** of this study was to determine the role of endogenous AII in the cardiovascular responses to acute hypoxia using the ACE inhibitor captopril.

Some of these results have already been published in abstract form (Green, McGarrigle, Bennet and Hanson, 1994c. see Appendix 8).

4.2 Methods

4.2.1 Surgical preparation

Fifteen fetuses aged between 113 and 121 days gestation were instrumented under general anaesthesia [2.3.2]. The lower half of the fetus was exteriorised through the uterine incision. An ultrasonic flow probe (3R: Transonic Systems Inc., Ithaca NY) was placed around a femoral artery and the transducer cable secured in 2 places to the fetal skin. The lower portion of the fetus was returned to the uterus. The upper portion was then exteriorised either through the same incision by rotating the fetus or through a second incision in the uterus following closure of the first. Two stainless steel electrodes were sewn onto the chest and an earthing electrode onto the back of the neck (or hindlimb at the time of femoral flow probe placement) to monitor ECG. A catheter was sewn onto the fetal skin for drug administration into the amniotic cavity and so that the amniotic pressure signal could be subtracted from arterial and venous pressures. Additional heparinised catheters were placed in a carotid artery and a jugular vein (as previously described in section 2.3.2) to monitor mean arterial and venous pressures and to administer antibiotics. In 5 out of the 9 CSD fetuses an extra catheter was placed in the right brachial vein specifically for the administration of drugs. A second ultrasonic flow probe (3 or 4R) was placed around the uncatheterised carotid artery.

Nine of these fetuses also underwent bilateral section of their CSNs (performed by Prof. M.A. Hanson, see 2.3.2 for details).

A maternal pedal vein was catheterised to administer antibiotics.

Five days of recovery were allowed before experimentation.

4.2.2 Experimental procedure

Measurements

MAP, MVP, FHR, CBF and FBF were recorded continuously onto chart paper [2.4.2].

2-3ml fetal arterial blood was collected during control (45 min), early and late hypoxaemia (75 and 105 min, respectively) and early and late recovery (135 and 165 min, respectively). Blood was transferred immediately to chilled EDTA tubes and spun at 4°C (3000 rpm) for 10 min. Plasma was then decanted into tubes and stored at -40°C for subsequent hormonal analysis. A further 0.6 ml arterial blood was collected at these times for the analysis of blood gases and electrolytes. In 3 intact and 5-6 CSD fetuses additional blood was collected for blood gas, electrolyte and AII analysis before the onset of vehicle or captopril infusion (-20 min).

Drug preparation

Captopril (Sigma Chemical Co., UK) was initially dissolved in sterile water and the volume made up with sterile saline (0.9%, Baxter Healthcare Ltd., UK) to give a final concentration of $1 mg.ml^{-1}$.

Angiotensin I (Sigma Chemical Co., UK) was dissolved in sterile saline to give a final concentration of $25 \mu g.ml^{-1}$.

Protocol

Three 3 h protocols were conducted on separate days: experiment 1, hypoxia with the infusion of saline vehicle; experiment 2, hypoxia with continuous captopril infusion and experiment 3, normoxia with continuous captopril infusion. In 1 CSD fetus the order of experiments 1 and 2 were reversed, and in 1 intact fetus the order of experiments 2 and 3 were reversed [see 2.6.3]. The hypoxia protocols comprised an initial 1 h normoxic control period, followed by 1 h fetal isocapnic hypoxia. Ewes were then returned to normoxic conditions and measurements continued for 1 h (recovery) [2.5.1]. In the normoxia protocol ewes were allowed to breathe room air throughout the 3 h protocol.

At -5 min an initial bolus of either captopril or vehicle was administered by a $12ml.h^{-1}$ infusion for 5 min (1mg captopril). This was followed by a $3ml.h^{-1}$ ($3mg.h^{-1}$ captopril) infusion for the duration of the 3 h protocol via the jugular or brachial (5 CSD fetuses only) vein.

In 4 CSD fetuses the response to two 5μg bolus doses of AI was investigated on all experimental days: first prior to the onset of vehicle or captopril infusion and second at the end of the 3 h protocol. The drug was administered i.v. to the fetus via the brachial vein catheter and flushed in with 2ml saline.

Plasma analysis

Plasma [AII] was analysed by Dr H.H.G. McGarrigle, Department of Obstetrics & Gynaecology, University College London. Details of the assay are given in full in Appendix 5, but briefly plasma [AII] was measured quantitatively using radioimmunoassay (RIA. Nichols Institiute Diagnostics Ltd.) following the separation of AII from plasma proteins by ethanol extraction. The sensitivity of the assay was 3.8 $pg.ml^{-1}$.

4.3 Data analysis

Grouped data is expressed as mean±S.E.M. Blood composition and cardiovascular measurements for individual fetuses were reduced to summary measures to describe normoxic, hypoxic and recovery hours [2.6.3]. Summary measures were then tested using Student's paired t-test to compare normoxia, hypoxia and recovery periods.

Student's paired t-test was used to test rapid transient changes in FHR.

Cardiovascular measurements made prior to the onset of vehicle or captopril infusion (-20 to -5 min) were reduced to a summary measure and this was compared by t-test to the mean of each variable over the subsequent 1 h normoxic period.

Statistical significance was accepted when $P<0.05$.

Student's t-test was used to compare plasma [AII] at 75 and 105 min with that at 45 min. The Bonferroni method of correction [2.6.3] was used so that these tests were performed at the $P<0.025$ level.

An unpaired t-test was substituted in the event of uneven sample groups and these instances are indicated in the text and/or figure legends.

Venous pressures were obtained for 7 out of 9 CSD and 2 out of 6 intact fetuses [4.5, methodological considerations], therefore for the sake of consistency within the study vascular resistances were calculated throughout from MAP ÷ flow.

4.4 Results

4.4.1 The response to angiotensin I

Figure 4.1 shows the response of one fetus to 5μg AI, prior to the onset of the 3 h protocol. There was a fall in FHR, as indicated in the figure by the reduction in the number of ECG waveforms recorded, a small rise in venous pressure, a large rise in arterial pressure and a fall in both CBF and FBF. In the 4 fetuses considered together (Figure 4.2), there was a rise in MAP in response to AI in the pre-infusion period in all experimental groups. Following the infusion of vehicle the response to AI remained (experiment 1) but was absent post-captopril infusion (experiment 2 and 3). The MAP response to AI pre-normoxia with captopril protocol (experiment 3) was significantly lower than that pre-hypoxia with captopril protocol (experiment 2). Angiotensin I caused a fall in FHR pre-hypoxia with vehicle protocol (experiment 1), however the FHR response to AI was not significant post-hypoxia with vehicle. AI reduced FBF pre-hypoxia with captopril (experiment 2) but this response was attenuated after captopril infusion.

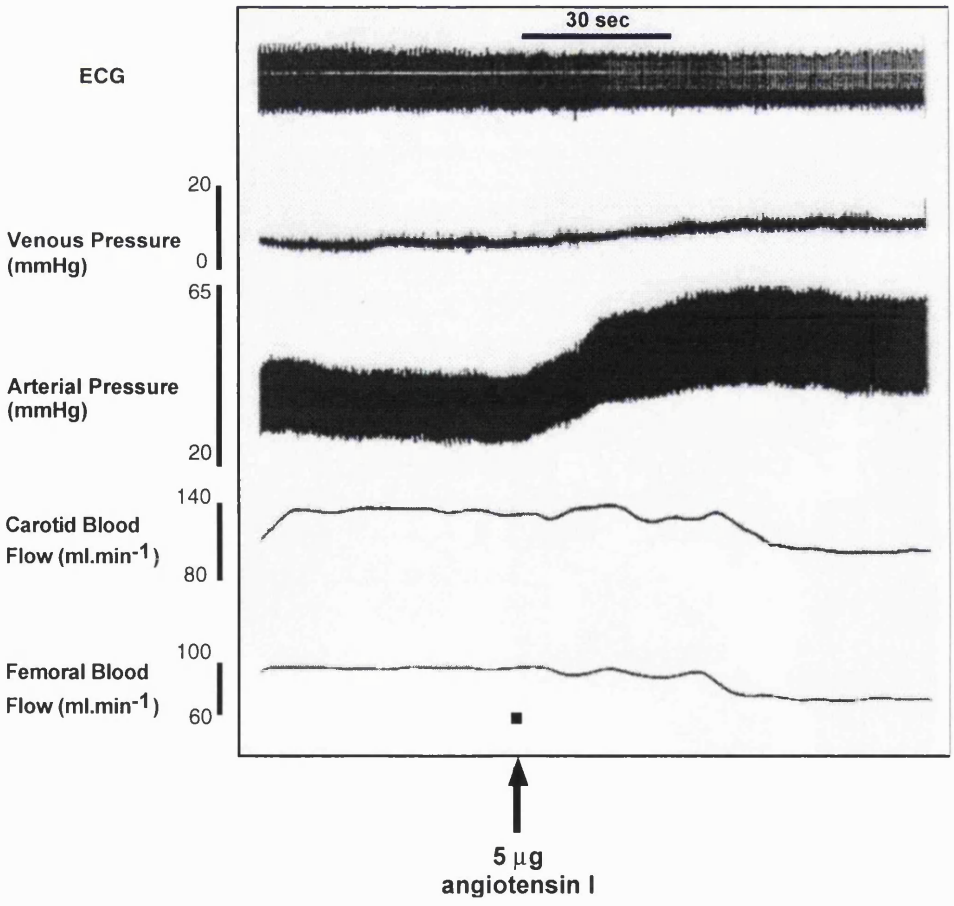


Figure 4.1 Cardiovascular responses of one CSD fetus to a bolus of 5μg AI (i.v.). The top trace shows the raw ECG signal in which the number of ECG waveforms decreases following the administration of AI, indicating a fall in FHR. There was a gradual rise in venous pressure and fall in CBF and FBF, and a large rise in arterial pressure following the AI bolus.

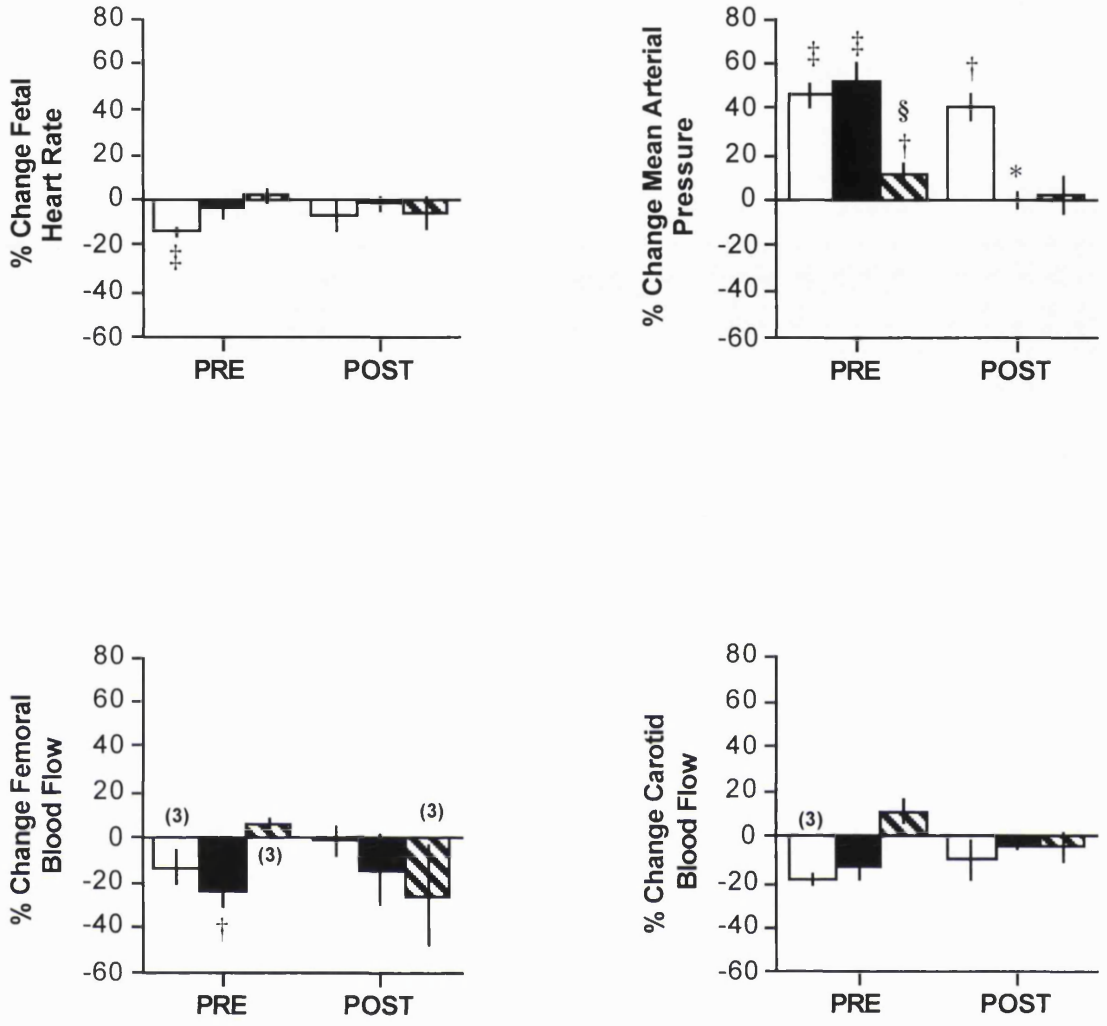


Figure 4.2 Cardiovascular responses of 4 CSD fetuses (unless shown otherwise by a bracketed number) to 5µg AI (i.v.) PRE- and POST- (abscissa) experiment 1 (hypoxia with vehicle, open bars), experiment 2 (hypoxia with captopril, closed bars) and experiment 3 (normoxia with captopril, striped bars). Values are shown as mean % change from pre-AI. * P<0.01 POST vs. PRE experiment values; † P<0.05 significant change from baseline values; ‡ P<0.01 significant change from baseline values; §P<0.01 compared to PRE-experiment 2. The rise in MAP in response to AI was blocked after the administration of captopril in experiments 2 & 3.

4.4.2 Plasma angiotensin II

There was no significant difference in plasma [AII] between intact and CSD fetuses throughout the protocol with vehicle (experiment 1) or captopril (experiment 2) infusion (Figure 4.3).

With the infusion of vehicle, plasma [AII] increased during hypoxia (75 and 105 min) in intact fetuses and after 15 min hypoxia in CSD fetuses (Figure 4.3). However this rise was absent in both groups of fetuses with the infusion of captopril.

During the normoxia protocol (experiment 3), after the onset of captopril infusion there was no significant change in plasma [AII] in intact or CSD fetuses (Figure 4.4). There

was no relationship between the magnitude of change between pre- (-20 min) and post- (45 min) captopril infusion and the initial plasma Na^+ level (-20 min, see Figure 4.9).

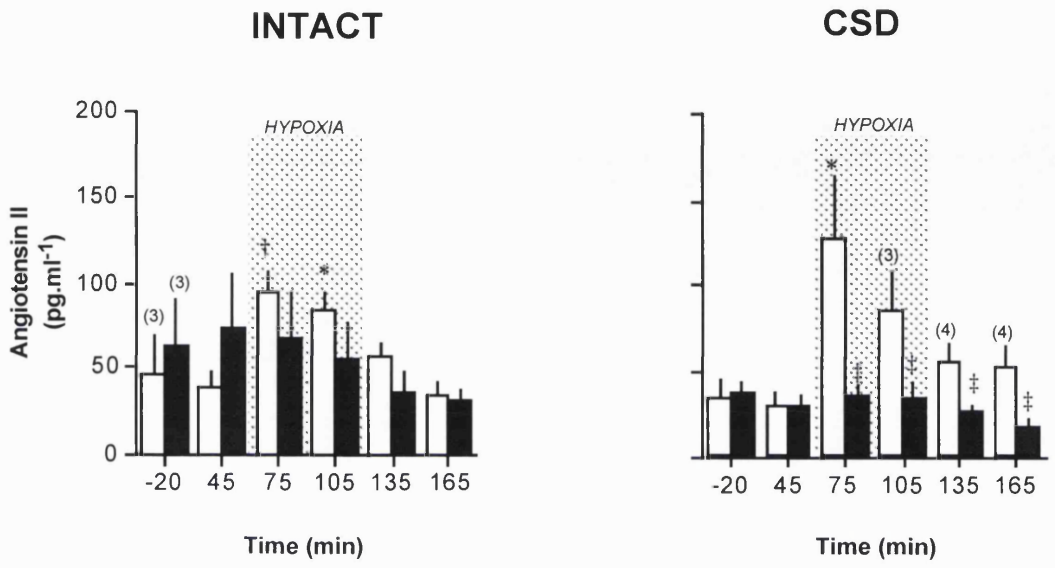


Figure 4.3 The effect of 1 h hypoxia (shaded area) on circulating [AII] in intact and CSD fetuses during vehicle (open bars, n=6 and 5, respectively) and captopril (closed bars, n=5 and 6, respectively) infusion. Values are shown as mean ± SEM. *P<0.025 significantly different from 45 min, † P<0.01 significantly different from 45 min and ‡P<0.05 significant difference between captopril and vehicle infused groups by unpaired t-test.

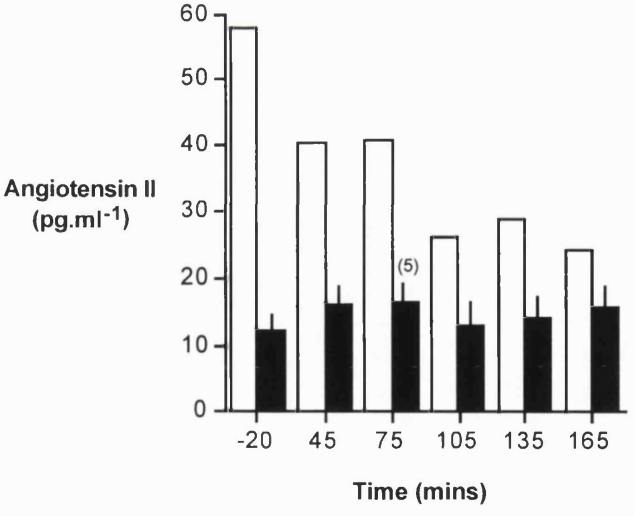


Figure 4.4 Plasma [AII] in intact (open bars. n=1) and CSD (closed bars. n=6, unless indicated otherwise by bracketed number) during normoxia with continuous captopril infusion.

fetuses

4.4.3 Blood gas, pH, lactate and glucose

PaO2

In intact and CSD fetuses, PaO_2 fell during hypoxia with the infusion of vehicle (○: P<0.01 (n=6) and P<0.01 (n=8), respectively) and captopril (●: P<0.01 (n=5) and

P<0.01 (n=7), respectively). During the recovery period PaO_2 returned to pre-hypoxic levels in all groups except captopril infused CSD fetuses in which PaO_2 was slightly lower than in normoxia (●: P<0.05 (n=7)). In the captopril infused normoxia protocol PaO_2 did not change throughout the course of the experiment in either intact or CSD fetuses (Figure 4.5).

%SaO2

Oxygen saturation fell in intact and CSD fetuses during hypoxia in both vehicle (○: P<0.01 (n=6) and P<0.01 (n=8), respectively) and captopril (●: P<0.01 (n=6) and P<0.01 (n=7), respectively) infused groups. During the recovery period $\%SaO_2$ returned to pre-hypoxic values in intact captopril infused fetuses, but was slightly depressed in intact vehicle (○: P<0.05 (n=6)) and captopril (●: P<0.01 (n=7)) infused fetuses, and captopril infused CSD fetuses (●: P<0.01 (n=7)). Captopril infusion during the normoxia protocol in intact fetuses produced a slight but significant fall in $\%SaO_2$ between 60 and 120 min compared to the previous hour (15-45 min) (▲: P<0.01 (n=6)) (Figure 4.5).

O2 content

During hypoxia, O_2ct fell in intact and CSD fetuses in vehicle (○: P<0.01 (n=6) and P<0.01 (n=8), respectively) infusion and captopril (●: P<0.01 (n=6) and P<0.01 (n=7), respectively) and returned to pre-hypoxic levels during the recovery period. During the normoxia protocol (▲) there was no change in O_2ct throughout the protocol (Figure 4.5).

Total haemoglobin

At the onset of captopril infusion there was a significant fall in haemoglobin in CSD involved in experiment 2 only (●: P<0.01 (n=7)). During hypoxia, Hb fell in intact and CSD fetuses infused with vehicle (○: P<0.01 (n=6) and P<0.01 (n=8), respectively) and captopril (●: P<0.01 (n=6) and P<0.01 (n=7), respectively) infusion, but returned to normoxic levels during recovery in all groups. The infusion of captopril during normoxia alone (▲) did not alter haemoglobin levels (Figure 4.5).

PaCO2

In intact fetuses with vehicle infusion $PaCO_2$ showed a transient fall after 30 min of hypoxia (45 min vs. 90 min: P<0.05), however from summary statistics there was no significant change in $PaCO_2$ in intact or CSD fetuses during hypoxia with vehicle (○), hypoxia with captopril (●), or normoxia with captopril (▲) experiments (Figure 4.6).

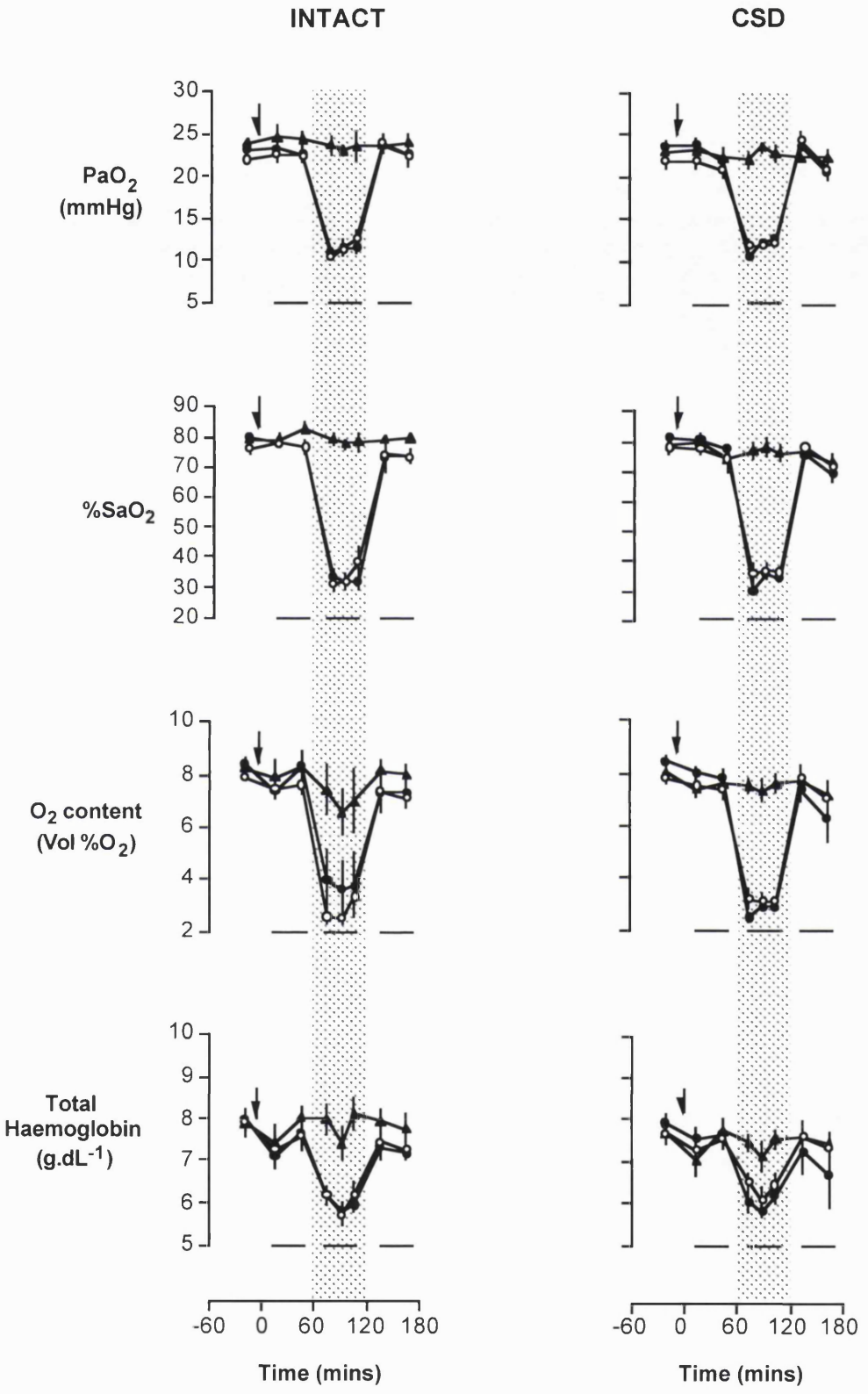


Figure 4.5 PaO_2, $\%SaO_2$, O_2 content and total haemoglobin of intact and CSD fetal arterial blood during experiments 1, 2 and 3. The shaded area denotes the 1 h of hypoxia for experiment 1 (○, vehicle infusion) and experiment 2 (●, captopril infusion) and the 1 h of normoxia in experiment 3 (▲, captopril infusion). The arrows show the start of infusion at time -5 min. The horizontal black bars show the time period over which values were reduced to summary measures. Summary statistics are given in the text. Values are shown as mean ± SEM. Where no error bars are visible they are contained within the plot symbol.

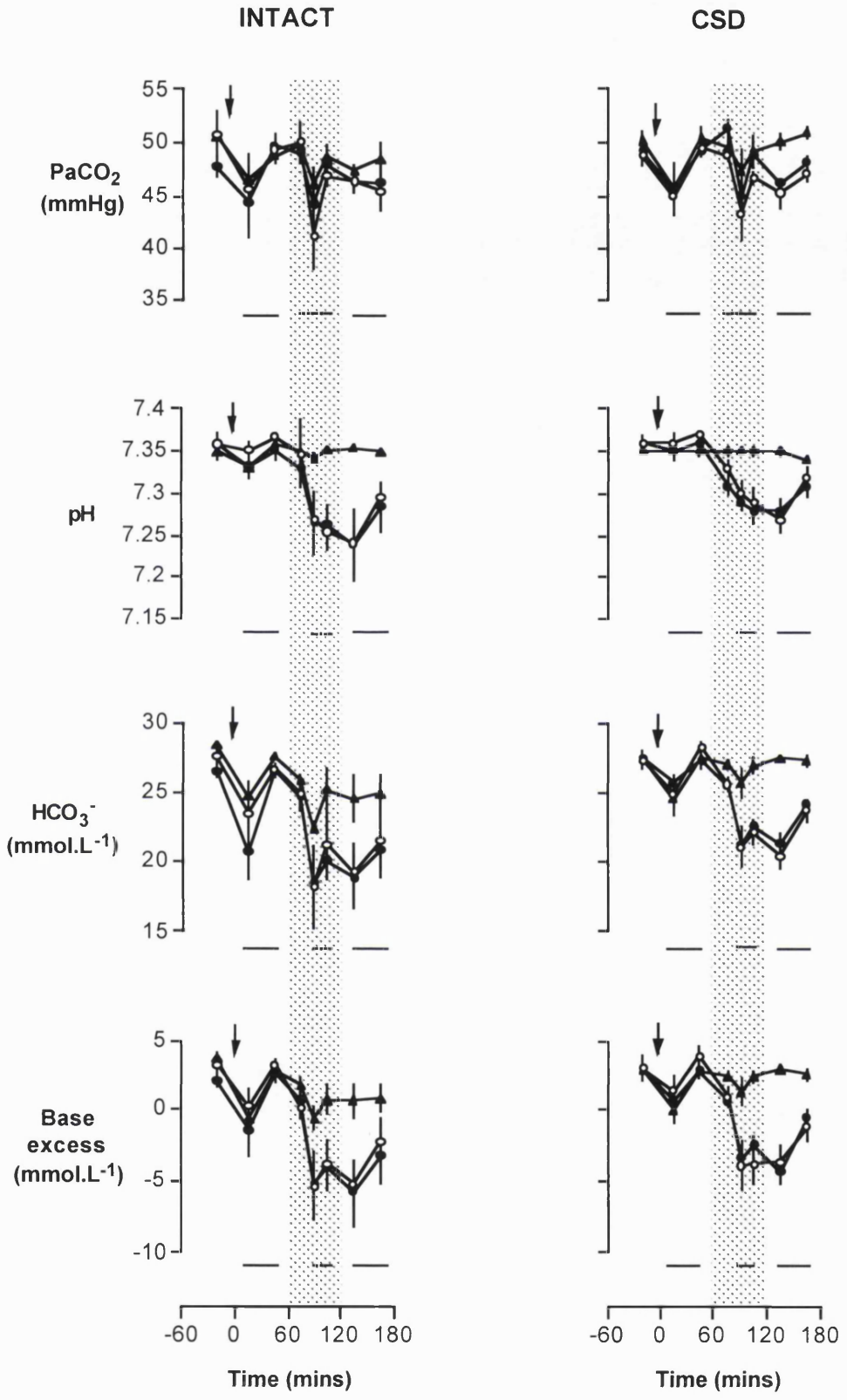


Figure 4.6 $PaCO_2$, pH, HCO_3^- and base excess of intact and CSD fetal arterial blood during experiments 1, 2 and 3. The shaded area denotes the 1 h of hypoxia for experiment 1 (○, vehicle infusion) and experiment 2 (●, captopril infusion), and the 1 h of normoxia in experiment 3 (▲, captopril infusion). The arrows show the start of infusion at time -5 min. The horizontal black bars show the time period over which values were reduced to summary measures. Summary statistics are given in the text. Values are shown as mean ± SEM. Where no error bars are visible they are contained within the plot symbol.

pH

In intact and CSD fetuses with vehicle infusion pH fell during hypoxia (○: P<0.01 (n=6) and P<0.01 (n=8), respectively) and remained lower than normoxia during the recovery period (○: P<0.01 (n=6) and P<0.01 (n=8), respectively). With captopril infusion pH also fell during hypoxia in CSD fetuses (●: P<0.01 (n=7)) and remained lower than normoxia during the recovery period (●: P<0.01 (n=7)), however this fall did not reach significance in intact fetuses (●: P=0.053). There was no alteration in pH during the normoxia with captopril protocol in intact or CSD groups (Figure 4.6).

$HCO3^-$ and base excess

The responses of HCO_3^- and base excess were of similar significance for all protocols of intact and CSD fetuses. In CSD fetuses, during normoxia, HCO_3^- and base excess fell by 15 min with the infusion of captopril in experiment 2 (-20 vs. 15 min. ●: P<0.05 (n=7 vs. n=6, unpaired t-test) and experiment 3 (▲: P<0.05 (n=8 vs. n=6, unpaired t-test), however this effect was not significant in intact fetuses (Figure 4.6). In intact and CSD fetuses, HCO_3^- and base excess levels fell during hypoxia with captopril (●: P<0.01 (n=6) and P<0.01 (n=6 vs. n=7, unpaired t-test), respectively) and vehicle infusion (○: P<0.05 (n=6) and P<0.01 (n=8), respectively), and remained lower than normoxia during recovery with vehicle (○: P<0.01 (n=6) and P<0.01 (n=8), respectively) and captopril (●: P<0.01 (n=6) and P<0.01 (n=6 vs. n=7, unpaired t-test), respectively) infusion.

Lactate and glucose

During hypoxia and recovery, lactate was significantly elevated from normoxia in intact and CSD fetuses with vehicle (○: P<0.01 (n=6) and P<0.01 (n=7), respectively) and captopril (●: P<0.01 (n=6) and P<0.01 (n=6)) infusion (Figure 4.7).

During hypoxia glucose was raised from normoxic levels in intact and CSD fetuses with vehicle (○: P<0.05 (n=6) and P<0.01 (n=7), respectively) and captopril (●: P<0.05 (n=5) and P<0.01 (n=6), respectively) infusion (Figure 4.7). In CSD, but not intact, fetuses glucose remained elevated during recovery with vehicle (○: P<0.01 (n=7)) and captopril (●: P<0.01 (n=6)) infusion .

The infusion of captopril in normoxia alone (experiment 3) did not alter lactate or glucose levels, apart from in intact fetuses during hour 3 (120-180 min) where glucose levels were elevated compared to the first hour (0-60 min) of normoxia (▲: P<0.05 (n=6). Figure 4.7).

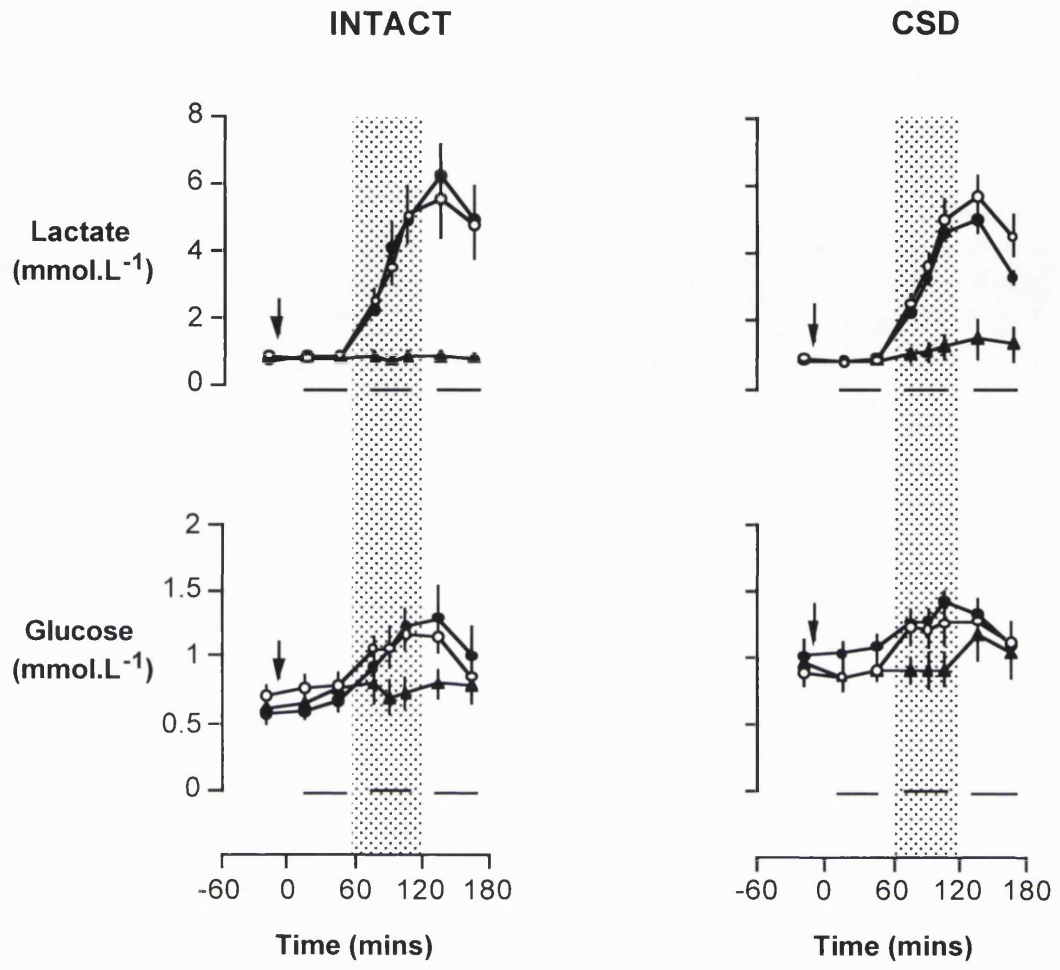


Figure 4.7 Lactate and glucose concentration of intact and CSD fetal arterial blood during experiments 1, 2 and 3. The shaded area denotes the 1 h of hypoxia for experiment 1 (○, vehicle infusion) and experiment 2 (●, captopril infusion), and the 1 h of normoxia in experiment 3 (▲, captopril infusion). The arrows show the start of infusion at time -5 min. The horizontal black bars show the time period over which values were reduced to summary measures. Summary statistics are given in the text. Values are shown as mean ± SEM. Where no error bars are visible they are contained within the plot symbol.

Sodium and potassium

Plasma Na^+ did not change from normoxia throughout the protocol, except for intact fetuses with vehicle infusion where Na^+ was below normoxia values during the recovery period (○: $P<0.01$ (n=6). Figure 4.8). K^+ was elevated during hypoxia in intact and CSD fetuses with vehicle infusion (○: $P<0.05$ (n=6) and $P<0.01$ (n=7), respectively), and remained higher than normoxia during recovery in CSD fetuses (○: $P<0.01$ (n=8)). Plasma K^+ rose during hypoxia in intact (●: $P<0.01$ (n=6)), but not CSD, fetuses with captopril infusion and remained greater than normoxia in the recovery period (●: $P<0.05$ (n=6)).

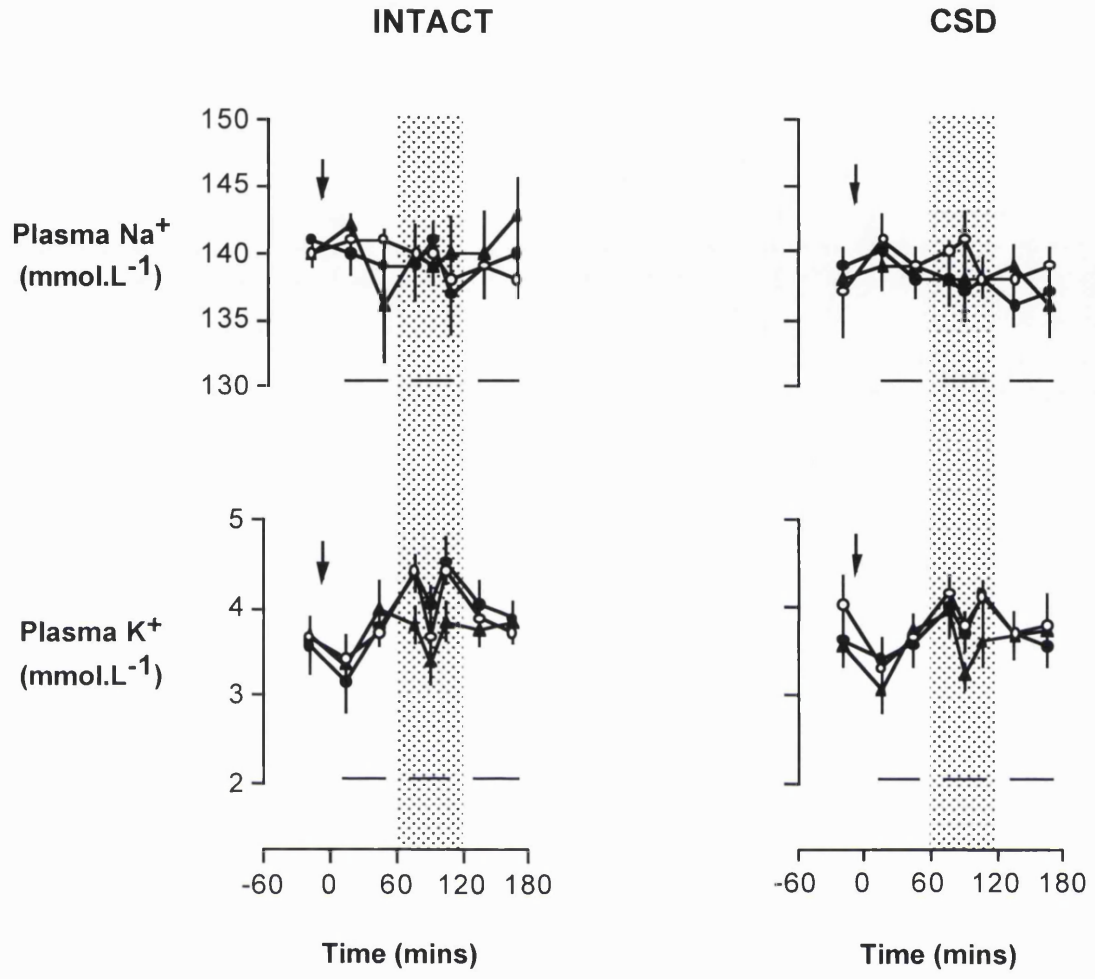


Figure 4.8 Plasma Na^+ and K^+ concentration of intact and CSD fetal arterial blood during experiments 1, 2 and 3. The shaded area denotes the 1 h of hypoxia for experiment 1 (○, vehicle infusion) and experiment 2 (●, captopril infusion), and the 1 h of normoxia in experiment 3 (▲, captopril infusion). The arrows show the start of infusion at time -5 min. The horizontal black bars show the time period over which values were reduced to summary measures. Summary statistics are given in the text. Values are shown as mean ± SEM.

4.4.4 Fetal heart rate

In CSD fetuses, FHR was lower during the 1 h normoxic period than pre-captopril infusion levels (experiment 2, Table 4.1). At the onset of hypoxia there was a rapid initial fall in FHR with the infusion of vehicle and captopril in both intact and CSD fetuses (see Figure 4.10). However the magnitude of the fall in FHR with vehicle infusion was less in CSD than in intact fetuses ($P<0.05$ (n=6 vs. n=9), unpaired t-test). As hypoxia proceeded FHR returned to prehypoxic levels in all groups. In intact fetuses, a tachycardia was apparent during recovery although this only reached significance with the infusion of captopril. In CSD fetuses, FHR was greater during recovery than during normoxia with vehicle and captopril infusion, although vehicle group values were significantly above those of the captopril group. In the normoxia protocol (Figure 4.11),

FHR did not change from levels found in the first hour of normoxia throughout the protocol in either intact or CSD fetuses.

4.4.5 Mean arterial and venous pressure

In intact fetuses there was a rise in MAP during hypoxia with both vehicle and captopril infusion (Figure 4.10). Furthermore, during the recovery period MAP remained significantly elevated compared to normoxia with vehicle but not with captopril infusion. In CSD fetuses, there was a rise in MAP with the infusion of vehicle however this rise was absent with the infusion of captopril (Figure 4.10). MAP remained above normoxic levels during recovery with vehicle infusion but lower than vehicle-infused *intact* fetuses during recovery ($P<0.05$ (n=5 vs. n=8), unpaired t-test). In the normoxia protocol with captopril infusion, MAP did not change from the first hour of normoxia in intact or CSD fetuses (Figure 4.11). In intact fetuses only, MAP fell after the onset of captopril infusion in experiment 3, but not experiment 2 (Table 4.1).

There was no relationship between the magnitude of change in MAP from pre- to post-captopril infusion and initial resting plasma Na^+ levels at -20 min (see Figure 4.9).

There was a tendency for MVP to rise during hypoxia in the 2 intact fetuses for which measurements were obtained (Figure 4.10). In CSD fetuses MVP tended to rise during hypoxia with vehicle infusion, but this did not reach significance. Moreover there was no change in MVP throughout the normoxia protocol with captopril infusion in CSD and the 2 intact fetuses (Figure 4.11).

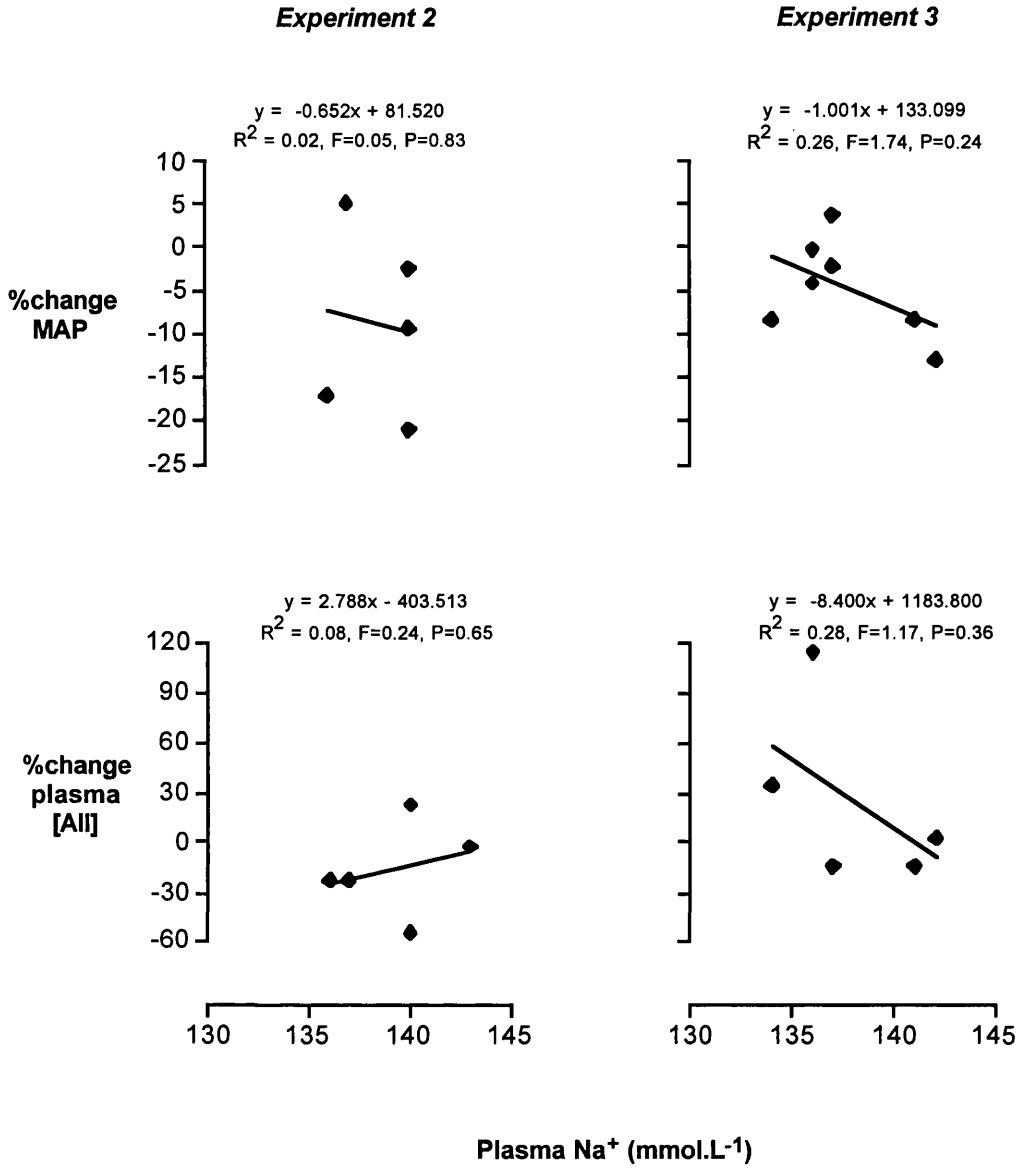


Figure 4.9 Scatter plots of %change MAP (difference between pre- and 1 h post-captopril infusion periods as a percentage of pre-infusion levels) and %change plasma [AII] (difference between -20 min and 45 min as a percentage of -20 min values) against pre-captopril plasma [Na^+] for experiment 2 (MAP: n=5; [AII]: n=5) and experiment 3 (MAP: n=7; [AII]:n=5) in CSD fetuses. Plots have been fitted with a regression line which is represented by an equation in the form 'y = mx + c'. 'R^2' is the square of the correlation coefficient, 'F' is the square of the test statistic and 'P' is the probability that the slope (m) is different from zero.

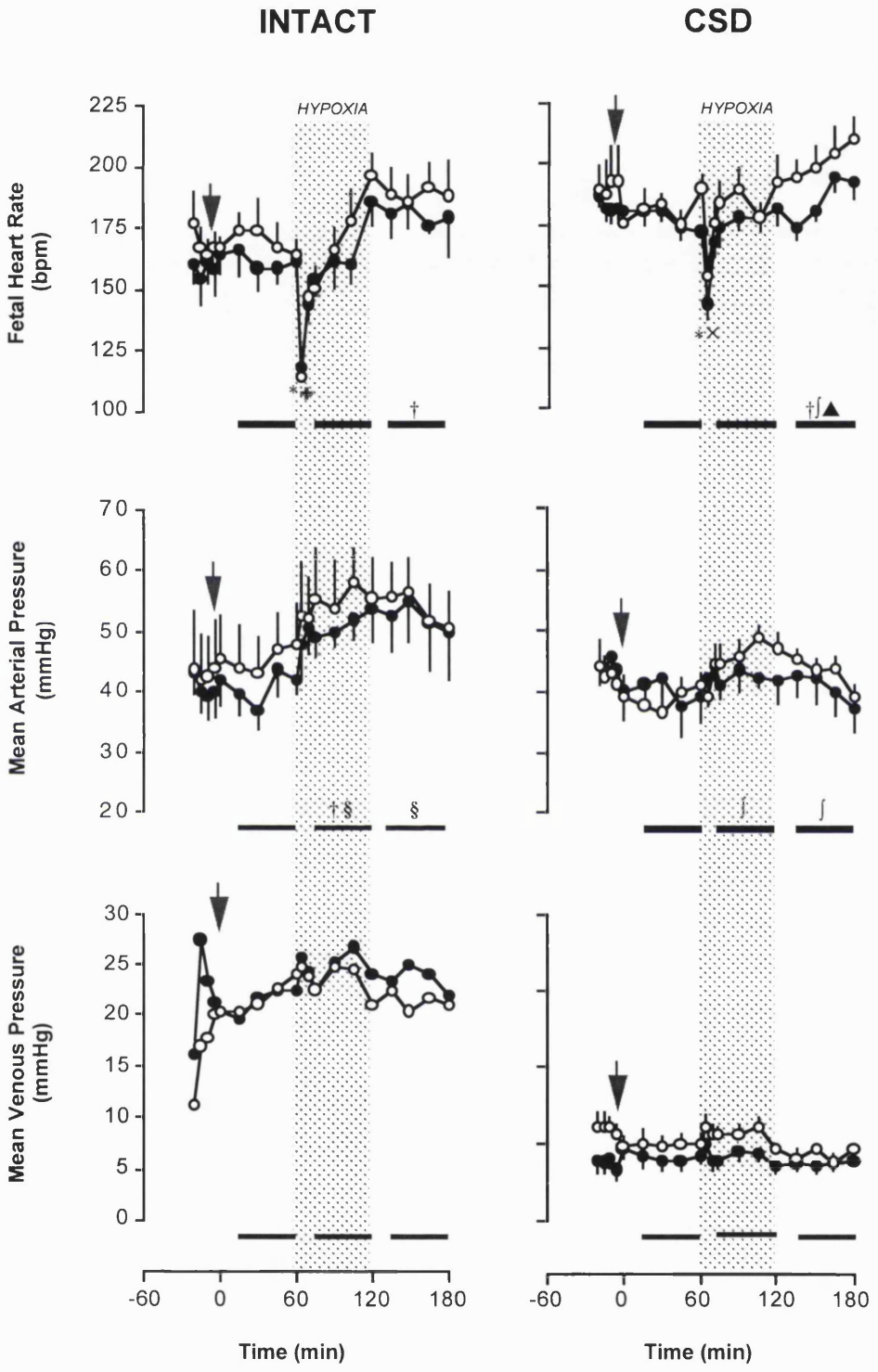


Figure 4.10 Intact and CSD fetal heart rate (FHR: n=6 and n=9, respectively), MAP (n=5 and n=8, respectively) and MVP (n=2 and n=8, respectively) responses to 1 h hypoxia (shaded region) during vehicle (○) and captopril infusion (●). The arrows show the onset of infusion at time -5 min. Values are shown as mean ± SEM. Horizontal bars show the time period over data was reduced to summary measures. *P<0.01, 60 vs. 65 min in vehicle group; ✖ P<0.05 and ✚P<0.01, 60 vs. 65 min in captopril group; †P<0.05, significantly different from normoxia in captopril group; ∫P<0.05 and §P<0.01, significantly different from normoxia in vehicle group; ▲P<0.05, vehicle vs. captopril group. Where no error bars are visible they are contained within the plot symbol, except in the case of intact MVP data in which no error bars were calculated due to the small sample size (n=2). 1 min measurements prior to infusion onset are summarised and compared to the subsequent infusion hour in Table 4.1.

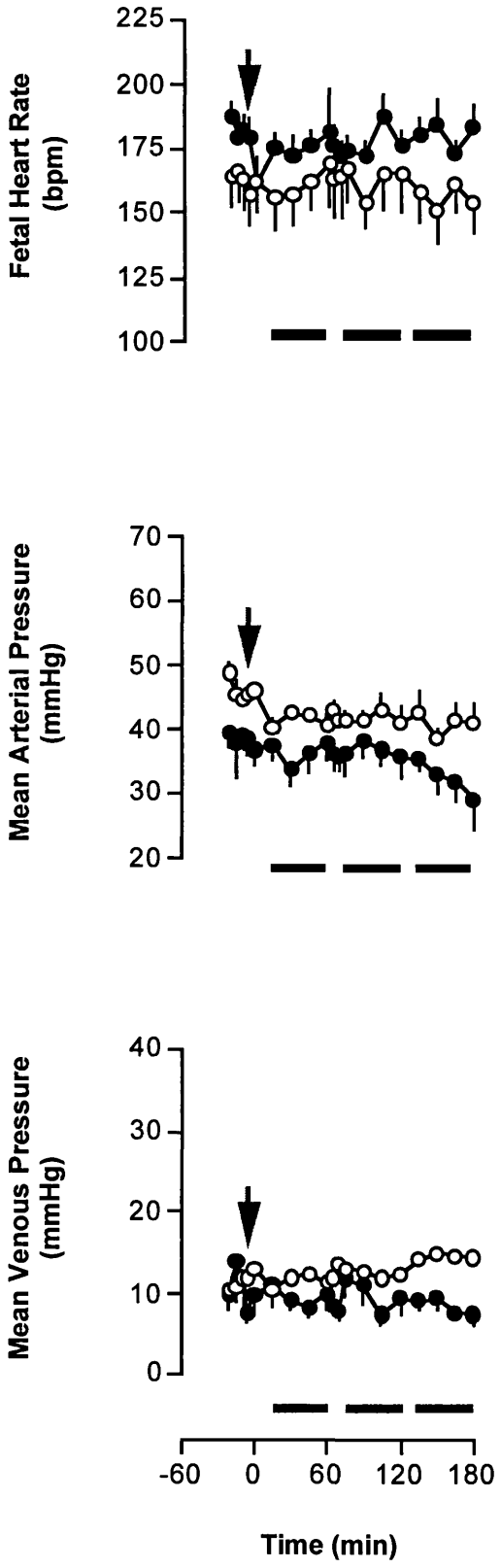


Figure 4.11 Intact (○) and CSD (●) fetal heart rate (FHR, n=6 and n=8, respectively), MAP (n=4 and n=7, respectively) and MVP (n=2 and n=7, respectively) responses to captopril infusion during 3 h of normoxia (experiment 3). The arrows show the time of onset of captopril infusion at time -5 min. The horizontal bars show the time periods over which variables were reduced to summary measures. Where no error bars are visible they are contained within the plot symbol, except in the case of intact MVP in which no error bars were calculated due to the small sample size (n=2). 1 min measurements prior to infusion onset are summarised and compared to the subsequent infusion hour in Table 4.1.

(60 vs. 65 min: vehicle and captopril $P<0.05$, by paired t-test)

4.4.6 Blood flow

Femoral bed

FBF fell during hypoxia in intact fetuses infused with vehicle and captopril (Figure 4.12). During the recovery period flow returned to normoxic levels. In the CSD group FBF fell at the onset of hypoxia with both vehicle and captopril infusion, but the magnitude of the fall *tended* to be less with captopril. Moreover, FBF remained below normoxic levels throughout hypoxia with vehicle but not with captopril infusion. During the recovery period FBF was elevated when compared to normoxia in CSD fetuses infused with captopril (Figure 4.12).

In the normoxia protocol FBF did not change from levels found in the first hour of normoxia throughout the protocol in either intact or CSD fetuses (Figure 4.14).

There was no change in FBF from pre-infusion levels during the first hour following the onset of captopril infusion, however FBF was raised during normoxia after the infusion of vehicle (experiment 1) in intact fetuses (Table 4.1).

Carotid bed

In intact fetuses there was a tendency for CBF to rise during hypoxia with both vehicle and captopril infusion, however this did not reach significance in either group (Figure 4.13). In CSD fetuses there was a tendency for, but no significant rise in CBF during hypoxia with vehicle infusion. In the captopril-infused CSD group however, CBF rose significantly during hypoxia, and remained elevated during the recovery period (Figure 4.13).

In the normoxia protocol CBF did not change from levels found in the first hour of normoxia throughout the protocol in either intact or CSD fetuses (Figure 4.15).

In the intact group CBF was greater following the infusion of vehicle (experiment 1) and captopril (experiment 2) (Table 4.1).

4.4.7 Vascular resistance

Femoral bed

In intact fetuses the initial rise in FVR at the onset of hypoxia was of similar magnitude with vehicle and captopril infusion (comparison of magnitude of the initial change in resistance at the onset of hypoxia (60 to 65 min). However from summary measures, while FVR rose significantly with vehicle infusion during hypoxia, the apparent rise in

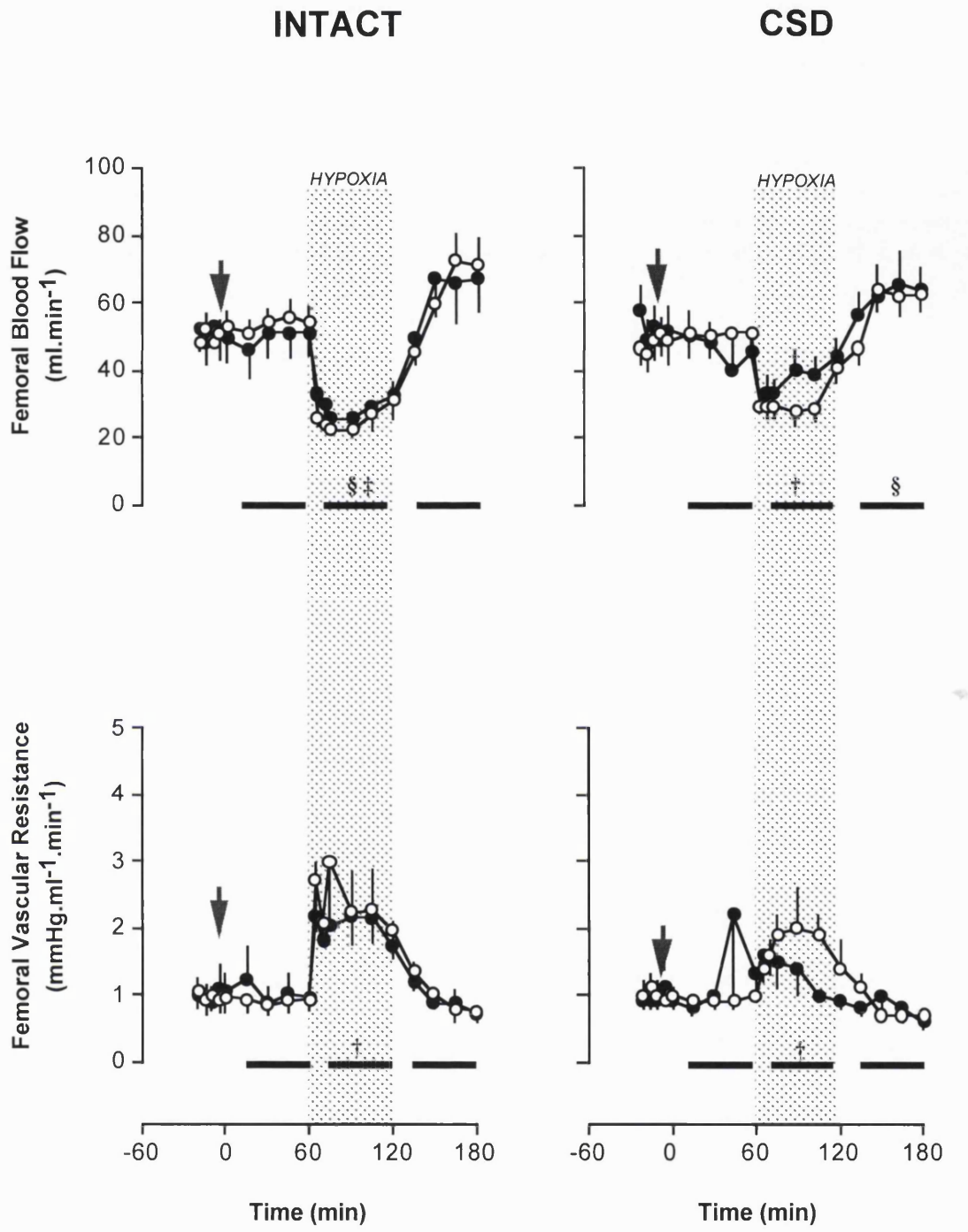


Figure 4.12 Intact and CSD fetal FBF (n=6 and n=9, respectively) and FVR (n=5 and n=8, respectively) responses to 1 h hypoxia (shaded region) during vehicle (❍) and captopril infusion (●). Values are shown as mean ± SEM. The arrows denote the onset of infusion. Horizontal bars show the time period over which summary measures were created. †P<0.05 and ‡P<0.01, significantly different from normoxia in vehicle group; §P<0.01, significantly different from normoxia in captopril group. Where no error bars are visible they are contained within the plot symbol. 1 min measurements prior to infusion onset are summarised and compared to the subsequent infusion hour in Table 4.1.

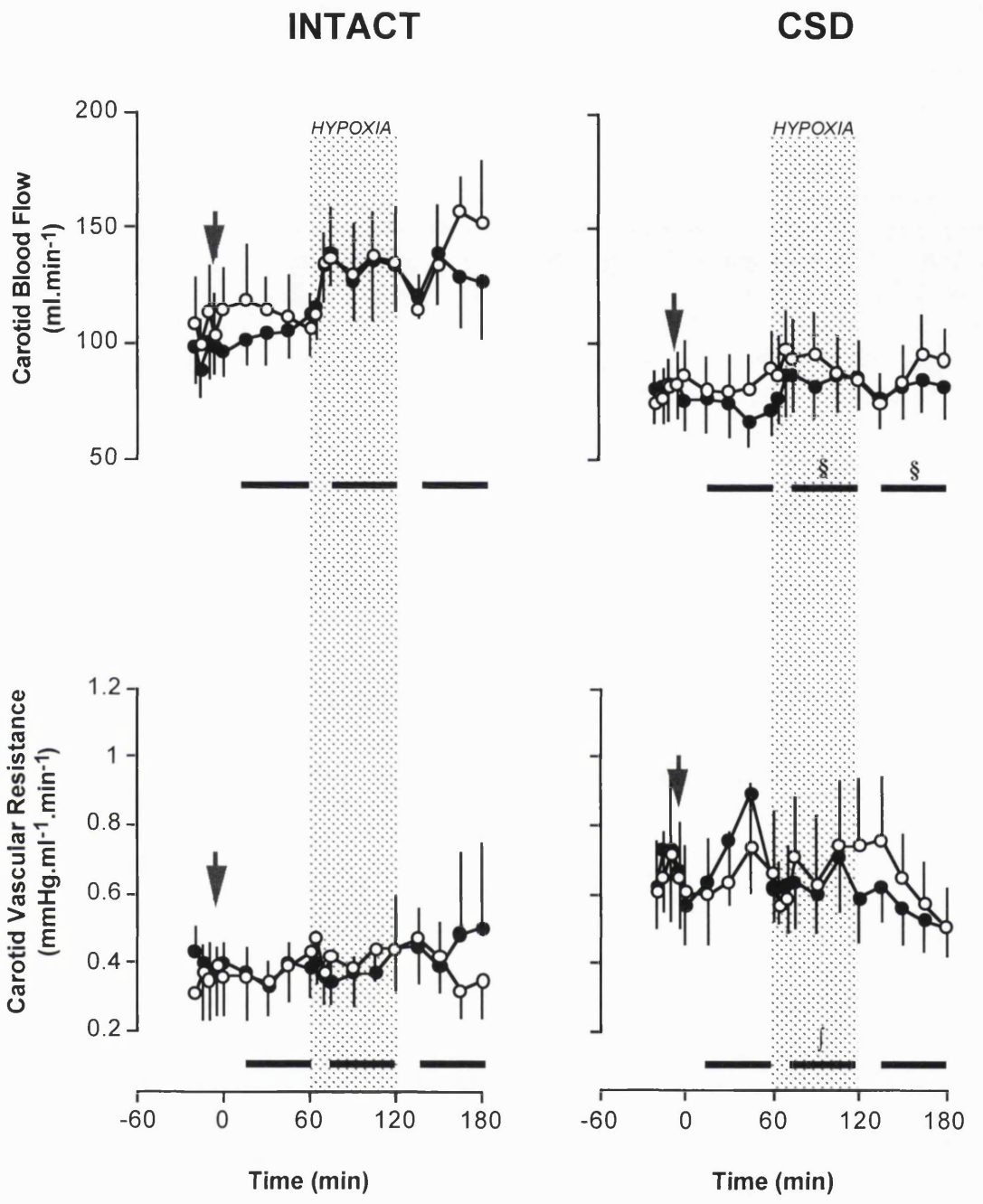


Figure 4.13 Intact and CSD fetal CBF (n=4 and n=8, respectively) and CVR (n=3 and n=7, respectively) responses to 1 h hypoxia (shaded region) during vehicle (❍) and captopril infusion (●). Values are shown as mean ± SEM. The arrows denote the onset of infusion at -5 min. Horizontal bars show the time period over which variables were reduced to summary measures. ∫P<0.05 and §P<0.01, significantly different from normoxia in captopril group. Where no error bars are visible they are contained within the plot symbol. 1 min measurements prior to infusion onset are summarised and compared to the subsequent infusion hour in Table 4.1.

resistance during hypoxia with captopril infusion did not reach significance (P=0.105) (Figure 4.12). The rise in FVR in CSD during hypoxia with vehicle infusion tended to be smaller than in intact fetuses, indeed the rapid rise seen in intact fetuses (60 vs. 65

min, $P<0.05$ by paired t-test) was absent in CSD fetuses. This rise in FVR during hypoxia in CSD with vehicle infusion was absent with captopril infusion (Figure 4.12).

In the normoxia protocol FVR remained unaltered, with relation to the first normoxic hour, throughout the course of the protocol (Figure 4.14).

In both groups of fetuses, the onset of captopril infusion did not alter tonic FVR (Table 4.1).

Carotid bed

In intact fetuses there was no apparent change in CVR during hypoxia with vehicle or captopril infusion (Figure 4.13). In the CSD group, while there was no change during hypoxia with vehicle infusion, CVR fell during hypoxia with captopril infusion (Figure 4.13).

In the normoxia protocol CVR remained unaltered throughout the course of the protocol (Figure 4.15).

In both groups of fetuses the onset of captopril infusion did not alter tonic CVR (Table 4.1).

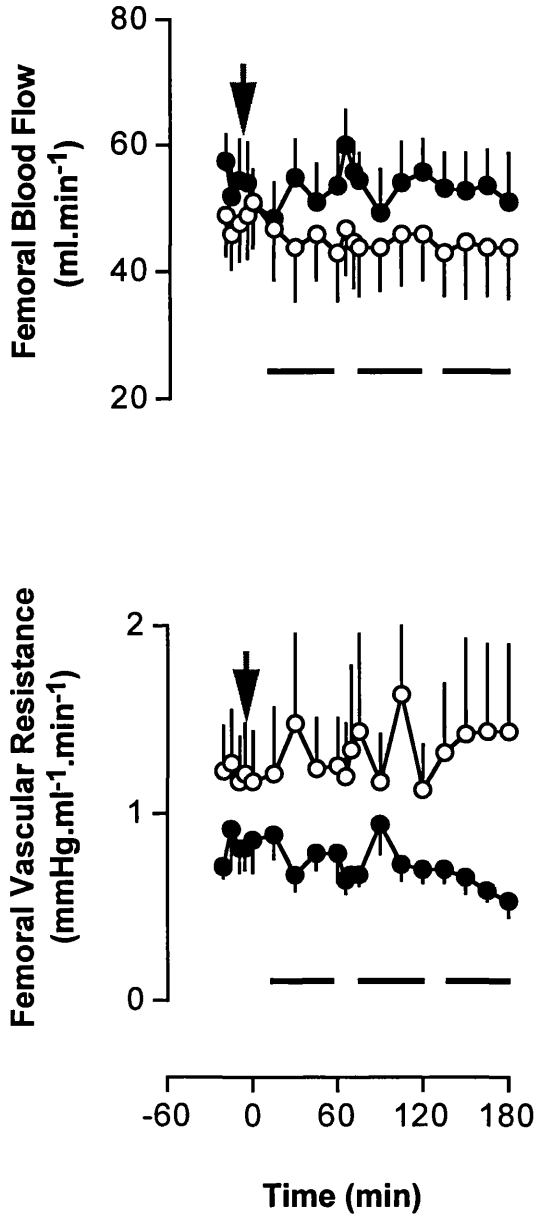


Figure 4.14 Intact (○) and CSD (●) fetal FBF (n=6 and n=7, respectively) and FVR (n=4 and n=6, respectively) responses to captopril infusion during 3 h of normoxia. The arrows show the time of onset of captopril infusion at time -5 min. The horizontal bars show the time periods over which variables were reduced to summary measures. Where no error bars are visible they are contained within the plot symbol. There was no change in FBF or FVR throughout the course of the protocol. 1 min measurements prior to infusion onset are summarised and compared to the subsequent infusion hour in Table 4.1.

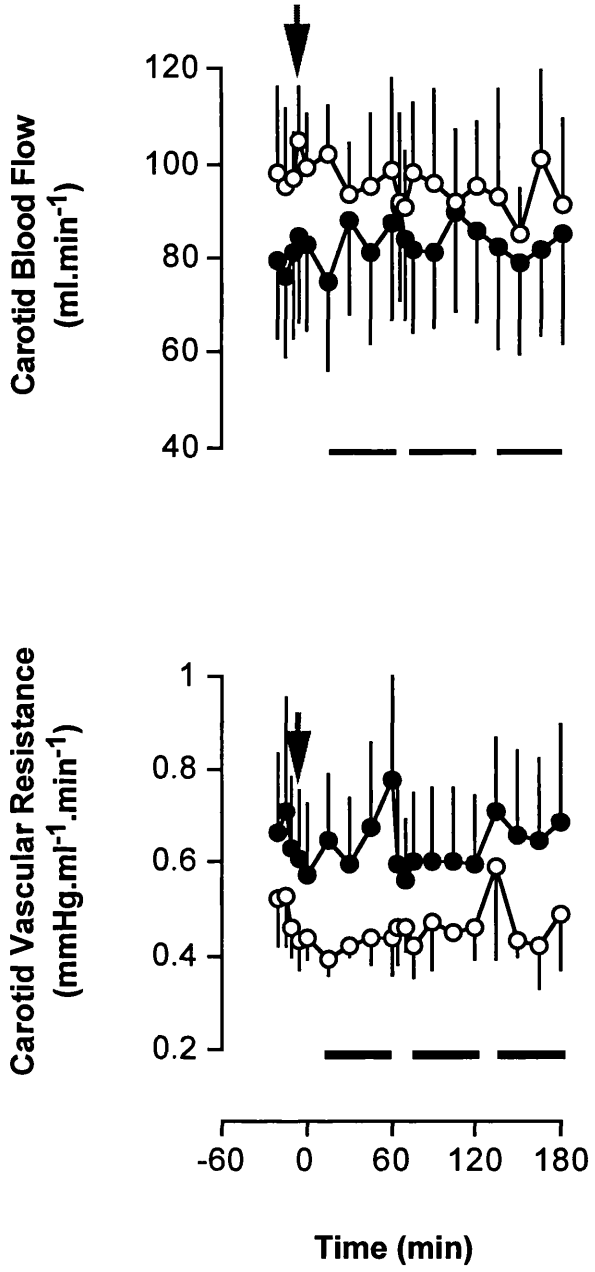


Figure 4.15 Intact (○) and CSD (●) fetal CBF (n=4 and n=7, respectively) and CVR (n=3 and n=6, respectively) responses to captopril infusion during 3 h of normoxia. The arrows show the time of onset of captopril infusion at time -5 min. The horizontal bars show the time periods over which variables were reduced to summary measures. Where no error bars are visible they are contained within the plot symbol. 1 min measurements prior to infusion onset are summarised and compared to the subsequent infusion hour in Table 4.1.

	INTACT						CSD					
	Experiment 1		Experiment 2		Experiment 3		Experiment 1		Experiment 2		Experiment 3	
	Pre	*Post*	*Pre*	*Post*	*Pre*	*Post*	*Pre*	*Post*	*Pre*	*Post*	*Pre*	*Post*
FHR (bpm)	168.20 ±7.53 (6)	169.20 ±7.04 (6)	158.60 ±8.56 (6)	162.00 ±7.97 (6)	162.40 ±11.10 (6)	161.10 ±11.77 (6)	190.75 ±13.02 (9)	181.04 ±2.55 (9)	183.03 ±4.39 (9)	177.16 ±3.84 (9)*	182.59 ±5.66 (8)	173.17 ±7.73 (8)
MAP (mmHg)	42.93 ±7.32 (5)	45.49 ±6.52 (5)	40.60 ±3.48 (5)	40.93 ±2.46 (5)	47.15 ±0.81 (4)	42.02 ±0.94 (4)*	42.62 ±3.53 (8)	38.65 ± 2.56 (8)	44.47 ±2.67 (8)	42.35 ±3.36 (8)	38.8 ±2.43 (7)	36.88 ±2.23 (7)
FBF ($ml.min^{-1}$)	50.00 ±3.75 (6)	53.80 ±3.91 (6)†	80.80 ±6.57 (6)	49.80 ±7.03 (6)	48.00 ±6.10 (6)	46.10 ±7.32 (6)	47.60 ±5.27 (9)	50.30 ±6.47 (9)	51.90 ±5.69 (9)	47.00 ±6.55 (9)	54.60 ±5.61 (7)	51.80 ±5.71 (7)
FVR ($mmHg.ml^{-1}.min^{-1}$)	0.95± 0.21 (5)	0.91± 0.18 (5)	0.97± 0.25 (5)	1.03± 0.29 (5)	1.21± 0.26 (4)	1.26± 0.32 (4)	1.02± 0.16 (8)	0.92± 0.12 (8)	0.94± 0.09 (8)	1.22± 0.32 (8)	0.81± 0.10 (6)	0.80± 0.11 (6)
CBF ($ml.min^{-1}$)	104.50 ±15.67 (4)	112.90 ±17.29 (4)*	96.06 ±12.62 (4)	103.80 ±12.19 (4)*	98.75 ±13.60 (4)	97.70 ±13.10 (4)	78.75 ±17.95 (8)	83.30 ±20.61 (8)	81.88 ±21.77 (8)	72.95 ±18.49 (8)	80.25 ±22.85 (7)	82.86 ±25.23 (7)
CVR ($mmHg.ml^{-1}.min^{-1}$)	0.37± 0.13 (3)	0.38± 0.11 (3)	0.39± 0.06 (3)	0.38± 0.05 (3)	0.49± 0.08 (3)	0.42± 0.05 (3)	0.67± 0.16 (7)	0.62± 0.16 (7)	0.69± 0.17 (7)	0.68± 0.13 (7)	0.64± 0.16 (6)	0.63± 0.16 (6)

Table 4.1 Table to compare pre-infusion (pre) with 1 h infusion (post) summary of measures cardiovascular variables in intact and CSD fetuses in experiment 1 (hypoxia with vehicle protocol), experiment 2 (hypoxia with captopril protocol) and experiment 3 (normoxia with captopril protocol). Values are shown as mean ± S.E.M. The bracketed numbers denote fetus number. *P<0.05 post-infusion significantly different from pre-infusion values by paired t-test, †P<0.01 post infusion significantly different from pre-infusion values by paired t-test.

4.5 Discussion

The results presented in this chapter confirm that plasma [AII] rises during hypoxia. However this rise was not significantly altered by CSD which suggests that the release of AII, during hypoxia, is not mediated by a carotid chemoreflex mechanism. Furthermore the results show that with the infusion of the ACE inhibitor, captopril, the rise in [AII] during hypoxia does not occur in either intact or CSD fetuses. In addition the fall in FBF observed during hypoxia is attenuated by the infusion of captopril in CSD but not intact fetuses. The implications of these findings are discussed below.

Methodological considerations

The use of captopril

The dose of captopril used in these experiments was based on studies in fetal sheep at term, in which the systemic arterial pressure response to AI was inhibited 5 min after the onset of captopril infusion (Davidson, 1987). In the present study, the pressor response

to a 5μg bolus of AI was investigated in 4 out of 9 CSD fetuses. This part of the protocol was introduced in an attempt to provide information on the efficacy of the dose of captopril used in blocking the RAS at the level of AI conversion to AII. However there is evidence to suggest that AI can itself cause a vasoconstriction via AII receptors (Ferrer, Encabo, Marin and Balfagon, 1992). Focusing on the MAP responses, it was clear that following captopril infusion the response to AI was blocked. These findings, combined with the results of the AII assay, in which no rise in [AII] was seen in either intact or CSD fetuses during hypoxia when captopril was infused, suggest that the dose of captopril used in these experiments was sufficient to significantly reduce the function of the RAS. The pre-infusion MAP response to AI, despite being significant on experimental day 3, was less than on experimental day 2. This suggests that not all the captopril administered during the previous day (experiment 2) had been cleared which would agree with previous fetal studies in which captopril effects remained for ca. 3 days (Broughton Pipkin, Symonds and Turner, 1982). Captopril is cleared predominantly by the kidneys, thus since fetal renal *function* [1.6.2 Lumbers, 1983] is immature compared to the adult this may have resulted in reduced captopril clearance in the present study and may account for the prolonged captopril effect on the AI pressor response. Accordingly, experiments were conducted in the same order in the majority of fetuses involved in the study. The 1 intact and 1 CSD fetus in which experimental order was different were included in the study nonetheless since their individual responses did not appear to differ from that of the rest of the group.

Captopril was chosen as a tool to investigate the RAS in the present study because of its cost, ease of solubility and the extensive number of fetal and adult studies which have already used it. However there is evidence to suggest that a component of the vasodilator effect of captopril is endothelium-dependent since its sulfhydryl group scavenges superoxide anions and thereby protects NO (Goldschmidt and Tallarida, 1991), and also due to its action of inhibiting the breakdown of bradykinin [1.5.3-bradykinin].

Animals involved in study

Nine CSD and 6 intact fetuses were initially involved in this study. Measurements of plasma [AII] were made in intact and CSD fetuses during hypoxia experiments with vehicle (6 and 5 fetuses, respectively) and with captopril (5 and 6 fetuses, respectively) infusion. During the normoxia protocol plasma [AII] measurements were made in one intact and 6 CSD fetuses. The small number of intact fetuses in which data was available was due to technical problems with the assay. Missing measurements were either because they were too low to be detected by the sensitivity of the assay or if insufficient blood was withdrawn at the time of experimentation to carry out the assay.

MAP measurements were not available for 1 intact fetus during vehicle infusion (experiment 1), thus for the sake of statistical comparisons MAP data from this fetus on the subsequent 2 experimental days were not included in grouped data. MAP was not available in another intact fetus during normoxia with captopril infusion (experiment 3). These missing data were due to problems with the pressure transducer connections and a blocked arterial line, respectively. MAP measurements were unavailable in 1 CSD fetus during experiments 1 and 2 (hypoxia with vehicle / captopril infusion) due to mechanical problems, and in another CSD fetus during the normoxia protocol with captopril infusion (experiment 3) because no experiment was carried out.

MVP pressure measurements were only made in 2 intact fetuses. MVP were possible in 7 out of the 9 CSD fetuses. It should be noted that pressure measurements were made from the same line that was used for the infusion of captopril or vehicle and AI, except in the case of 5 of the CSD fetuses in which a separate brachial vein catheter was implanted specifically for the administration of drugs. All measured MVP's were considered together as a group (Figure 4.10) however, due to the methodological problems outlined above, the measurements made, in intact fetuses in particular, are unphysiologically high and therefore MVP was not used in the calculation of vascular resistances.

CBF measurements, thought to be a good estimate of cerebral blood flow (van Bel, Roman, Klautz, Teitel and Rudolph, 1994, see 1.4.7) were available in 4 intact and 8 CSD fetuses. In the 1 CSD fetus removed from analysis, CBF was deemed to be abnormal and was attributed to an electrical connection problem within the flow probe itself. One intact fetuses flow was excluded from the study because it was considered unreasonably low. The reason for low flow was not obvious but may have been due either to partial occlusion of the vessel by the flow probe or the signal strength of probe itself was low probably because the probe sensor was old/damaged, or there was increased resistance due to repair of the probe cable. CBF measurements were unavailable for the second intact animal because of total absence of signal, which may have been caused by insufficient peritoneal fluid surrounding the vessel.

Angiotensin II is not chemoreflexly released during hypoxia

Plasma [AII] is already known to rise during hypoxia in the late gestation sheep fetus (Broughton Pipkin, Lumbers and Mott, 1974), although the response is less in fetuses below 120 days gestation (Gomez and Robillard, 1984). While PRA has been shown to rise to a similar extent in response to haemorrhage in bilaterally vago-sympathectomized fetal sheep (Wood, Chen and Bell, 1989), it has been suggested that peripheral arterial chemoreceptors may mediate the AII response to hypoxia *with* hypercapnia in the late gestation fetus (Wood, Kane and Raff, 1990). The extent to which the rise in [AII]

during hypoxia is mediated by *carotid* chemo- and baroreflexes was investigated in the present study by measuring plasma [AII] in intact and CSD fetuses. The results of this Chapter show that plasma [AII] rose significantly in both intact and CSD fetuses during hypoxia. Mean baseline and hypoxic levels of plasma [AII] were similar to those measured in previous fetal sheep studies (Broughton Pipkin, Lumbers and Mott, 1974). Furthermore I observed that there was no difference in [AII] between intact and denervated fetuses throughout the protocol. Thus these results indicate that the release of AII during hypoxia is not carotid chemoreflexly mediated. It is possible that the aortic chemoreceptors play a more significant part in the release of AII. However AII is not a suitable candidate for the, as yet unidentified, reflexly released substance which is thought to exert rapid vasoconstrictor effects, alongside those of α-adrenergic mechanisms, at the onset of hypoxia [see 1.5.2 and Figure 1.2].

As mentioned earlier, the rise in [AII], seen during hypoxia in intact and CSD fetuses, was absent with the infusion of captopril. It was interesting to note, however, that there was no difference in [AII] levels between pre-infusion values and those seen normoxia after the onset of captopril infusion (normoxia 45 min). Plasma [renin] in the near term fetus is greater than that seen in the mother (Broughton-Pipkin, Lumbers and Mott, 1974; Raimbach and Thomas, 1990), although relative to this high [renin], fetal [AII] is low. The major site of conversion of AI to AII is the pulmonary vascular bed, however in the fetus not only is pulmonary blood flow low (Rudolph and Heymann, 1970), but so are pulmonary levels of ACE (Raimbach and Thomas, 1990) compared to the adult. Thus the lack of effect of captopril on *resting* [AII] in the present study is consistent with these findings. In contrast, earlier studies have shown [AII] to be reduced by captopril in the late gestation fetus (Broughton Pipkin, Symonds and Turner, 1982). While the reason for this discrepancy is not obvious it is possible that the 48 h allowed for recovery from surgery in the previous study was not time enough for the RAS to return to baseline activity following any rise in its activity due to surgery. Studies in the *lamb* (4-38 days) in which administration of much lower doses of captopril (approximately 450 $\mu g.h^{-1}$) resulted in a large fall in plasma [AII] (Weismann, Herrig, McWeeny and Robillard, 1983) are consistent with the results presented in this Chapter since the rise in pulmonary blood flow seen at birth [1.4.8], along with greater pulmonary ACE activity in the first few postnatal days in the rat (Costerousse, Allegrini, Huang, Bounik and Alhenc-Gelas, 1994) provide a much greater exposure of the blood to ACE.

ACE-inhibition does not alter baseline haemodynamics

The role of RAS in the maintenance of baseline fetal MAP is not clear. In the present study ACE inhibition did not cause any change in resting MAP (normoxia, experiments 2 and 3) which is consistent with the observed lack of effect of captopril on resting plasma [AII] and which agrees with previous studies (Mattioli, Chien, Vassenon, Crist and Lynn, 1979). However, my results contrast with those of Broughton Pipkin *et al.* (1982) and Robillard *et al.* (1983) who saw a fall in basal fetal systemic blood pressure with captopril administration, although unlike the present study this was in conjunction with reduced plasma [AII]. Inhibition of ACE not only reduces the conversion of AI to AII but also prevents the breakdown of the potent vasodilator bradykinin. Therefore it is possible that a component of any fall seen in MAP with captopril administration is attributable to bradykinin accumulation (Clappison, Anderson and Johnston, 1981). In the lamb the fall in MAP following captopril infusion (Weismann, Herrig, McWeeny and Robillard, 1983) is consistent with greater pulmonary ACE activity and blood flow relative to the fetus. In the adult, the effect of RAS blockade is dependent on the initial state of activation of the system (Keeton, Pettinger and Campbell, 1976), e.g. a more pronounced increase in PRA and fall in MAP is observed in response to AII receptor blockade when the animal is Na^+-depleted. Similarly studies in fetal sheep suggest that raised plasma Na^+ inhibits renin release (Lumbers and Stevens, 1983), and show that AII receptor blockade has minimal effect on arterial blood pressure until the RAS is activated, for example by furosemide stimulation of renin release (Lumbers and Stevens, 1987). In the present study plasma Na^+ measurements were made prior to the onset of captopril infusion in intact (experiment 2 (n=2) and experiment 3 (n=3)) and CSD (experiment 2 (n=7) and experiment 3 (n=8)) fetuses. Plasma [Na^+] ranged between 136-143mmol.L^{-1} in agreement with other chronic fetal sheep studies (Broughton Pipkin, Lumbers and Mott, 1974; Robillard, Weismann, Gomez, Ayers, Lawton and VanOrden, 1983). Fetuses were therefore considered to be sodium replete prior to the ACE inhibition and there was no relationship between the effect of captopril on plasma [AII] and the initial level of Na^+.

Exogenous AII has a positive chronotropic action in the sheep fetus which has been suggested to act via a direct action on the heart (Iwamoto and Rudolph, 1981). In this study I saw a fall in FHR after captopril infusion to CSD fetuses. This occurred without a rise in MAP and implies direct action of AII on the heart. In previous fetal (Broughton Pipkin, Symonds and Turner, 1982; Iwamoto and Rudolph, 1979; Robillard *et al.*, 1983) and neonatal (Weismann *et al.*, 1983) studies no change in FHR was observed with captopril, even in the face of lowered MAP. Indeed the results in this Chapter showed no change in FHR in intact fetuses, where MAP also did not change, following captopril

infusion. The difference between intact and CSD fetuses in this study is not easily explained particularly in view of the similar baseline FHR in the two groups with vehicle infusion. Furthermore a fall in FHR was not seen following captopril infusion in experiment 3 in CSD fetuses, but this would not be surprising if ACE activity was already reduced as a result of the previous days captopril administration (see methodological considerations).

Previous fetal sheep studies in which AII receptors were blocked by saralasin revealed no change in baseline pulmonary vascular resistance (Hyman, Heymann, Levin and Rudolph, 1975), while other studies showed endogenous AII to exert a tonic vasoconstriction in the peripheral (Iwamoto and Rudolph, 1979) and renal (Robillard *et al.*, 1983) circulation. The rise in FBF in intact fetuses with vehicle infusion is not easily explained from the present study. In addition the rise in CBF seen in intact fetuses following captopril infusion implies a carotid vasodilatation, but this is difficult to assess because of the similar rise in flow which occurred subsequent to vehicle infusion.

Cardiovascular responses to hypoxia with the infusion of vehicle

During hypoxia, the rapid initial components (e.g. bradycardia at hypoxia onset) and slower components (e.g. rise in MAP) of the fetal cardiovascular responses to hypoxia seen in vehicle infused intact fetuses in the present study agree with observations of previous studies (Giussani, Spencer, Moore, Bennet and Hanson, 1993; Bartelds, van Bel, Teitel and Rudolph, 1993). These results also show that although a rapid bradycardia occurs in CSD vehicle infused fetuses, the magnitude of this fall was significantly less than in intact fetuses. Thus these findings support a role for peripheral chemoreceptors in the rapid reflex changes in heart rate during hypoxia in agreement with previous studies (Giussani *et al.*, 1993; Bartelds *et al.*, 1993). The initial rapid rise in FVR was absent in CSD fetuses, but during hypoxia FBF fell and FVR rose to a similar level in intact and CSD fetuses. These results are qualitatively similar to those of previous investigations (Giussani *et al.*, 1993; Bartelds *et al.*, 1993), although Giussani *et al.* (1993) attributed a larger component of the rapid changes in peripheral blood flow during hypoxia to carotid chemoreflex mechanisms. The reason for this is not obvious since the level of PaO_2 reached during hypoxia in the present study (ca. 12mmHg) was similar to that of the previous studies, and any decrease in pH during hypoxia was not rapid enough in onset to contribute to reflex changes. Also it is possible that aortic chemoreceptors, which would have been unaffected by cutting the CSN, play a more significant role in the reflex changes at the onset of hypoxia than has been indicated from other CSD studies (Giussani *et al.*, 1993; Bartelds *et al.*, 1993).

Captopril alters cardiovascular responses to hypoxia in CSD but not intact fetuses

The absence of an effect of captopril infusion on the rapid changes seen in response to hypoxia in intact fetuses in this study provides further evidence that AII does not play a major part in the reflex responses to hypoxia. Adult studies have implicated AII and AVP in the maintained rise in MAP seen during acute hypoxia with acidosis (see Rose, Vance, Dacus, Brashers, Peach and Carey, 1991 for discussion), so it might have been expected that the rise in plasma [AII] seen during hypoxia in intact and CSD in the present study would contribute to the slower cardiovascular responses that are observed in hypoxia. Angiotensin II is implicated in splanchnic responses to hypoxia in the maturing lamb (Weismann, Herrig, McWeeny and Robillard, 1983), and blockade of AII action with saralasin blunts the fetal hypertensive response to hypoxia (Mattioli, Chien, Vassenon, Crist and Lynn, 1979), although AII does not appear to mediate fetal pulmonary vasoconstriction induced by hypoxia (Hyman, Heymann, Levin and Rudolph, 1975). While the results presented in this chapter do not support a large role for AII in cardiovascular responses to hypoxia in the *intact* late gestation fetus, in the *CSD* group captopril blunted the hypertensive response to hypoxia, and attenuated the fall in FBF and rise in FVR during hypoxia. This suggests that chemoreceptor mediated mechanisms exert a major influence on cardiovascular control during hypoxia, and that it is only once these are removed that other mechanisms, such as the action of AII, are un-masked. Studies in the adult rat indicate the importance of the *combined* effort of three mechanisms in the maintenance of basal MAP, namely AII, the α-adrenergic system and AVP (Paller and Linas, 1984). The results of the present study support this concept since CSD, or captopril infusion, alone does not alter baseline haemodynamics. On the other hand, under pathological conditions, such as sodium depletion, a single disruption may have a large effect on MAP (Keeton, Pettinger and Campbell, 1976). Similarly in this Chapter, during hypoxia a large neuronal influence on cardiovascular control was indicated, since it was not until the CSNs were cut that the effect of captopril on peripheral blood flow changes was seen.

4.6 Conclusion

The results of this study confirm a rise in plasma [AII] during hypoxia but show that this rise is not mediated by carotid chemoreflex mechanisms. The inhibition of ACE does not appear to alter baseline [AII], and AII does not appear to contribute to cardiovascular control in normoxia. During hypoxia the cardiovascular changes in intact fetuses are not altered by the infusion of captopril, however once carotid reflex mechanisms are removed a role for AII in the regulation of peripheral blood flow becomes apparent.

CHAPTER 5

THE ROLE OF ENDOTHELIN-1 IN THE CARDIOVASCULAR RESPONSES TO HYPOXIA

5.1 Introduction

Results presented in the previous Chapter show that the rise in circulating [AII] during hypoxia is not initiated by a chemoreflex mechanism. Indeed a role for AII in fetal cardiovascular responses to acute hypoxia only becomes apparent after carotid chemoreceptor input has been removed. Thus it seems possible that the neuronal mechanisms initiated during hypoxia are operating against a background of other hormonal mechanisms. In the case of the RAS, this is probably by an *endocrine* action of a rise in circulating [AII], although there is substantial evidence of a local endothelium-based RAS (Dzau, 1984b; Kifor and Dzau, 1987) whereby AII acts in a *paracrine* fashion on the underlying vascular smooth muscle [1.5.3]. However it was not possible from the previous study to distinguish between the humoral and local-vascular RAS. The vascular endothelium produces a number of other vasoactive factors such as PGI_2, NO and ET-1 and plays an important part in the regulation of vascular tone [1.5.3]. It was therefore the aim of Chapters 5 and 6 to focus on the mechanisms which hypoxia brings into action at a local tissue level, in particular the role played by ET-1 and NO in the fetal cardiovascular responses to hypoxia.

The potent vasoconstrictor ET-1 [1.5.3] is the only one of a family of three isopeptides (ET-1, -2 and -3) to be synthesised by vascular endothelial cells. The two main ET receptors subtypes, designated ET_A and ET_B, are located on smooth muscle and endothelial cells, respectively. ET_B receptors show equal affinity for all three ET-subtypes and mediate the release of vasodilators such as NO, while ET_A receptors show a high affinity for ET-1 and ET-2 and mediate vasoconstriction (Masaki, 1993).

One approach employed in a number of animal studies to investigate the role of ET-1 in cardiovascular control has been the administration of exogenous ET-1. In adult sheep i.v. administration of ET-1 produces a potent arterial vasoconstriction (Scoggins, Spence, Parkes, McDonald, Wade and Coghlan, 1989). This systemic vasoconstrictor action of ET-1 is also seen in the neonate (Bradley, Czaja and Goldstein, 1990) as well as a pulmonary vasoconstriction which increases with postnatal maturation (Wong, Vanderford, Fineman and Soifer, 1994). There is evidence to suggest that the fetus

possesses a functional ET system since administration of the ET-1 precursor, big ET-1, causes a systemic and pulmonary hypertension in the late gestation fetus which can be blocked by an inhibitor of ECE (Jones and Abman, 1994). Other fetal studies have shown that ET-1 causes a pulmonary vasodilatation in the fetus (Chatfield, McMurtry, Hall and Abman, 1991) which is suggested to be tone-dependent from *in vivo* (Cassin, Kristova, Davis, Kadowitz & Gause, 1991 [1.5.3]), but not *in vitro* (Wang and Coceani, 1992) studies.

The secretion of ET-1 is localised to the abluminal surface of the endothelial cell (Wagner *et al.*, 1992) and influences the underlying smooth muscle in a paracrine manner. Furthermore, smooth muscle cells are able to produce ET-1 which may then cause short-term vasoconstriction in an autocrine manner (Hahn, Resink, Scott-Burden, Powell, Dohi and Buhler, 1990). Thus this evidence supports a local (paracrine or autocrine) rather than circulatory (endocrine) action of ET-1. This mechanism of action would limit the physiological significance of studies in which exogenous ET-1 is administered, although it is possible that during hypoxia the release of ET-1 from the endothelium is of sufficient magnitude to increase circulating [ET-1]. Endothelin-1 is released from rat resistance vessels in response to hypoxia *in vitro* (Rakugi *et al.*, 1990) and elevated plasma [ET-1] has been observed during hypoxia in adult rats (Shirakami *et al.*, 1991; Horio *et al.*, 1991) and with prolonged but not acute episodes of hypoxia in the sheep fetus (Jones, Abman and Wilkening, 1994). Endothelin-1 is present in fetal plasma at greater concentrations than in maternal plasma (Haegerstrand, Hemsen, Gillis, Larsson and Lundberg, 1989; Nakamura, Kasai, Konuma, Emoto, Banba, Ishikawa and Shimoda, 1990). One possibility is that a rise in plasma [ET-1], if rapid enough, may contribute to the rise in FVR seen during acute (1 h) hypoxia. Thus the ***first*** aim of this Chapter was to measure plasma [ET-1] during normoxia and 1 h of isocapnic hypoxia in the fetus.

ET_A receptors have been located on and around the adult carotid body, and exogenous ET-1 increases chemoreceptor discharge (Spyer, McQueen, Dashwood, Sykes, Daly and Muddle, 1991). Thus it is possible that a feed-forward mechanism could operate whereby a rise in plasma [ET-1] could stimulate carotid chemoreceptor discharge and thus augment reflex vasoconstriction. This vasoconstriction could then increase the release of ET-1 from the vascular endothelium by increased shear stress as indicated by *in vitro* studies (Yoshizumi *et al.*, 1989). Thus the ***second*** aim in this Chapter was to measure circulating [ET-1] in the vehicle infused intact and CSD fetuses presented in Chapter 4.

The development of selective ET receptor blockers has provided a new approach to determining a physiological role for ET-1 in the fetus (Ihara *et al.*, 1992). ET_A receptor activation is implicated in the regulation of basal arterial pressure and renal vascular tone

in adult anaesthetised rats (Pollock and Opgenorth, 1993). ET-1 is implicated in the maintenance of the high basal pulmonary vascular tone *in vivo* (Ivy, Kinsella and Abman, 1994; Wong, Fineman and Heymann, 1994) but apparently has no role in determining basal systemic vascular resistance in the fetus *in vivo* (Ivy *et al.*, 1994). A role for ET-1 is implicated in the fetal pulmonary vasoconstriction response to hypoxia *in vitro* (Wang, Coe, Toyoda and Coceani, 1995) and preliminary evidence suggests that ET-1 contributes to the rise in fetal systemic vascular resistance in response to 3 h of hypoxia produced by maternal common iliac artery occlusion (Jones, 1995). Thus the ***third*** aim of this study was to use a specific ET_A receptor bocker to investigate the role for endogenous ET-1 in the fetal *systemic* cardiovascular responses to acute isocapnic hypoxia.

In view of the potential interaction between mechanisms of fetal cardiovascular control [1.5.4] I have also measured plasma [AII], [ACTH] and [cortisol] in samples collected in this Chapter.

Some of these data have already been presented in abstract form (Green, Bennet and Hanson, 1994a; Green, McGarrigle, Bennet and Hanson, 1995d/e) and are shown in Appendices 10,12 and 13.

5.2 Methods

5.2.1 Surgical Preparation

Seven fetuses aged between 114 and 121 days gestation were instrumented under general anaesthesia [2.3.2]. The lower half of the fetus was exteriorised through a uterine incision. An ultrasonic flow probe (3R: Transonic Systems Inc., Ithaca NY) was placed around a femoral artery and the transducer cable secured in 2 places to the fetal skin. The lower portion of the fetus was returned to the uterus. The upper portion was then exteriorised either through the same incision by rotating the fetus or through a second incision in the uterus following closure of the first. Two stainless steel electrodes were sewn onto the chest and an earthing electrode onto the back of the neck to monitor ECG. A catheter was sewn onto the fetal skin for drug administration into the amniotic cavity. Additional heparinised catheters were placed in a carotid artery and a jugular vein to monitor MAP and MVP, and in the right brachial vein to administer antibiotics and experimental drugs. A second ultrasonic flow probe (3 or 4R) was placed around the uncatheterised carotid artery. A maternal pedal vein was catheterised to administer antibiotics.

A tracheal catheter and diaphragm EMG electrodes were implanted to record FBMs. Stainless-steel electrodes were implanted bilaterally on the parietal dura to measure ECoG [2.3.2].

Five to 8 days of recovery were allowed before experimentation.

5.2.2 Experimental Procedure

Measurements

MAP, MVP, tracheal pressure, FHR, CBF, FBF, ECoG activity and diaphragm EMG were recorded continuously onto chart paper and MacLab Chart Software (AD Instruments Pty. Ltd.) using a Macintosh LCIII computer (Apple computers Inc.) [2.4.2].

2-3ml fetal arterial blood was collected prior to the onset of vehicle or FR139317 infusion (time zero), during control (15 and 45 min), hypoxia (75 and 105 min) and recovery (135 and 165 min). Blood was transferred immediately to chilled EDTA tubes and spun at 4°C (3000 rpm) for 10 min. Plasma was then decanted into tubes and stored at -20°C for subsequent hormonal analysis. A further 0.6 ml arterial blood was collected at these times and also at 90 min (hypoxia) for the analysis of blood gases and electrolytes.

Drug Preparation

50μg ET-1 (Human, Porcine. Sigma Chemical Co. Ltd. UK) was dissolved in 10ml degassed-saline with 2 drops of glacial acetic acid (Fisons, UK). 10 ml of 2mg.ml^{-1} sheep albumin (Fraction V powder, Sigma Chemical Co. Ltd. UK) was added to give a final ET-1 concentration of 2.5μg.ml^{-1}. This stock solution was divided into 200μl aliquots, each containing 500ng ET-1, which were stored at -20°C (shelf-life of approximately 3 months). On the day of study the required aliquots were resuspended in 2ml saline.

On the day of experimentation approximately 7mg FR139317 (a gift from Fujisawa Pharmaceutical Co., Ltd., Japan) was dissolved in saline and equimolar NaOH (Sigma Chemical Co. Ltd. UK. 5 drops per 10ml saline) to give a final solution of 700μg.ml^{-1} (Nirei, Hamada, Shoubo, Sogabe, Notsu and Ono, 1993).

The vehicle solution was composed of saline with equimolar NaOH (5 drops per 10ml).

Protocol

In one fetus (125 days gestation) the cardiovascular responses to 4 different doses (500ng, 1μg, 1.5μg and 2μg) of ET-1 (i.v.) were investigated. On the following day the same fetus (126 days gestation) was used to establish a dose of the ET-1 receptor antagonist (FR139317) which would block the response to 1.5μg ET-1. Three dose regimes of FR139317 were investigated: dose 1, 3μg bolus followed by 0.5μg.min^{-1} infusion; dose 2, 30μg bolus followed by 5μg.min^{-1} infusion; dose 3, 300μg bolus followed by 50μg.min^{-1} infusion. In another fetus (133 days gestation), which was not subsequently involved in the hypoxia study, the response to a maximal dose of FR139317 was investigated (3mg bolus followed by 500μg.min^{-1} infusion).

In the seven fetuses, two 3 h protocols were conducted on separate days: day 1, hypoxia with the infusion of vehicle (125±0.8 days gestation); day 2, hypoxia with infusion of FR139317 (126±0.7 days gestation). In 2 fetuses the order of experiments on days 1 and 2 were reversed. The hypoxia protocols comprised an initial 1 h normoxic control period, followed by 1 h fetal isocapnic hypoxia. Ewes were then returned to normoxic gas mixture and measurements continued for 1 h (recovery) [2.5.1].

At 0 min an initial bolus of either vehicle (2ml) or FR139317 (300μg in 2ml) was administered via the brachial vein and flushed in with 2ml saline. This was followed by a 4ml.h^{-1} infusion of vehicle or FR139317 (50μg.min^{-1}). FR139317 infusion was stopped at approximately 125 min (Recovery period. See methodological considerations).

Plasma Analysis

Plasma [ET-1], [ACTH], [cortisol] and [AII] were analysed by Dr H.H.G. McGarrigle, Department of Obstetrics & Gynaecology, University College London. Details of these assay are given in full in Appendix 5.

Plasma [ET-1] was quantitatively measured using an ET1-21 specific [^{125}I] RIA (Nichols Institute, UK) using a synthetic ET-1 standard, rabbit anti-endothelin serum, [^{125}I]ET-3 (synthetic) tracer and donkey anti-rabbit serum. The sensitivity of the assay was 8pg.ml^{-1}.

The ET-1 assay was also carried out on samples from the intact and CSD vehicle infused fetuses from Chapter 4. The assay results from these fetuses are presented in the results section of the present Chapter.

Plasma [cortisol] was measured by RIA using a ^{125}I cortisol (sensitivity of the assay was 30fmol.ml^{-1}), and ACTH was measured using a double-antibody ^{125}I RIA (sensitivity of the assay was 8pg.ml^{-1}).

Plasma [AII] was measured quantitatively using RIA (Nichols Institute, UK) following the separation of AII from plasma proteins by methanol extraction and chromatography (sensitivity of the assay was 3.8pg.ml^{-1}).

5.3 Data Analysis

Grouped data is expressed as mean ± S.E.M. Blood composition and cardiovascular measurements for individual fetuses were reduced to summary measures to describe normoxic, hypoxic and recovery hours [2.6.3] as indicated by horizontal bars in e.g. Figure 5.13. Summary measures were then tested using Student's paired t-test to compare hypoxic and recovery periods to normoxia.

Student's paired t-test was used to test rapid transient changes in FHR and in $PaCO_2$ during hypoxia.

Cardiovascular measurements made prior to the onset of vehicle or FR139317 infusion (-5 to 0 min) were reduced to a summary measure and this was compared by t-test to the subsequent 1 h normoxic period (Table 5.1).

Student's t-test was used to compare plasma [AII], [ET-1], [ACTH] and [cortisol] in hypoxia and recovery with normoxia (45 min), and between pre-drug and normoxia (45 min). The Bonferroni method of correction [2.6.3] was used where multiple comparisons were necessary.

Time spent breathing and in LV-ECoG activity was expressed as a percentage of the hours of normoxia, hypoxia and recovery. Paired t-test was then used to compare hypoxia and recovery to normoxia.

An unpaired t-test was substituted in the event of uneven sample groups, and these instances are indicated in the text and figure legends.

In this chapter venous pressure measurements were available in all fetuses during part of the hours of normoxia and hypoxia and therefore vascular resistances were calculated during these periods from (MAP-MVP) ÷ flow.

Statistical significance was accepted when $P<0.05$.

5.4 Results

5.4.1 Response to ET-1

There was a rapid rise in MAP in response to ET-1 (Figure 5.1) which was of a similar magnitude at all the doses investigated (Figure 5.2). ET-1 caused a fall in FHR (Figure 5.1) which was greatest at the highest dose of ET-1 (Figure 5.2). Exogenous ET-1 also caused a fall in FBF and CBF (Figure 5.1). The size of the fall in flow appeared to be greater the higher the dose of ET-1 (Figure 5.2).

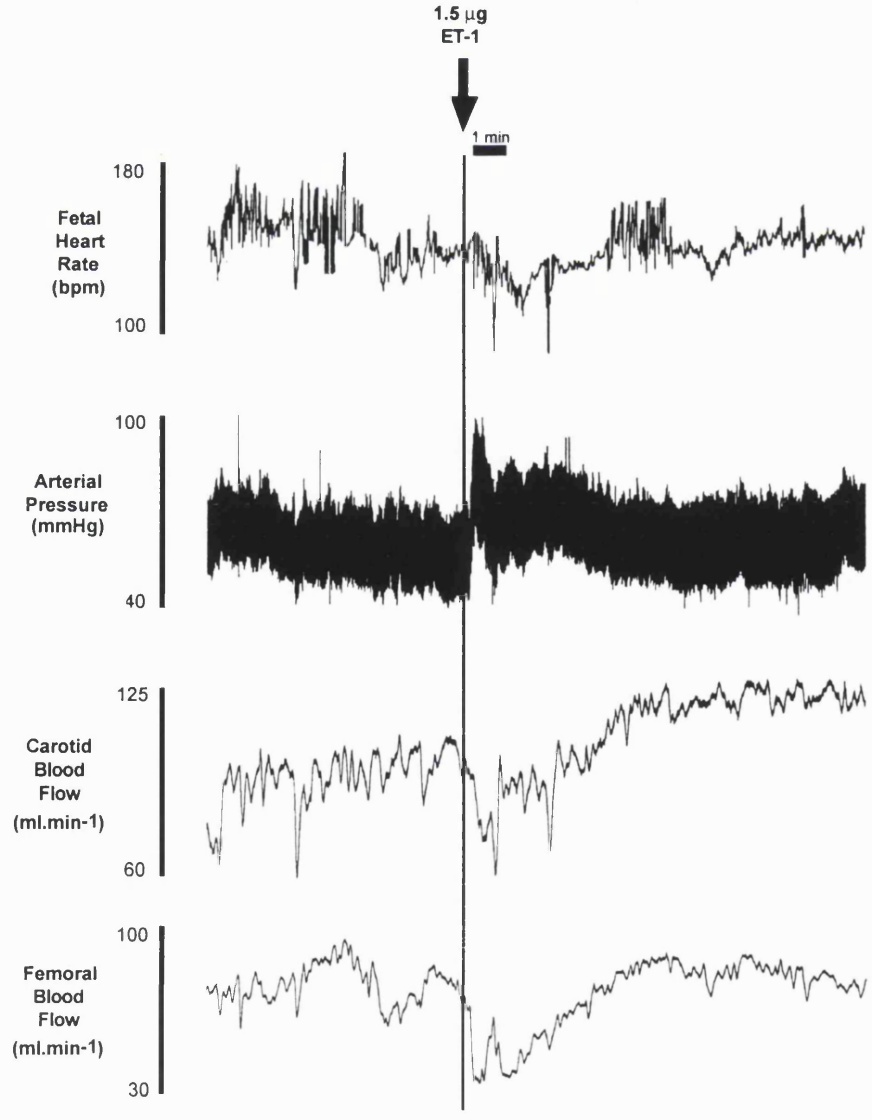


Figure 5.1 Raw trace demonstrating the response of one fetus (132 days gestation) to 1.5µg ET-1 i.v. (denoted by arrow).

5.4.2 Blockade of ET-1 pressor response by FR139317

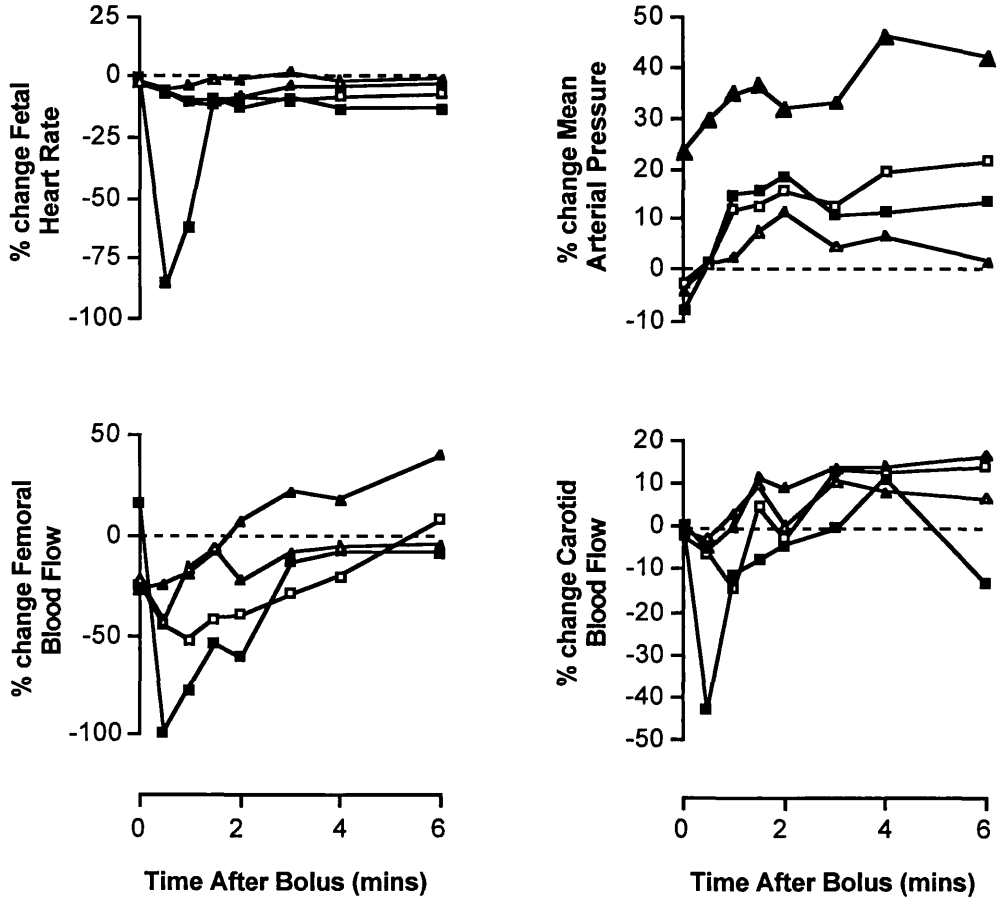


Figure 5.2 Percentage change from baseline of FHR, MAP, FBF and CBF in response to i.v bolus doses of 500ng (Δ), 1μg (▲), 1.5μg (□) and 2μg (■) ET-1 in one fetus (125 days gestation).

In the one fetus investigated, the fall in FBF in response to ET-1 was markedly attenuated by the infusion of all doses of FR139317 investigated (Figure 5.3). The FHR response to ET-1 was increased during dose 1 of the antagonist, no different from the pre-antagonist response during dose 2 of the antagonist and appeared to be attenuated after the highest dose of the antagonist. The pressor response to ET-1 observed prior to antagonist infusion was reversed after dose 1 of the antagonist and lower than pre-antagonist levels with doses 2 and 3. Before antagonist infusion CBF fell in response to the ET-1 bolus, however in the presence of FR139317 ET-1 caused a *rise* in CBF.

The one fetus to which a maximal dose of FR139317 was administered responded with an initial rapid peripheral vasoconstriction shown by a fall in FBF, rise in MAP and transient fall in FHR. This was followed by a more gradual fall in CBF and fall in MAP

and FHR. Approximately 30 min after the onset of infusion the fetus was dead (Figure 5.4).

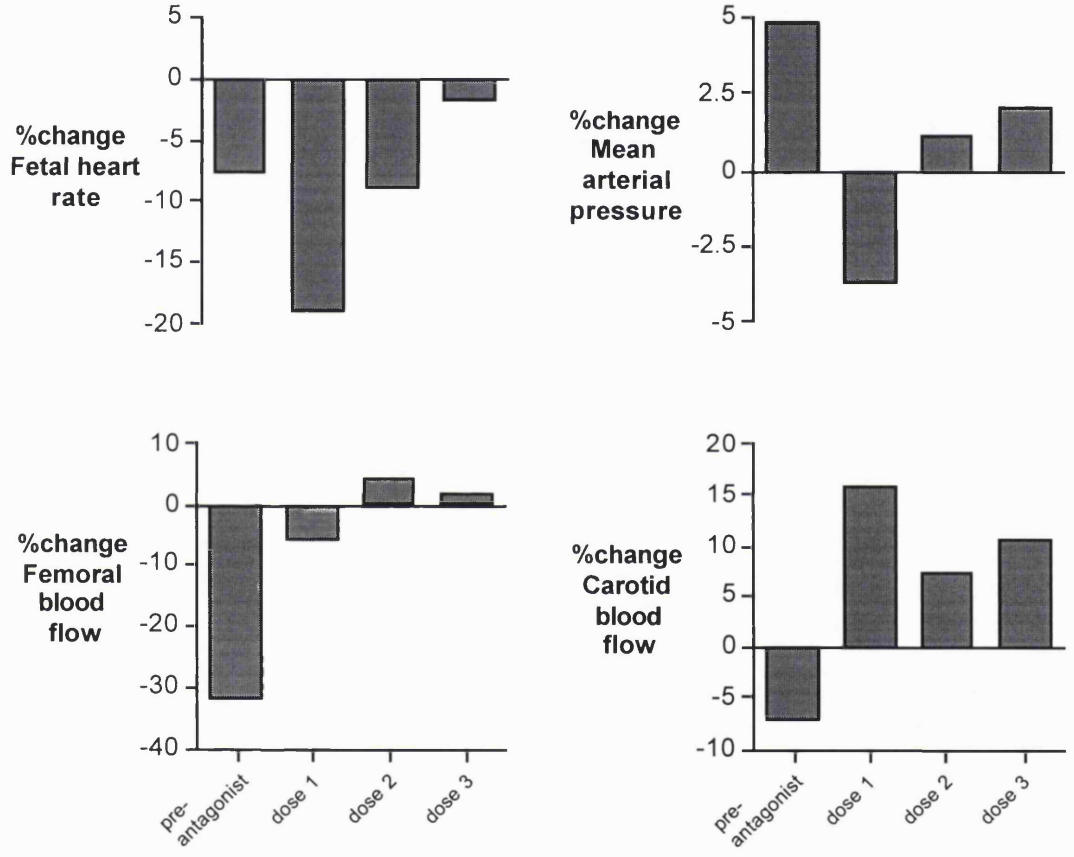


Figure 5.3 Graphs demonstrating the percentage change from baseline of FHR, MAP, FBF and CBF in response to 1.5μg ET-1 in one fetus (126 days gestation), before and during the infusion of the ET_A receptor antagonist FR139317 at three doses: (dose1) 3μg bolus + 0.5μg.min^{-1} infusion; (dose2) 30μg bolus + 5μg.min^{-1} infusion; and (dose3) 300μg bolus + 50μg.min^{-1} infusion.

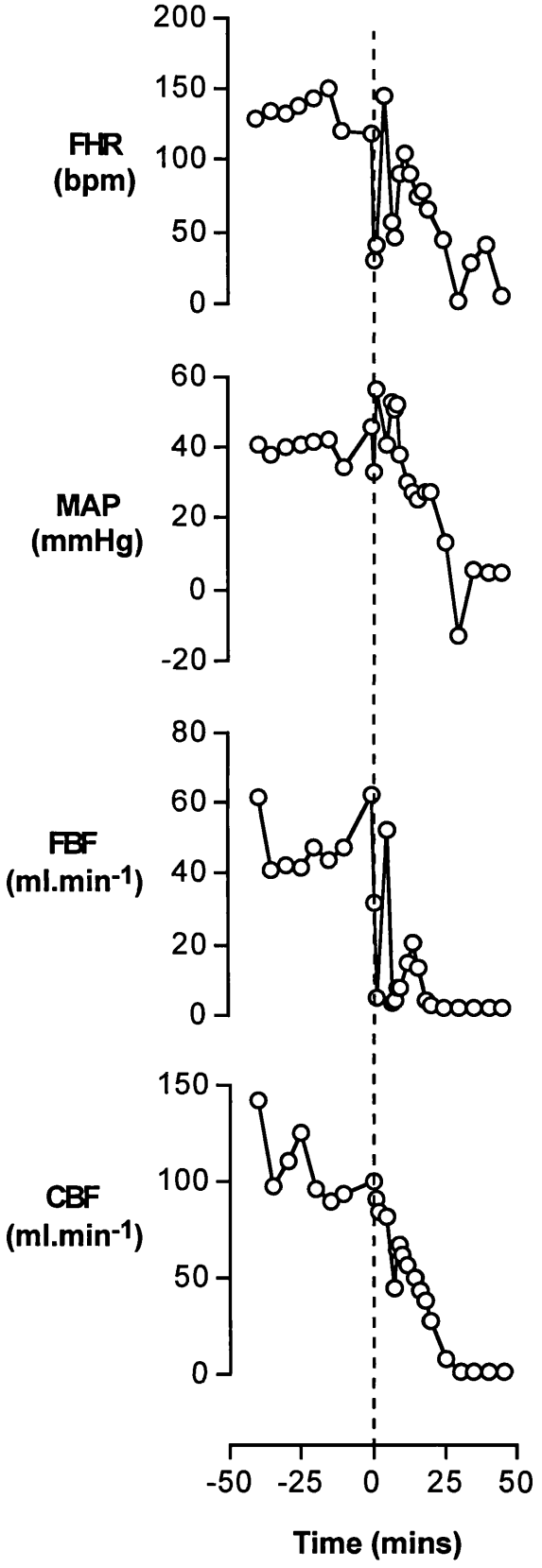


Figure 5.4 FHR, MAP, FBF and CBF responses of one fetus (133 days gestation) to a maximal dose regime of FR139317 (i.v.). The dotted line shows the time (0 min) at which a bolus dose of 3mg followed by a continuous infusion of 500μg.min^{-1} of FR139317 was given to the fetus.

5.4.3 Blood gases, electrolytes, glucose and lactate

Arterial Oxygenation

During hypoxia there was a fall in PaO_2 from ca. 22 to 12mmHg, in $\%SaO_2$ from ca. 80 to 40%, in O_2ct from ca. 8 to 4 $vol\%O_2$, and in total Hb from ca. 8 to 6 $g.dL^{-1}$ in both vehicle and FR139317 infused fetuses. O_2ct and $\%SaO_2$ during normoxia, and haemoglobin during recovery, were lower with antagonist than with vehicle infusion. O_2ct and Hb were lower after 15 min vehicle, and PaO_2, O_2ct and Hb were lower after 15 min FR139317 than pre-infusion onset (Figure 5.5).

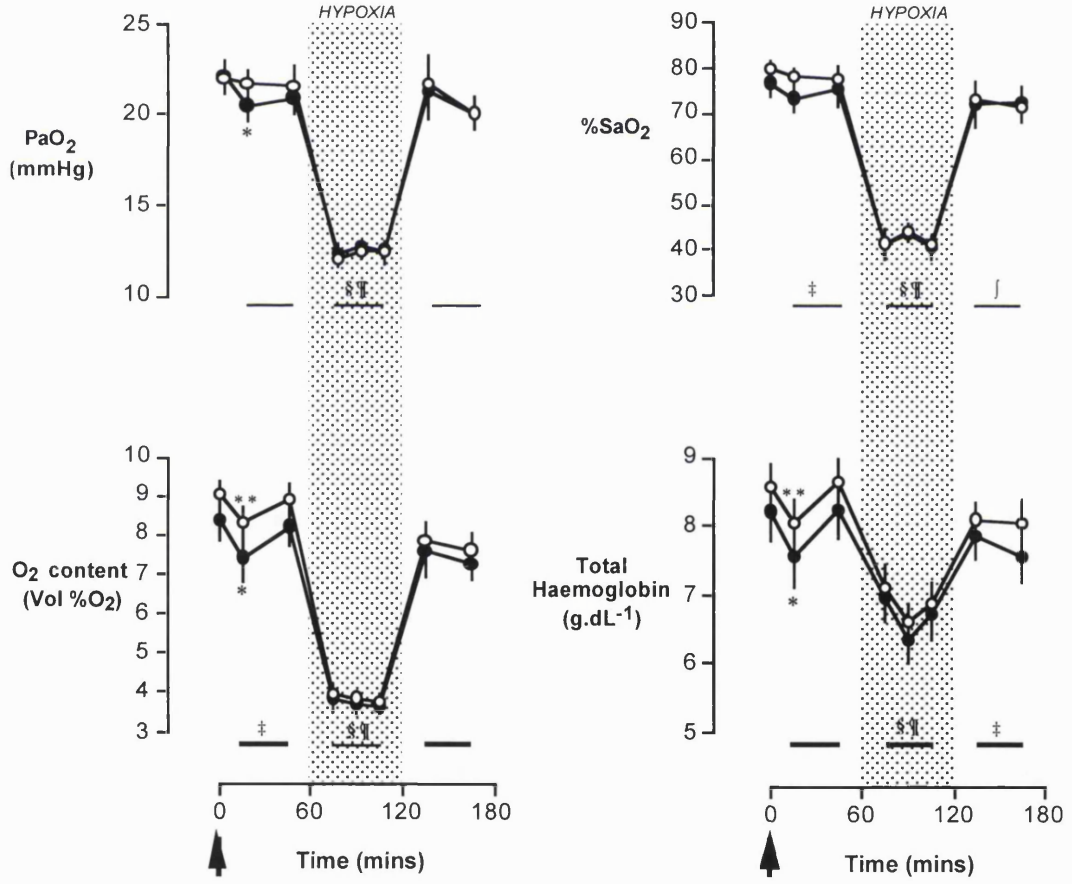


Figure 5.5 PaO_2, $\%SaO_2$, O_2 content and total haemoglobin in vehicle (○) and FR139317 (●) infused fetuses (n=7) during normoxia, hypoxia (shaded area) and recovery. The first sample was taken just prior to the onset of infusion at time zero (arrow). The horizontal bars show the time period over which values were reduced to summary measures. §P<0.01 and ∫P<0.05, significantly different from normoxia in vehicle group by paired t-test; ¶P<0.01, significantly different from normoxia in FR139317 group by paired t-test; ‡P<0.05, FR139317 significantly different from vehicle group by paired t-test; **P<0.01 and *P<0.05, significantly different from pre-infusion by paired t-test.

Acid-base status

After 15 min of FR139317 infusion, $PaCO_2$, HCO_3^- and base excess fell. A fall in $PaCO_2$ and HCO_3^- was also seen after 15 min vehicle infusion (Figure 5.6). During hypoxia pH, HCO_3^- and base excess fell and remained lower than normoxic levels during recovery in both vehicle and FR139317 groups. There was a transient fall in $PaCO_2$ at 90 min (hypoxia) in both groups, however over the hour of hypoxia as a whole there was no change from normoxic levels.

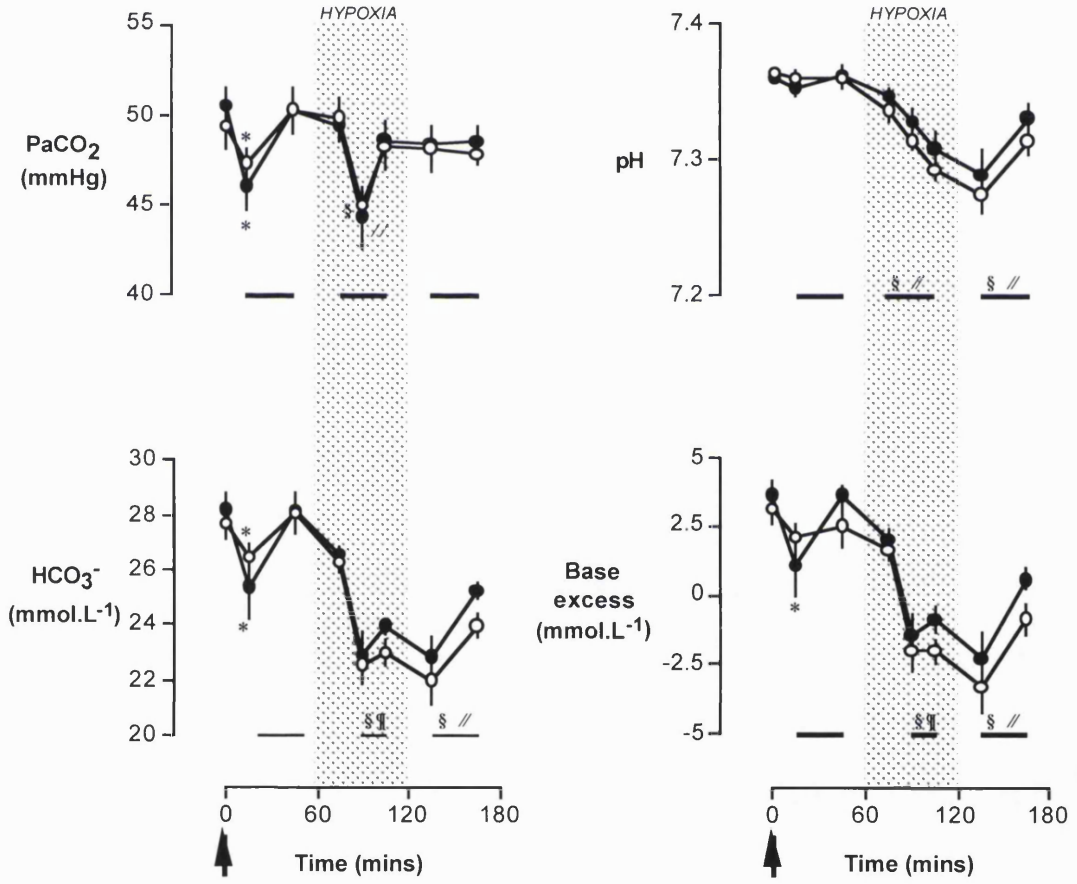


Figure 5.6 $PaCO_2$, pH, HCO_3^- and base excess during normoxia, hypoxia (shaded area) and recovery in vehicle (○) and FR139317 (●) infused fetuses (n=7). The first sample was taken just prior to the onset of infusion at time zero (arrow). The horizontal bars show the time period over which values were reduced to summary measures. §P<0.01, significantly different from normoxia in vehicle group by paired t-test; ||P<0.05 and ¶P<0.01, significantly different from normoxia in FR139317 group by paired t-test; *P<0.05, significantly different from pre-infusion by paired t-test.

Lactate and Glucose

Lactate rose significantly during hypoxia in both vehicle and FR139317 groups, and remained elevated in both groups during the recovery period. Similarly, glucose rose significantly during hypoxia and tended to remain elevated during the subsequent recovery period, but this only reached significance in the antagonist infused group (Figure 5.7).

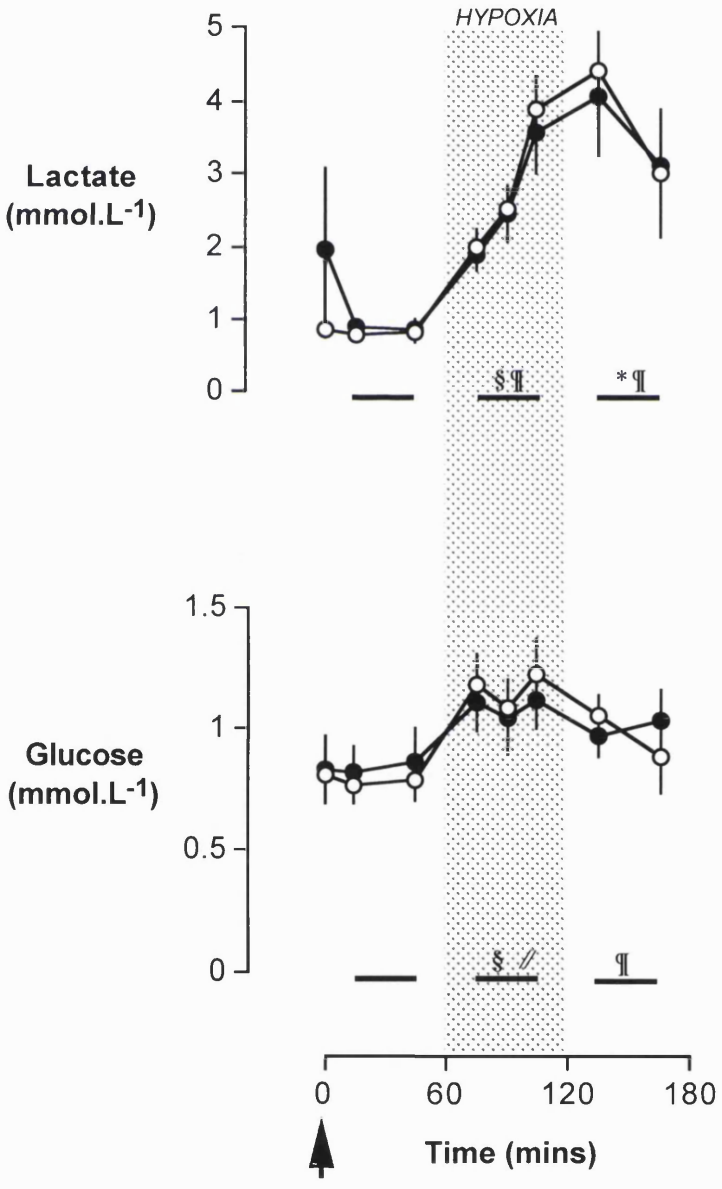


Figure 5.7 Lactate and glucose during normoxia, hypoxia (shaded area) and recovery in vehicle (○) and FR139317 (●) infused fetuses (n=7). The first sample was taken just prior to the onset of infusion at time zero (arrow). The horizontal bars show the time period over which values were reduced to summary measures. *P<0.01 (unpaired t-test) and §P<0.05 (paired t-test), significantly different from normoxia in vehicle group; //P<0.05 and ¶P<0.01, significantly different from normoxia in FR139317 group by paired t-test.

Sodium and potassium

While sodium levels remained unchanged throughout the course of the protocol, potassium rose significantly during hypoxia in both vehicle and FR139317 groups. Furthermore potassium values remained elevated compared to pre-hypoxic levels during recovery in the vehicle group (Figure 5.8).

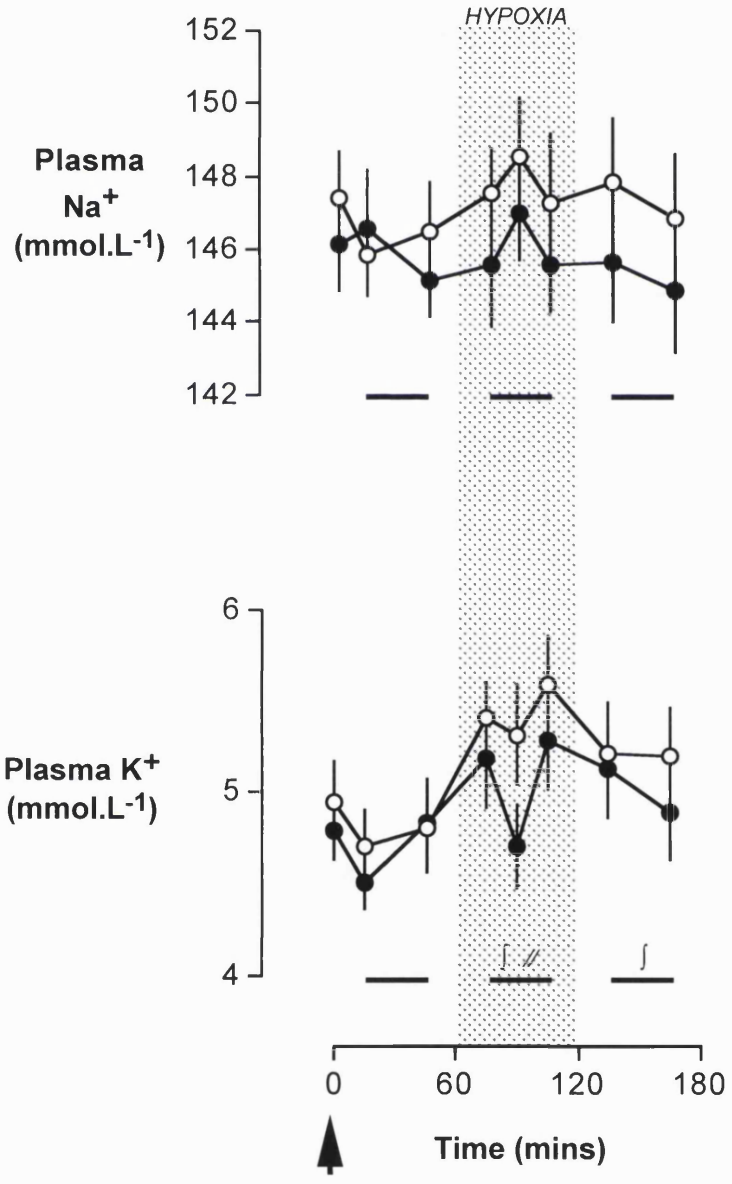


Figure 5.8 Plasma sodium and potassium during normoxia, hypoxia (shaded area) and recovery in vehicle (○) and FR139317 (●) infused fetuses (n=7). The first sample was taken just prior to the onset of infusion at time zero (arrow). The horizontal bars show the time period over which values were reduced to summary measures. ‖P<0.05, significantly different from normoxia in FR139317 group by paired t-test; ∫P<0.05, significantly different from normoxia in vehicle group by paired t-test.

5.4.4 Plasma endothelin

Plasma [ET-1] were not altered from control levels of ca. 35pg.ml^{-1} by the administration of FR139317. Furthermore there was no change in [ET-1] levels during 1 h hypoxia in either vehicle or FR139317 infused groups (Figure 5.9).

Neither intact nor CSD group plasma [ET-1] was changed from control (ca. 60pg.ml^{-1}) during hypoxia (Figure 5.10). Furthermore there was no difference in [ET-1] between intact and CSD groups, except for after 15 min hypoxia when intact were lower than CSD values.

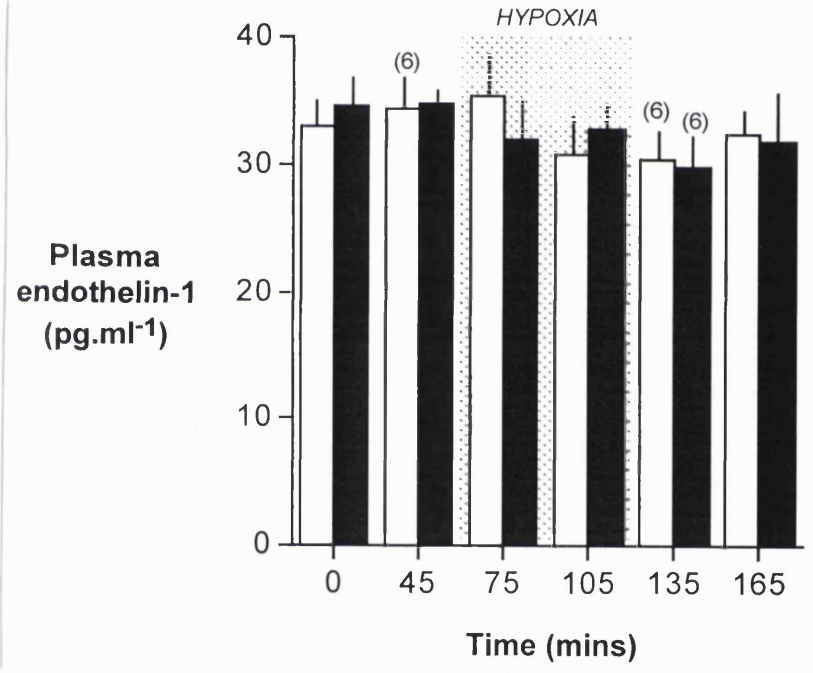


Figure 5.9 Plasma [ET-1] in response to 1 h hypoxia (shaded area) in vehicle (open bars) and FR139317 (closed bars) infused fetuses (n=7, unless otherwise indicated by bracketed number).

5.4.5 Plasma angiotensin II

During hypoxia plasma [AII] tended to rise but this only reached significance in the vehicle group (75 min, Figure 5.11). There was no significant difference in [AII] between vehicle and FR139317 infused groups throughout the protocol.

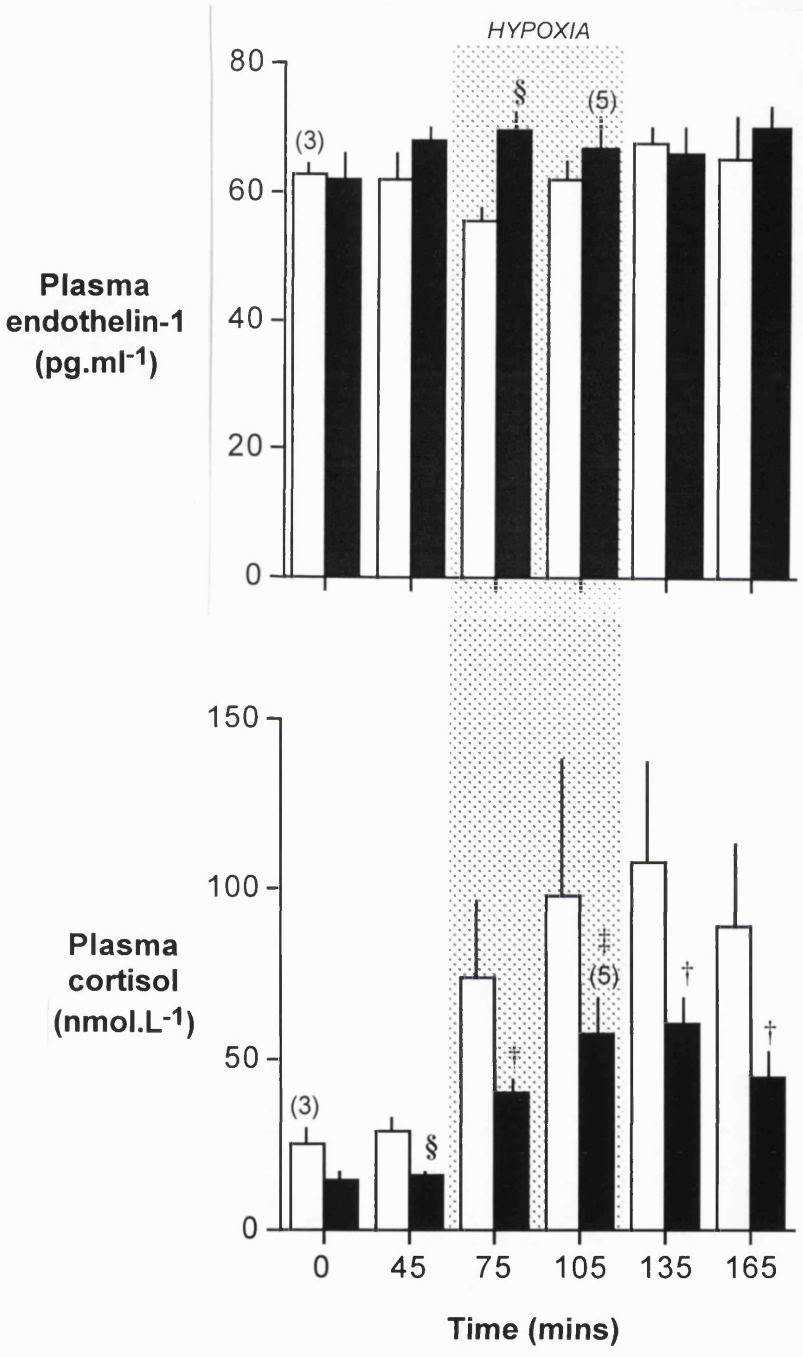


Figure 5.10 Plasma [ET-1] and [cortisol] in response to 1 h hypoxia (shaded area) in 6 intact (open bars) and 6 CSD (closed bars) fetuses (unless otherwise indicated by bracketed number). ‡(unpaired t-test) and †(paired t-test) $P<0.0125$, significantly different from 45 min (normoxia); §$P<0.05$, significantly different from intact group.

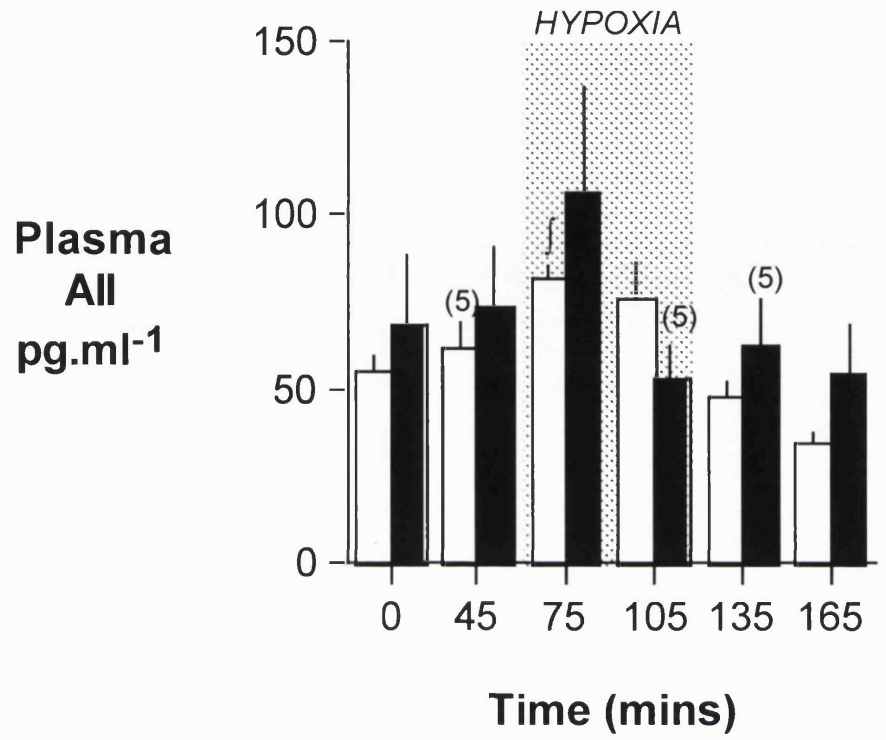


Figure 5.11 Plasma [AII] in response to 1 h hypoxia (shaded area) pre- (time 0) and during vehicle (open bars) and FR139317 (closed bars) infusion (n=6, unless otherwise indicated by bracketed numbers). ∫P<0.0125, significantly different from 45 min by unpaired t-test.

5.4.6 Plasma ACTH and cortisol

Plasma [ACTH] rose during hypoxia in both vehicle and FR139317 infused groups and remained above normoxic levels during the recovery period (Figure 5.12). During late hypoxia (105 min) and recovery (135 and 165 min) plasma [ACTH] was greater with vehicle than with FR139317 infusion.

During hypoxia plasma [cortisol] tended to rise in both groups, but this only reached significance in the FR139317 group (75 min. Figure 5.12).

During normoxia (45 min) plasma [cortisol] was lower in CSD than in intact fetuses (Figure 5.10). Cortisol rose in CSD, but not intact, fetuses during hypoxia and remained elevated during the recovery period (Figure 5.10).

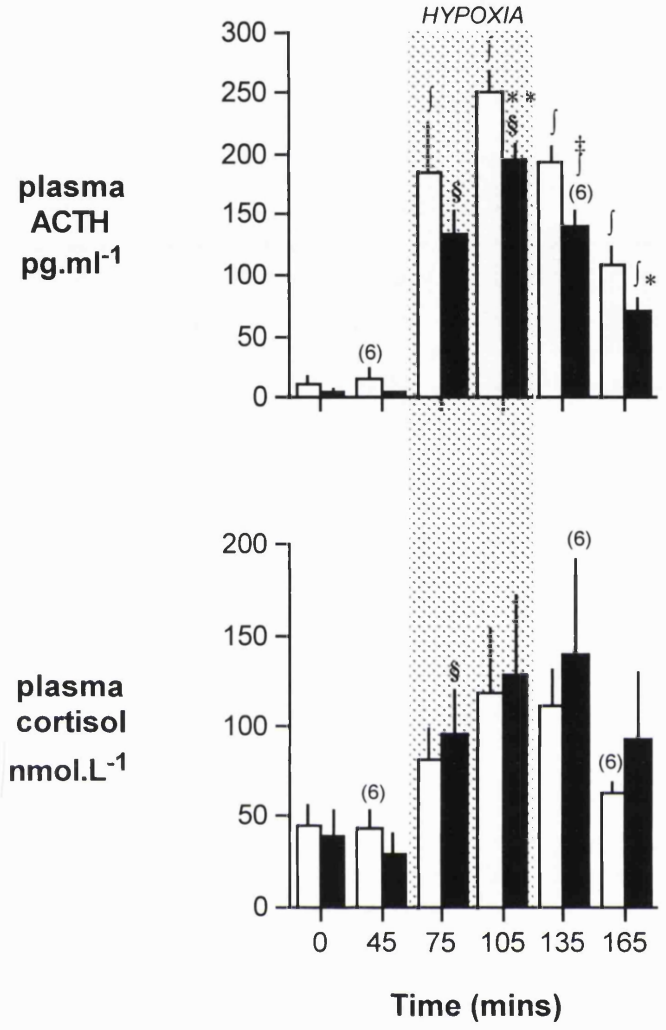


Figure 5.12 Plasma [ACTH] and [cortisol] in response to 1 h hypoxia (shaded area) pre- (time 0) and during vehicle (open bars) and FR139317 (closed bars) infusion (n=7, unless otherwise indicated by bracketed number). **P<0.01 and *P<0.05 significantly different from vehicle group by paired t-test; ‡P<0.05, significantly different from vehicle group by unpaired t-test; § (paired t-test) and ∫ (unpaired t-test) P<0.0125, significantly different from 45 min.

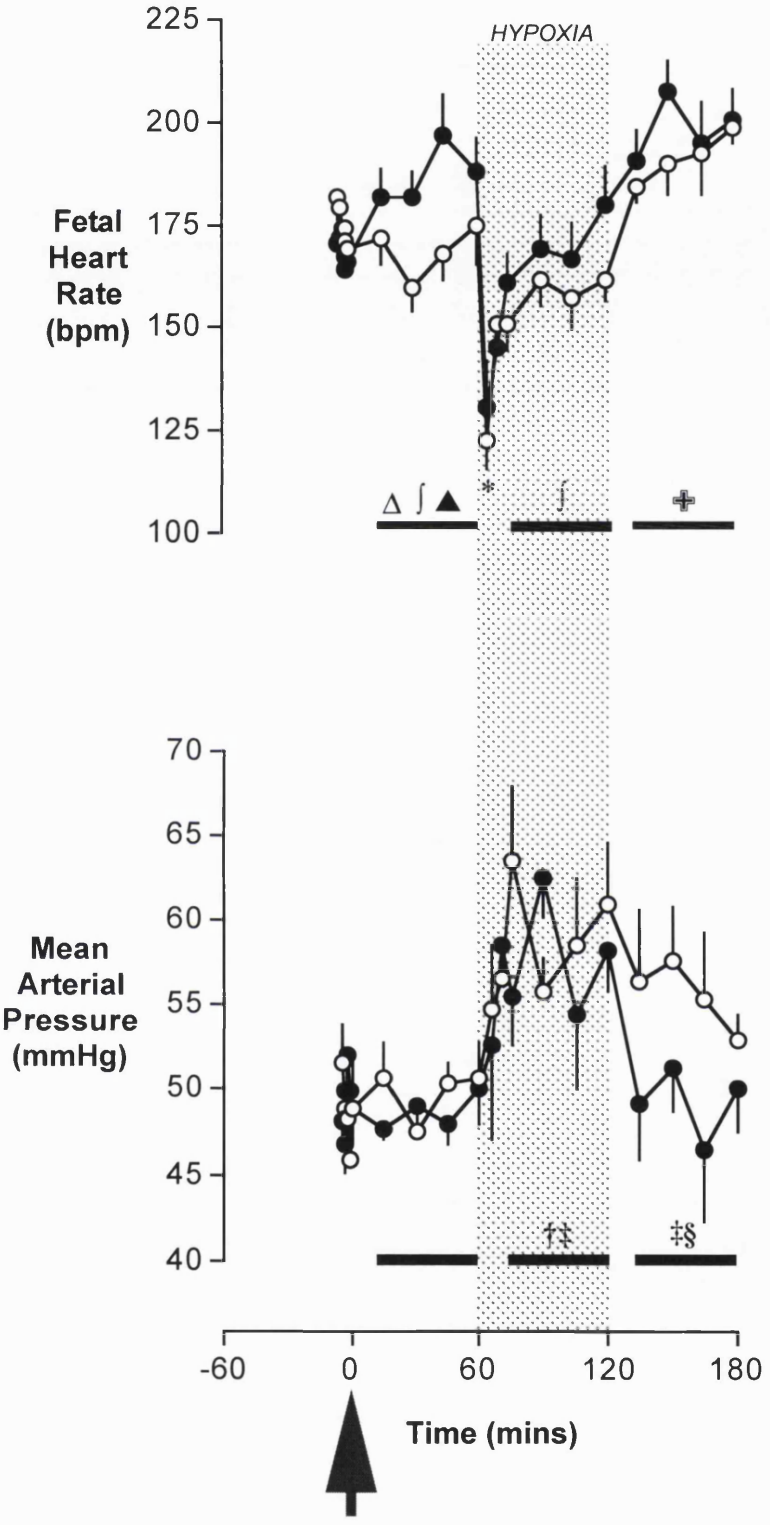


Figure 5.13 FHR and MAP responses to 1 h hypoxia (shaded area) with vehicle (○: n=7 and n=6, respectively) and FR139317 antagonist (●: n=7 and n=6, respectively) infusion. The onset of infusion is shown by the arrow at time zero. The horizontal bars show the time period over which data was reduced to summary measures. *P<0.01, 60 vs. 65 min in both groups, by paired t-test; ✙P<0.01, vs. normoxia in vehicle group, by paired t-test; †P<0.01, vs. normoxia in FR139317 group, by paired t-test; ‡P<0.05, vs. normoxia in vehicle group, by paired t-test; ∫P<0.05, vehicle vs. FR139317 group, by paired t-test; §P<0.01, vehicle vs. FR139317 group, by paired t-test; ΔP<0.05, vs. pre-infusion in vehicle group, by paired t-test (see table 5.1); ▲P<0.05, vs. pre-infusion in FR139317 group (see Table 5.1).

5.4.7 Fetal heart rate

FHR fell following the onset of vehicle and rose following the onset of FR139317 infusion (Table 5.1). Moreover, while there was no difference between the two groups during the pre-infusion period, FHR was significantly greater with antagonist than with vehicle infusion in the one hour following infusion onset (Figure 5.13). At the onset of hypoxia there was a rapid bradycardia which was of a similar magnitude in both groups. As hypoxia proceeded, FHR returned to pre-hypoxic levels in both groups, although once more heart rate during this time was greater in the FR139317 group. A tachycardia developed during the recovery period in the vehicle but not in the FR139317 infused group (Figure 5.13).

5.4.8 Mean arterial pressure

There was no change in MAP after the onset of FR139317 infusion (Table 5.1). During hypoxia MAP rose significantly and by the same magnitude with vehicle and FR139317 infusion. Pressure remained elevated compared to normoxia during the recovery period with vehicle but not FR139317 infusion (Figure 5.13).

5.4.9 Blood flow

Femoral bed

Despite a tendency for FBF to rise after the onset of FR139317 infusion this did not reach significance (Table 5.1). At the onset of hypoxia, flow fell to a similar level in both groups and remained low for the hypoxic hour. FBF returned to pre-hypoxic levels during recovery in both groups (Figure 5.14).

Carotid bed

After the onset of FR139317 infusion CBF tended to rise although this did not reach significance, although during normoxia, CBF was significantly higher with the infusion of FR-antagonist than with vehicle infusion (Figure 5.14 and Table 5.1). At the onset of hypoxia flow rose significantly in the vehicle, but not in the FR139317 infused group.

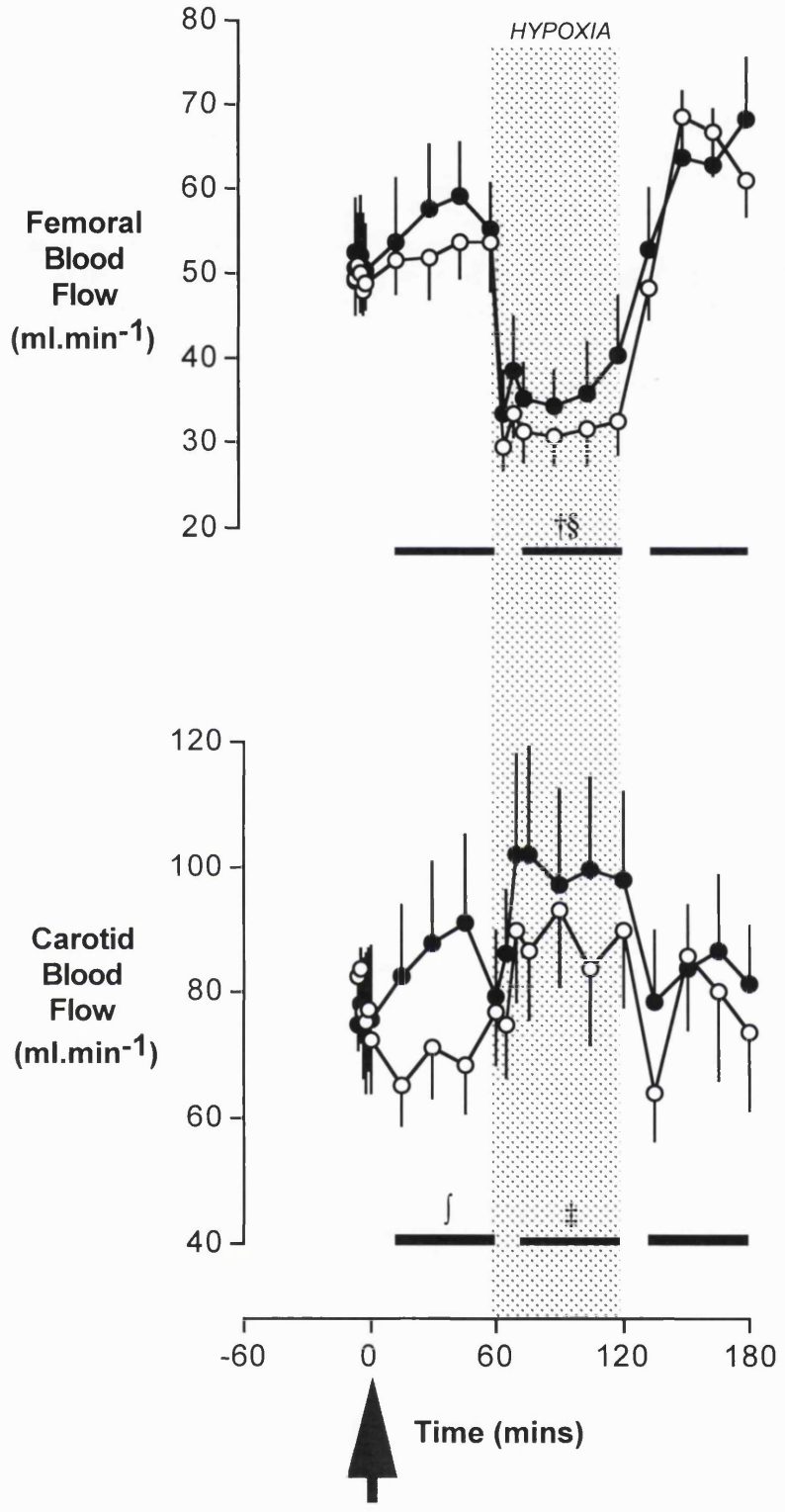


Figure 5.14 FBF and CBF responses to 1 h hypoxia (shaded area) with vehicle (n=6) and FR139317 (n=6) infusion. The arrow denotes the onset of infusion at time zero. The horizontal bars show the time period over which data was reduced to summary measures. †P<0.01, vs. normoxia in vehicle group, by paired t-test; ‡P<0.05, vs. normoxia in vehicle group, by paired t-test; ∫P<0.05, vehicle vs. FR139317 group, by paired t-test; §P<0.01, vs. normoxia in FR139317 group, by paired t-test.

	VEHICLE		FR139317	
	PRE	POST	PRE	POST
FHR (n=7)	175±5.8	168±5.6 §	168±5.5	187±7.0 †§
MAP (n=6)	48.97±1.89	49.82±1.37	49.44±1.40	48.63±0.85
FBF (n=6)	49.60±3.37	52.72±4.42	51.04±6.03	56.43±6.57
CBF (n=6)	78.48±9.86	70.50±7.30	76.29±9.82	85.20±11.30 †

Table 5.1 Cardiovascular responses to vehicle and FR139317 infusion during normoxia. Values are given as mean±S.E.M. of summary measures of data before the onset (PRE: -5 to 0 min) and during the first hour of (POST: 15-60 min) infusion. †P<0.05, significantly different from vehicle group by paired t-test; §P<0.05, significantly different from pre-infusion, by paired t-test.

5.4.10 Vascular resistance

Femoral bed

During hypoxia FVR rose in both vehicle and FR139317 groups. However there was no difference in resistance between groups throughout normoxia or hypoxia (Figure 5.15).

Carotid bed

and hypoxia

During normoxia CVR was lower with FR139317 than with vehicle infusion. During hypoxia resistance fell in the vehicle group, but this fall was absent with the infusion of the FR-antagonist (Figure 5.15).

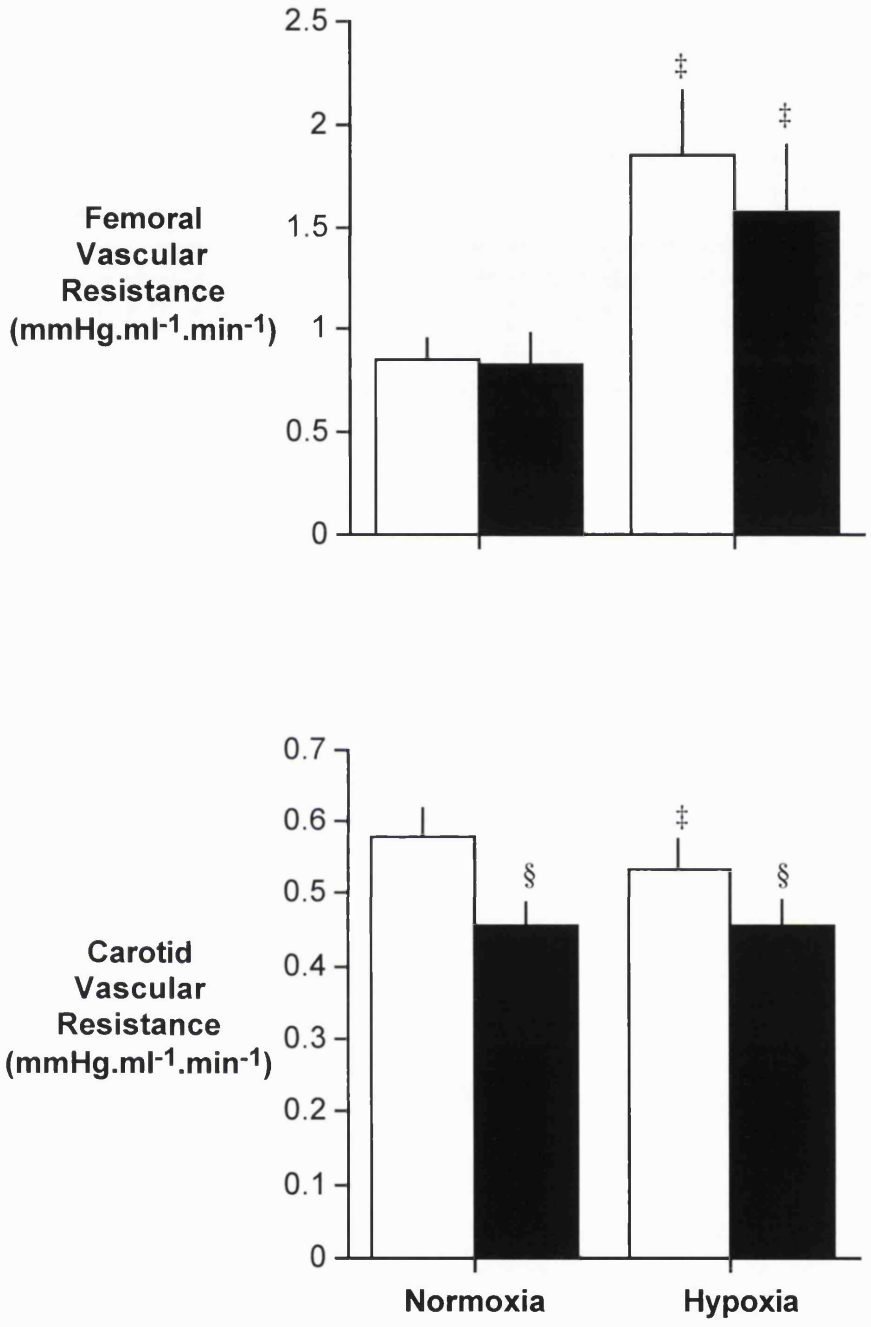


Figure 5.15 FVR (n=5) and CVR (n=5) during normoxia and hypoxia with vehicle (open bars) and FR139317 (filled bars) infusion. Recovery and pre-infusion periods were not calculated due to total absence of venous pressure readings. ‡P<0.05, significantly different from normoxia by paired t-test; §P<0.01, FR139317 significantly different from vehicle group by paired t-test.

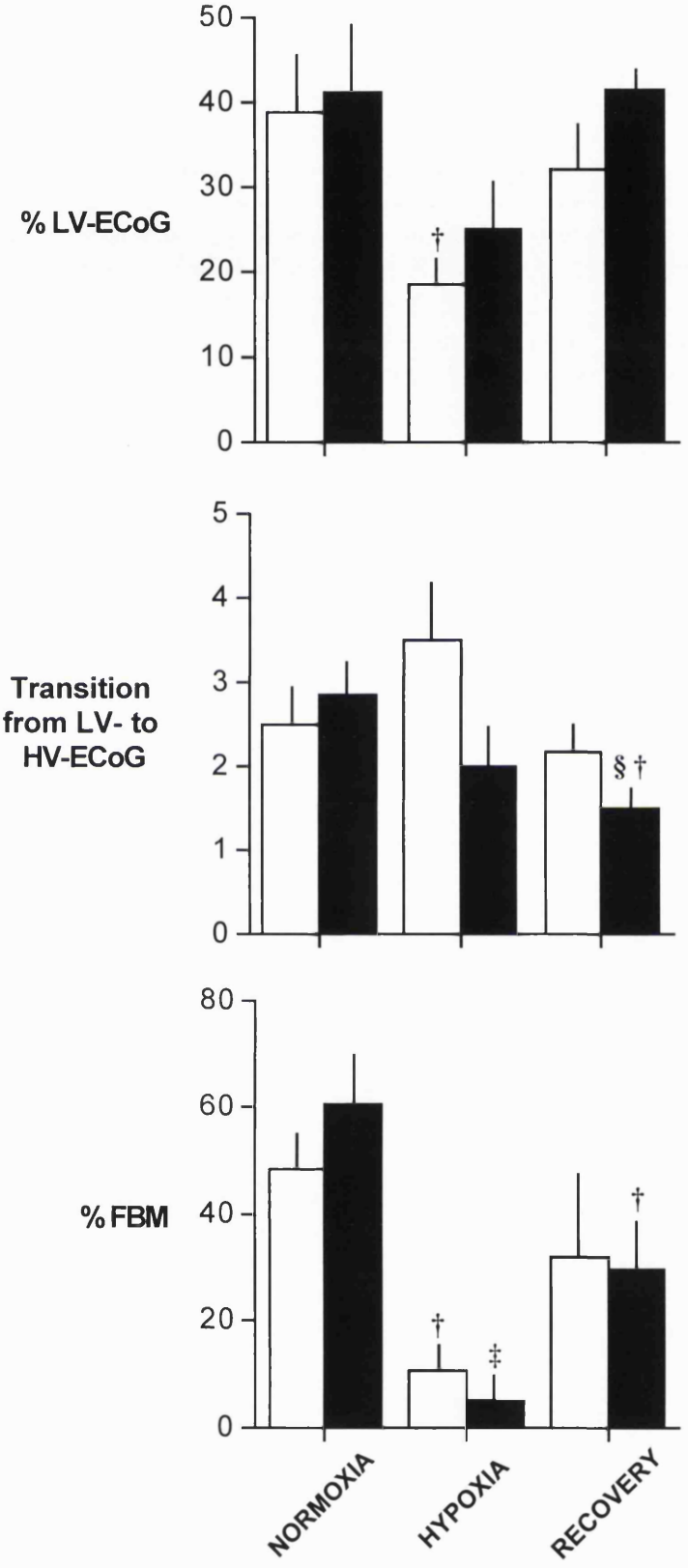


Figure 5.16 Percent LV-ECoG (n=6), number of transitions from LV- to HV-ECoG (n=6) and percent FBMs (n=4) during normoxic, hypoxic and recovery hours in vehicle (open bars) and FR139317 (closed bars) infused groups. Data are expressed as mean±S.E.M. †P<0.05 and ‡P<0.01, significantly different from normoxia by paired t-test; §P<0.05, significantly different from vehicle group by paired t-test.

5.4.11 ECoG and Fetal Breathing Movements

The time spent in LV-ECoG (% LV-ECoG) was significantly reduced from normoxia during hypoxia in vehicle but not FR139317 (P=0.051) infused groups (Figure 5.16). Furthermore the number of transitions from LV- to HV-ECoG activity tended to be greater during hypoxia in the vehicle group, although this did not reach significance. In the FR-antagonist group the number of such transitions was lower during recovery than during normoxia (Figure 5.16).

During hypoxia there was a large fall in FBMs in vehicle and FR-antagonist infused fetuses (Figure 5.16). During recovery, the proportion of time spent making breathing movements tended to return towards normoxic levels in both groups, although was still significantly lower than normoxia in the FR139317 group.

5.5 Discussion

The results presented in this Chapter suggest that ET-1 alters basal FHR, CBF and CVR but has no effect on peripheral vascular tone. The modulation of FHR and CVR persists during hypoxia.

Methodological considerations

The use of FR139317

FR139317 and BQ123 are ET_A-receptor antagonists. While both compounds are able to block ET-1-induced contractions of the guinea-pig iliac artery, the potency of FR139317 is ca. one order of magnitude less than that of BQ123, but unlike BQ123 has the advantage of being a competitive-antagonist (Schoeffter, Randriantsoa, Jost and Bruttel, 1993). FR139317 (Gardiner, Kemp, March, Bennett, Davenport and Edvinsson, 1994), but not BQ123 (Pollock and Opgenorth, 1993) has been shown to attenuate the renal vasoconstrictor effects of ET-1 in rats. It has been suggested that the physiochemical properties of a modified linear tripeptide such as FR139317 may make it more stable, or increase its bioavailability, in comparison to BQ123. FR139317 has been shown, *in vitro* and *in vivo*, to be a selective ET_A receptor antagonist (Sogabe *et al.*, 1992) able to prevent ET-1 binding to coronary vessels *in vitro* in a concentration-dependent manner (Dashwood, Alen, Luu and Muddle, 1994) and ET-1 induced renal vasoconstriction in adult rats *in vivo* (Gardiner *et al.*, 1994). The dose of FR139317 used in these experiments was based on a dose response curve constructed in one fetus involved in the study. In this fetus a 300μg bolus followed by 50μg.min^{-1} infusion of FR139317 blocked the pressor response, as shown by absence of change in FBF and

MAP, to 1.5μg ET-1. Moreover, following the administration of the lowest dose of the FR-antagonist an increase in CBF and decrease in MAP was seen in response to ET-1 rather than the fall in flow and rise in pressure seen without antagonist. These observations agree with studies in the anaesthetised adult rabbit in which pressor, but not depressor, responses to ET-1 were inhibited by FR139317 (McMurdo, Lidbury, Thiemermann and Vane, 1993). Thus it seems reasonable to speculate that the ET-1 induced vasoconstriction observed in the present study is mediated by ET_A receptors while other receptors subtypes, such as ET_B, may mediate the depressor actions of exogenous ET-1. These findings suggest that the dose of FR139317 used in these experiments was sufficient to antagonise the action of 1.5μg exogenous ET-1 via ET_A receptors. The large dose of FR139317 investigated in a different fetus had massive pressor effects and ultimately caused its death. This pressor effect has been observed before with high FR139317 doses in the conscious adult rat (Gardiner *et al.*, 1994) but it is not possible to explain this effect without further pharmacological investigation beyond the scope of this thesis. One possibility is that FR139317 possesses partial agonist properties.

The half-life of FR139317 in the circulation has been shown to be 0.38±0.13 h following i.v. administration of $1mg.kg^{-1}$ to the adult rat, and 0.85±0.26 h following i.v. administration of $10mg.kg^{-1}$ to the adult dog (Product Development Research Laboratories, Fujisawa Pharmaceutical Co., *unpublished observations*). On the basis of this information, the two fetuses in which the order of experiments were reversed were included in the analysis.

FR139317 infusion ceased at ca. 125 min (recovery period). This was necessary because of the limited quantity of the antagonist which was available for the entire study. While it is likely that some FR139317 remained in the circulation for the rest of the recovery period, cardiovascular and endocrine measurements made during this time have been interpreted with caution.

Animals involved in study

Seven fetuses were initially involved in this study. Measurement of plasma [ET-1], [ACTH] and [cortisol] was made in all of these fetuses, and [AII] measurement in 6 fetuses. Missing measurements were either because levels were too low to be detected by the sensitivity of the assay or if insufficient blood was withdrawn at the time of experimentation to carry out the assay.

MAP measurements were not available for one fetus during FR139317 infusion due to equipment problems. Therefore for the sake of statistical comparison this fetus was excluded from the grouped MAP vehicle group measurements.

CBF data was not available in one fetus of the vehicle group due to a bad electrical connection within the flow probe, therefore measurements taken on the subsequent antagonist infused day were not included in the analysis. FBF was considered to be below normal physiological levels in one fetus and it was therefore not included in the analysis. The reason for low flow was not obvious but may have been due either to partial occlusion of the vessel by the flow probe or that the signal strength of probe itself was low.

Hypoxia does not alter circulating levels of ET-1

Baseline [ET-1] is higher in fetal than in maternal plasma which would seem to rule out the passive transfer of maternal ET-1 to the fetus. The higher levels are probably not due to active transport of ET-1 since there is no umbilical arterio-venous difference. This implies that the fetus is able to actively secrete ET-1 (Nakamura *et al.*, 1990). A range of fetal baseline [ET-1] has been reported in previous studies: normal human umbilical venous serum, ca. 0.027pg.ml^{-1} (Schiff, Weiner, Zalel, Mashiach, Sibai and Shalev, 1994); mixed human umbilical plasma, ca. 23pg.ml^{-1} (Haegerstrand, Hemsen, Gillis, Larsson & Lundburg, 1989); human umbilical artery plasma, ca. 8pg.ml^{-1} (Nakamura *et al.*, 1990); late-gestation ovine fetal plasma, ca. 8pg.ml^{-1} (Jones, Abman and Wilkening, 1994). In the present Chapter baseline plasma [ET-1] was ca. 60pg.ml^{-1} (CSD study) and ca. 30pg.ml^{-1} (FR139317 study).

Previous adult *in vivo* and *in vitro* studies have suggested that hypoxia stimulates ET-1 production (Rakugi *et al.*, 1990; Shirakami *et al.*, 1991). In this chapter I have shown that during 1 h isocapnic hypoxia there is no change in circulating plasma [ET-1], in agreement with preliminary fetal sheep studies (Jones *et al.*, 1994). Thus it is possible that with the hypoxic insult used in the present study ET-1 production is not altered. Furthermore the administration of FR139317 did not have any effect on circulating [ET-1], which does not support a positive-feedback mechanism in ET-1 production. In adult cats binding sites for [^{125}I]ET-1 have been found in the carotid bifurcation, on smooth muscle of the common and external carotid artery, the carotid sinus region and within the carotid body (Spyer, McQueen, Dashwood, Sykes, Daly and Muddle, 1991) and, while the number of binding sites is not altered by chronic chemoreceptor stimulation (Dashwood, McQueen, Sykes, Muddle, Daly, Evrard and Spyer, 1993), ET-1 is known to cause chemoexcitation (Spyer *et al.*, 1991; McQueen, Dashwood, Cobb and Marr, 1994). In CSD fetuses there was no evidence to support the suggestion that carotid chemoreceptors control the release of ET-1 since there was no difference in baseline

plasma [ET-1] between intact and CSD fetuses. [ET-1] did not rise during hypoxia and contrary to the hypothesised feed-forward mechanism during hypoxia, ET-1 in the CSD was actually lower after 15 min hypoxia than in the intact group.

These results suggest that the paracrine actions of ET-1 are likely to be more important. Endothelial cells in culture release the majority of ET-1 towards the abluminal surface, therefore the absence of an effect of the various manipulations on plasma [ET-1] in this study may simply indicate that circulating [ET-1] does not reflect the local concentration of the peptide within the blood vessel wall (Wagner *et al.*, 1992), rather than implying minimal activity of the peptide. A method of measuring local ET-1 production or ET-1 gene expression would be more appropriate (Li *et al.*, 1994) but was beyond the scope of the present study.

FR139317 does not affect basal plasma AII levels

Co-culture of mouse renal juxtaglomerular cells with bovine microvascular endothelial cells suggest that endothelial cells exert some tonic inhibitory effect on the release of renin, perhaps via the release of ET-1 (Kurtz, Kaissling, Busse and Baier, 1991). An interaction of ET-1 with the RAS has been suggested in conscious adult rats since ET-1 induced hypertension, attributed to an increase in peripheral resistance, is prevented by ACE-inhibition (Mortensen and Fink, 1992). They suggested that ET-1 action may be mediated by AII either at the local tissue level [see 1.5.3] or through the renal RAS. Indeed intrarenally administered ET-1 will inhibit renal arterial constriction-stimulated renin release from denervated kidneys of anaesthetised adult dog, perhaps by a direct action on the juxtaglomerular cells (Naess, Christensen and Kiil, 1993). On the other hand ET-1 production from cultured endothelial cells is stimulated by AII (see Yanagisawa *et al.*, 1988), and studies in perfused adult rat mesenteric resistance arteries suggest that AII-stimulated ET-1 production amplifies the pressor effects of the RAS (Dohi, Hahn, Boulanger, Buhler and Luscher, 1992). Other conscious adult rat studies suggest that AII and ET-1 may act in synergy to elevate MAP, since AII alone or ET-1 alone failed to produce significant changes in MAP (Yoshida, Yasujima, Kohzuki, Kanazawa, Yoshinaga and Abe, 1992). In the present Chapter plasma [AII] rose after 15 min hypoxia with vehicle infusion, and tended to rise in the FR139317-infused group, although this did not reach significance. However throughout the course of the protocol there was no difference in [AII] between the two groups. Thus these results do not tend to support an interaction between ET-1 and AII. It is possible however that changes in [ET-1] and [AII] at a local level would have provided a better reflection of any interaction.

This study shows that there was no difference in plasma cortisol levels during normoxia or hypoxia between the vehicle and FR139317 groups. While plasma [ACTH] rose significantly in both groups during hypoxia, [ACTH] was significantly lower with

FR139317 than with vehicle during late hypoxia (105 min) and recovery which raises the possibility that ET-1 could exert an endogenous stimulatory action on the production of ACTH *in vivo*, although the mechanism of this effect will require further investigation.

Endogenous ET-1 alters basal heart rate and carotid vascular tone

In the present study the infusion of FR139317 caused a rise in FHR during normoxia. This finding implies a tonic negative-chronotropic effect of endogenous ET-1 on FHR, but differs from other fetal studies in which the ET_A receptor antagonist, BQ123 did not change resting FHR (Ivy, Kinsella and Abman, 1994; Wong, Fineman and Heymann, 1994). The tachycardia observed following FR139317 infusion is in keeping with the bradycardia observed after the *exogenous* ET-1 in this (Figure 5.1) and other adult (Scoggins, Spence, Parkes, McDonald, Wade and Coghlan, 1989) and neonatal (Wong, Vanderford, Fineman and Soifer, 1994) studies. It seems quite likely that the bradycardia in response to exogenous ET-1 is a baroreflex response because it is preceded by a large rise in MAP, however the positive chronotropic effect seen after FR-antagonist infusion was not accompanied by a change in MAP and is therefore more likely to be due to a direct action on the fetal heart. Indeed ET_A and ET_B receptor subtypes have been localised to adult atrial and ventricular myocardium, the atrioventricular conducting system and endocardial cells, with relatively higher density and proportion of ET_B receptors in the AV-node (see Golfman, Hata, Beamish and Dhalla, 1993 for discussion).

FR139317 had no effect on peripheral vascular resistance, as shown by the lack of a difference in FBF and MAP between vehicle and FR139317 groups after the onset of infusion. However during normoxia, CBF was higher and CVR was lower in the FR139317 than the vehicle group which indicates a tonic vasoconstrictory action of ET-1 in the carotid vascular bed. This agrees with adult studies in which the abluminal administration of ET-1 causes vasoconstriction of microvasculature in the cerebral cortex (Willette and Sauermelch, 1990). These results imply a degree of vascular bed selectivity in the action of endogenous ET-1, which may reflect heterogeneity in the receptor population [see 1.5.3].

ET-1 does not mediate systemic cardiovascular changes in hypoxia

During hypoxia, the characteristic rapid bradycardia at the onset of hypoxia was of a similar magnitude in both vehicle and FR139317 infused groups. This does not support a role for ET-1 in augmenting chemoreceptor discharge in the fetus. However the modulation of FHR seen during normoxia persisted throughout the late-hypoxic period.

A role for ET-1 has been implicated in the fall in fetal hind-limb blood flow during 3 h maternal common iliac artery occlusion-induced hypoxia (Jones, 1995), and in the pulmonary hypertensive response of the lamb (Wang, Coe, Toyoda and Coceani, 1995). However in the present study the FR-antagonist did not affect the MAP response to hypoxia since pressure rose to a similar level in both vehicle and FR139317 groups. Furthermore while FBF during hypoxia tended to be greater with antagonist infusion this did not reach significance. Thus the results presented in this Chapter do not suggest a large role for ET-1 in the fetal peripheral vascular changes during acute hypoxia. However there is some evidence of a small number of ET_B receptors on porcine aortic smooth muscle cells which mediate vasoconstriction, and if present these would not have been antagonised by FR139317 (Shetty, Okada, Webb, DelGrande and Lappe, 1993). During recovery, MAP remained elevated compared to normoxia with vehicle but not with FR139317 infusion. Despite the methodological problem of the curtailed-FR139317 infusion, it is possible that by the recovery period hypoxia had stimulated the production of adequate ET-1 to sustain a raised MAP, evident in the vehicle but not in the antagonist-infused group.

The results in this Chapter show that the rise in CBF and fall in CVR with vehicle infusion during hypoxia did not reach significance in the FR139317 group. There are three possible explanations for this finding: first, that the reduction in CVR seen during normoxia with FR139317 infusion was so great that vascular resistance could not fall any lower during hypoxia; secondly, that a component of the fall in CVR during hypoxia is normally due to the removal of ET_A-induced vasoconstrictor tone so that, when this has already been achieved by FR139317 infusion during normoxia, no further fall in CVR is seen during hypoxia; thirdly, that FR139317 partially effects ET_B receptors. Of these the last explanation seems least likely since FR139317 has been shown to be a specific ET_A antagonist (Sogabe *et al.*, 1993). One approach to investigating the first explanation would be to try to cause a further decrease in CVR by challenging with CO_2 after FR139317 infusion.

Exogenous ET-1 (i.v.) administration to the adult anaesthetised rat, caused a hyperventilation due to increased activity of the carotid arterial chemoreceptors, probably mediated via stimulation of ET_A receptors in the carotid body, rather than ET_B receptor stimulation or NO release (McQueen, Dashwood, Cobb and Marr, 1994). The results of the present study did not implicate a role for ET-1 in basal ECoG activity or the incidence of FBMs. With vehicle infusion the time spent in LV-ECoG was lower during hypoxia,

but this did not reach significance in the FR139317 group, however the incidence of FBMs fell to a similar extent during hypoxia in both groups.

5.6 Conclusion

Blockade of ET_A receptors implicates ET-1 in the regulation of baseline FHR and CVR. While this study shows no evidence of ET-1-stimulation of chemoreflex responses, the modulation of FHR and CVR with FR139317 persists during hypoxia. ET-1 does not appear to mediate the rise in CBF during moderate isocapnic hypoxia, nor does it significantly alter the fall in peripheral blood flow during hypoxia. This would seem to be supported by the lack of change in plasma [ET-1] during hypoxia, however future study will need to address the effect of hypoxia on ET-1 production and action in the fetus at the local tissue level before its role can be fully defined.

CHAPTER 6

THE EFFECT OF NITRIC OXIDE SYNTHESIS INHIBITION ON CARDIOVASCULAR RESPONSES TO HYPOXIA

6.1 Introduction

In the previous Chapter the results suggested a role for the endothelial-derived constricting factor, ET-1, in baseline fetal cardiovascular control. In the present Chapter the study was extended to investigate the part played by NO in cardiovascular control in normoxia and in response to isocapnic hypoxia.

Nitric oxide is released in both luminal and abluminal directions and is able to penetrate the entire vascular wall, although it may rely on mechanisms in addition to diffusion to do so (Bassenge, Busse and Pohl, 1987). Nitric oxide is rapidly inactivated by reacting with oxygen and superoxide anion radicals in vascular tissues to form nitrate and nitrite. It may be, therefore, that NO is stored transiently (<1sec) in a complex with ferrous iron-dithiolate (Busse, Mulsch, Fleming and Hecker, 1993). It is by the very nature of its extremely short half-life that NO would seem to be a particularly suitable candidate for the continual modulation of cardiovascular tone (Holden, 1994).

There is controversy as to whether hypoxia inhibits (Shaul, Farrar and Zellers, 1992; McQueston, Cornfield, McMurtry and Abman, 1993) or stimulates NO-release (Pohl and Busse, 1989; Tenney, 1990). The paradox is that while NOS requires O_2 as a substrate, its survival is enhanced in hypoxia due to reduced oxygen radical production. Furthermore during hypoxia there is a rise in intracellular Ca^{2+} ($[Ca^{2+}]i$) which is correlated to the release of NO *in vitro* (see Busse, Mulsch, Fleming and Hecker, 1993; Bredt and Snyder, 1994). Thus this rise in $[Ca_2{}^{+}]i$ during reduced, but nonetheless physiological, PaO_2 may be the crucial factor in increasing NO-synthesis (Shaul and Wells, 1994).

The mechanisms by which NO and guanylate cyclase produce relaxation appear to be fully functional in cerebral arteries of the term fetus (Pearce and Longo, 1991). Indeed L-NAME has been shown to contract cerebral vessels of both pre-term (105 days gestation) and late-gestation (125-130 days gestation) ovine fetuses *in vitro* (Wagerle, Moliken and Russo, 1995). It has also been shown that there is a marked developmental increase in

basal NO production in pulmonary endothelium around 125-135 days gestation in the fetal sheep (Shaul, Farrar and Magness, 1993), at which time the pulmonary vasoconstriction response to hypoxia develops (Lewis, Heymann and Rudolph, 1976).

To date, studies concerning the role of NO in the fetal circulation *in vivo* have focused on the pulmonary vasculature. Low NO-activity may account for the high basal fetal pulmonary vascular resistance (Moore, Velvis, Fineman, Soifer and Heymann, 1992) and NO synthesis partly mediates the normal transition of the pulmonary circulation in response to oxygenation at birth (Fineman, Wong, Morin, Wild and Soifer, 1994). In addition there is some preliminary evidence to suggest that NO-synthesis is involved in the fetal cerebral vasodilatation after 6 h of reduced uterine blood flow (McCrabb and Harding, 1994). However the role of NO in the regulation of fetal *systemic* vasculature has not been investigated. Thus the aim of this study was to investigate the part played by NO-synthesis in fetal systemic cardiovascular control during normoxia and hypoxia.

In view of the potential interaction between mechanisms of fetal cardiovascular control [1.5.4] I have also measured plasma [AII], [ACTH] and [cortisol] in samples collected in this Chapter.

Some of these results have previously been published in abstract form (Green, Bennet and Hanson, 1994b and 1995a, see Appendices 9 and 11).

6.2 Methods

6.2.1 Surgical preparation

The 7 fetuses involved in the present Chapter were the same as those presented in Chapter 5. Briefly, fetuses aged between 114 and 121 days gestation were instrumented with femoral and carotid arterial ultrasound flow probes, catheters in a carotid artery, the right brachial vein, a jugular vein, amniotic cavity and the trachea, and stainless-steel electrodes to monitor ECG, diaphragmatic activity and ECoG [see 5.2.1].

Five to 8 days of recovery were allowed prior to experimentation.

6.2.2 Experimental procedure

Measurements

MAP, MVP, tracheal pressure, FHR, CBF, FBF, ECoG activity and diaphragm EMG were recorded continuously onto chart paper (Gould ES1000) and MacLab Chart Software (AD Instruments Pty. Ltd.) using a Macintosh LCIII computer (Apple computers Inc.) [2.4.2].

2-3ml fetal arterial blood was collected prior to the onset of vehicle or L-NAME infusion (time zero), during normoxia (15 and 45 min), hypoxia (75 and 105 min) and recovery (135 and 165 min). Blood was transferred immediately to chilled EDTA tubes and spun at 4°C (3000 rpm) for 10 min. Plasma was then decanted into tubes and stored at -20°C for subsequent hormonal analysis. A further 0.6 ml arterial blood was collected at these times and also at 90 min (hypoxia) for the analysis of blood gases and electrolytes.

Drug Preparation

On the day of experimentation approximately 400mg L-NAME (Sigma Chemical Co. Ltd. UK) was dissolved in sterile saline to give a final concentration of 33mg.ml^{-1}.

The vehicle solution was composed of saline with equimolar NaOH (5 drops per 10ml) [see 5.2.2].

Protocol

In all fetuses, two 3 h protocols were conducted on separate days: day 1, hypoxia with the infusion of vehicle (125±0.8 days gestation); day 2, hypoxia with infusion of L-NAME (127±0.9 days gestation). The hypoxia protocols comprised an initial one hour normoxic control period, followed by one hour fetal isocapnic hypoxia. Ewes were then returned to normoxic gas mixture and measurements continued for one hour (recovery) [2.5.1].

At 0 min an initial bolus of either vehicle (2ml) or L-NAME (20mg in 2ml) was administered via the brachial vein and flushed in with 2ml saline. This was followed by infusion of vehicle (4ml.h^{-1}) or L-NAME (3ml.h^{-1}; 99mg.h^{-1}) for the duration of the 3 h protocol.

Plasma hormonal analysis

Plasma samples taken during vehicle infusion were analysed for [ET-1], [cortisol] and [AII] as described in Chapter 5. Plasma [AII], [cortisol] and [ACTH] was measured in samples collected during L-NAME infusion and are presented in this Chapter. Details of the assay are given in Appendix 5.

6.3 Data analysis

Grouped data are expressed as mean ± S.E.M. Blood composition and cardiovascular measurements for individual fetuses were reduced to summary measures to describe normoxic, hypoxic and recovery hours [2.6.3] as shown by horizontal bars in e.g.

Figure 6.1. Summary measures were then tested using Student's paired t-test to compare hypoxic and recovery periods to normoxia.

Student's paired t-test was used to test rapid transient changes in FHR and in $PaCO_2$ during hypoxia, and to test blood compostition values pre- vs. post-vehicle or L-NAME infusion (0 vs 15 min).

Cardiovascular measurements made prior to the onset of vehicle or L-NAME infusion (-5 to 0 min), were reduced to a summary measure and this was compared by t-test to the subsequent 1 h normoxic period (Table 6.1).

Time spent making breathing movements and in LV-ECoG activity was expressed as a percentage of the hours of normoxia, hypoxia and recovery. Paired t-test was then used to compare hypoxia and recovery to normoxia.

An unpaired t-test was substituted in the event of uneven sample groups and these instances are indicated in the figure legends.

In this chapter venous pressure measurements were available in all fetuses for part of the normoxia and hypoxia hours, and therefore vascular resistances were calculated during these periods from (MAP-MVP) ÷ flow.

Statistical significance was accepted when $P<0.05$.

6.4 Results

6.4.1 Blood gases, electrolytes, glucose and lactate

Arterial oxygenation

During hypoxia there was a fall in PaO_2, SaO_2, O_2ct, total Hb, oxyhaemoglobin and MetHb (oxidised haemoglobin), and a rise in Hct (Figures 6.1 and 6.2) in both vehicle and L-NAME groups. Furthermore there was no difference between vehicle and L-NAME infused groups throughout the course of the protocol. In the recovery period arterial oxygenation returned to pre-hypoxic levels, apart from SaO_2 and oxyhaemoglobin which remained slightly lower than normoxic values in the vehicle infused group.

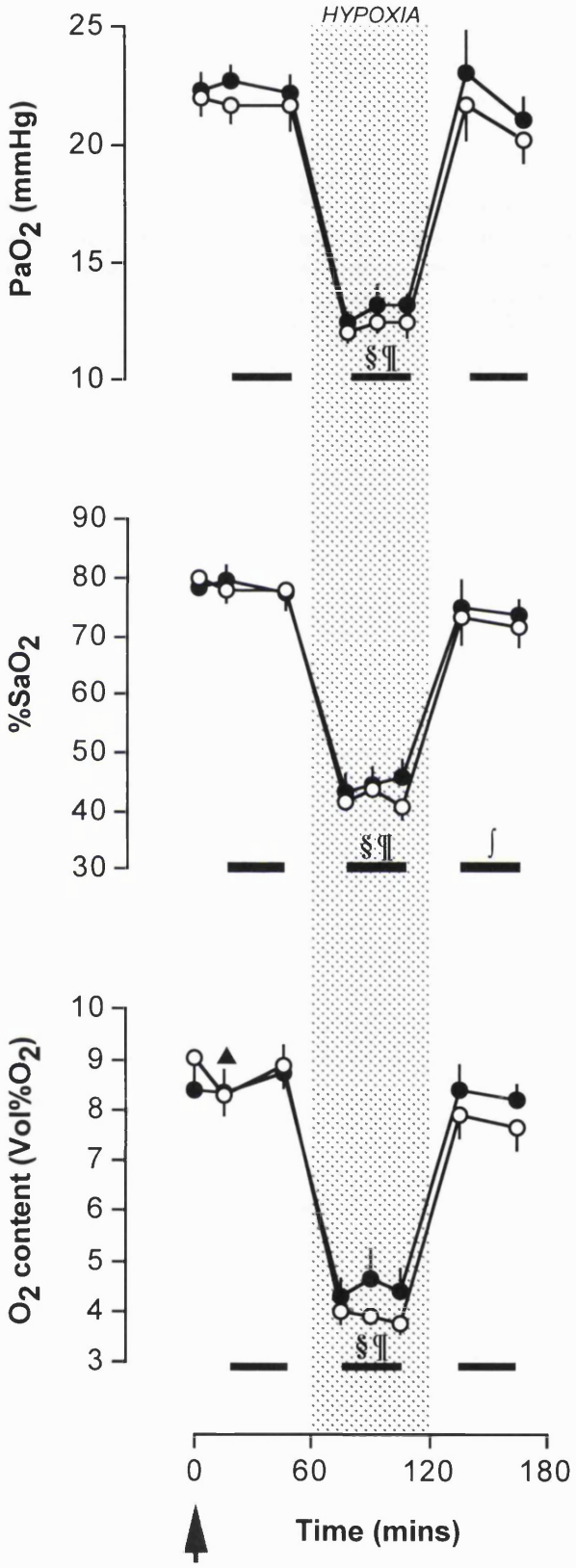


Figure 6.1 PaO_2, $\%SaO_2$ and O_2 content during normoxia, hypoxia (shaded area) and recovery with vehicle (○) and L-NAME (●) infusion (n=7). The first sample was taken just prior to the onset of infusion at time zero (arrow). The horizontal bars show the time period over which values were reduced to summary measures. ∫P<0.05 and §P<0.01, significantly different from normoxia in vehicle group by paired t-test; ¶P<0.01, significantly different from normoxia in L-NAME group by paired t-test; ▲P<0.01, significantly different from pre-vehicle infusion by paired t-test.

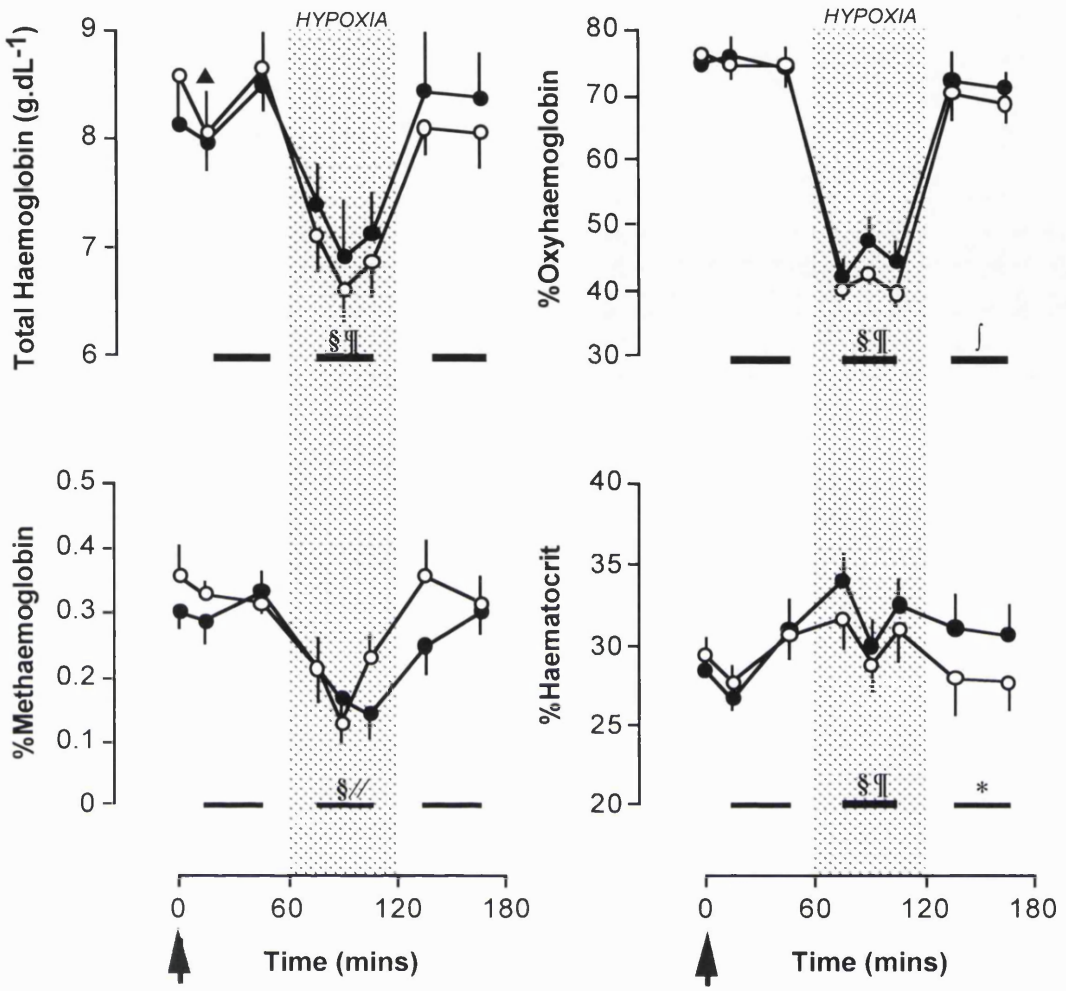


Figure 6.2 Total haemoglobin, %oxyhaemoglobin, %methaemoglobin and %haematocrit during normoxia, hypoxia (shaded area) and recovery with vehicle (○) and L-NAME (●) infusion (n=7). The first sample was taken just prior to the onset of infusion at time zero (arrow). The horizontal bars show the time period over which values were reduced to summary measures. ∫P<0.05 and §P<0.01, significantly different from normoxia in vehicle group by paired t-test; ¶P<0.01, significantly different from normoxia in L-NAME group by paired t-test; ▲P<0.01, significantly different from pre-vehicle infusion by paired t-test; *P<0.01 L-NAME significantly different from vehicle group by paired t-test.

Acid-base status

At 15 min normoxia $PaCO_2$ and HCO_3^- were lower than pre-infusion values in both vehicle and L-NAME groups (Figure 6.3). During hypoxia pH, HCO_3^- and base excess fell, and remained lower throughout the subsequent recovery period in both vehicle and L-NAME infused groups (Figure 6.3). There was a transient fall in $PaCO_2$ at 90 min (hypoxia) in the vehicle group, however over the hour of hypoxia as a whole there was no significant change from normoxia.

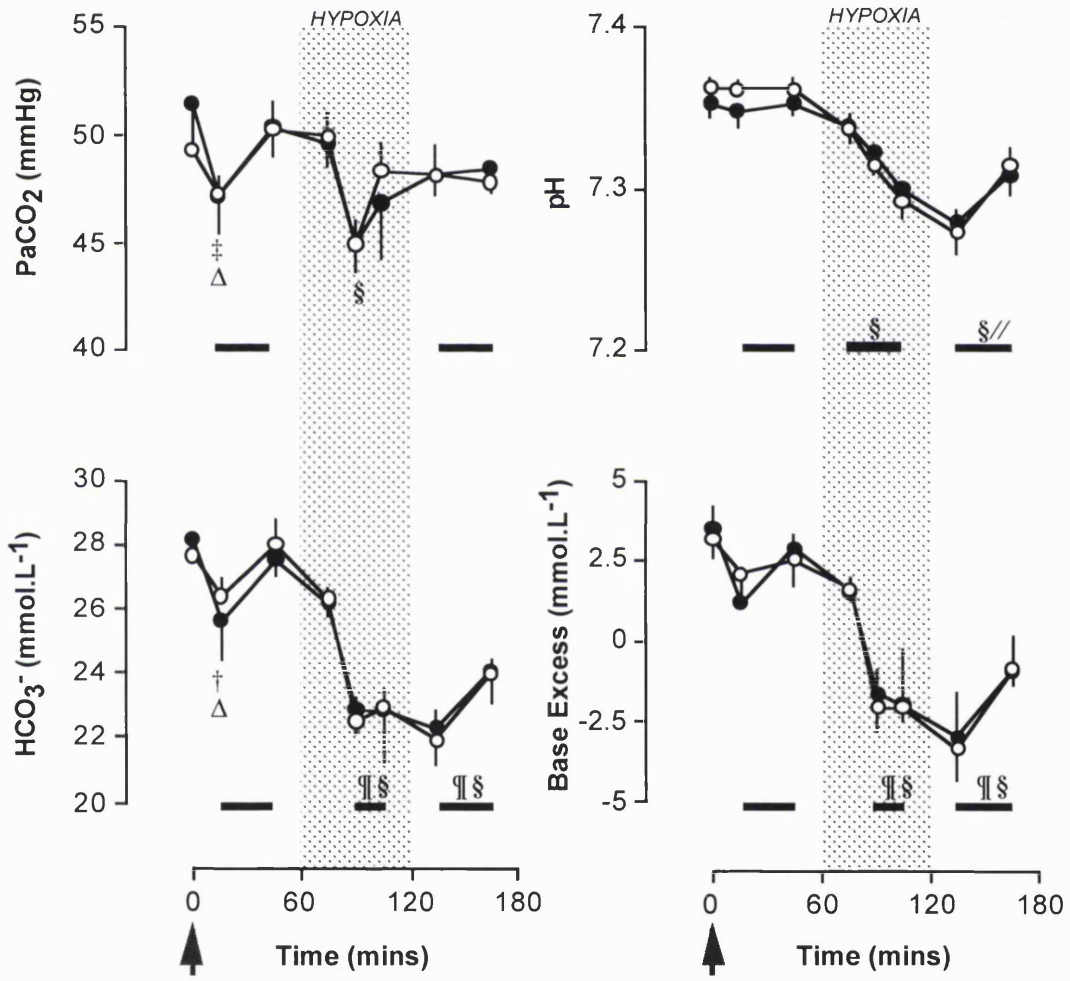


Figure 6.3 $PaCO_2$, pH, HCO_3^- and base excess during normoxia, hypoxia (shaded area) and recovery with vehicle (○) and L-NAME (●) infusion (n=7). The first sample was taken just prior to the onset of infusion at time zero (arrow). The horizontal bars show the time period over which values were reduced to summary measures. §P<0.01, significantly different from normoxia in vehicle group by paired t-test; ΔP<0.05, significantly different from pre-vehicle infusion by paired t-test; †P<0,05 and ‡P<0.01, significantly different from pre-L-NAME infusion by paired t-test; //P<0.05 and ¶P<0.01, significantly different from normoxia in L-NAME group by paired t-test.

Lactate and Glucose

Lactate rose during hypoxia and remained elevated during the recovery period in both vehicle and L-NAME groups (Figure 6.4). However during hypoxia lactate levels were lower with L-NAME than with vehicle infusion.

During hypoxia, glucose rose with vehicle but not L-NAME infusion, and glucose was higher throughout hypoxia with vehicle than with L-NAME infusion (Figure 6.4).

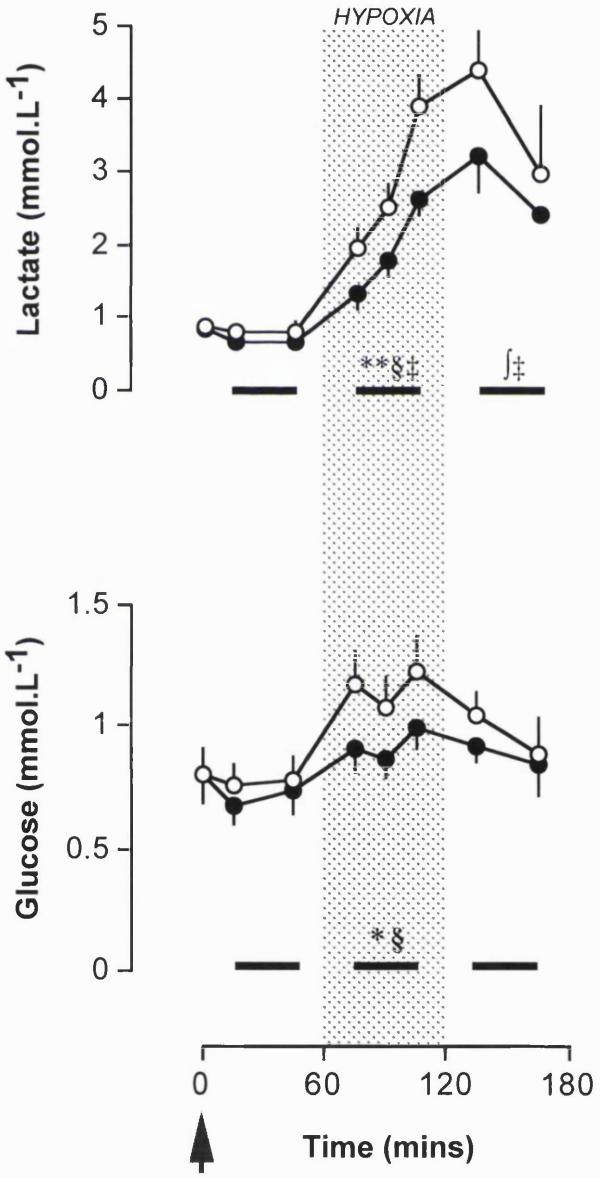


Figure 6.4 Lactate and glucose during normoxia, hypoxia (shaded area) and recovery with vehicle (○) and L-NAME (●) infusion (n=7). The first sample was taken just prior to the onset of infusion at time zero (arrow). The horizontal bars show the time period over which values were reduced to summary measures. §P<0.01, significantly different from normoxia in vehicle group by paired t-test; ∫P<0.01, significantly different from normoxia in vehicle group by un-paired t-test; ‡P<0.01, significantly different from normoxia in L-NAME group by un-paired t-test; *P<0.05 and **P<0.01 L-NAME significantly different from vehicle group by paired t-test.

Sodium and potassium

There was a rise in plasma sodium following the onset of L-NAME but not vehicle infusion (Figure 6.5). However sodium did not change from normoxic levels throughout the protocol with either vehicle or L-NAME infusion.

Plasma potassium rose during hypoxia with both vehicle and L-NAME infusion (Figure 6.5). While levels remained elevated in the recovery period this was only significantly different from normoxia in the vehicle group.

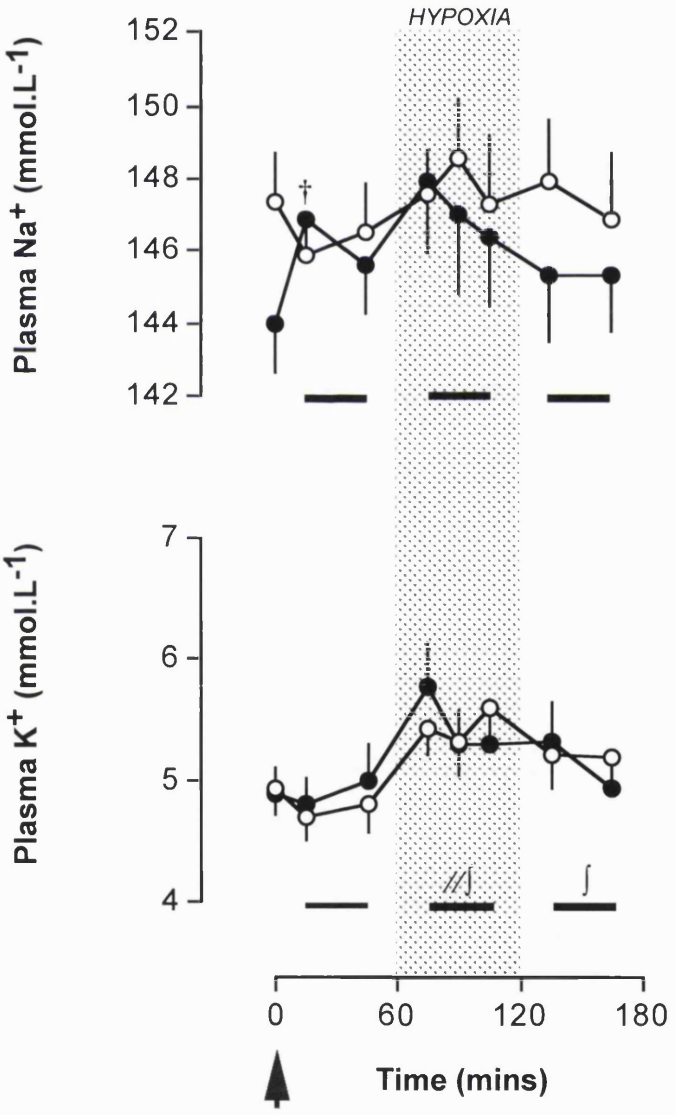


Figure 6.5 Plasma Na^+ and K^+ during normoxia, hypoxia (shaded area) and recovery with vehicle (○) and L-NAME (●) infusion (n=7). The first sample was taken just prior to the onset of infusion at time zero (arrow). The horizontal bars show the time period over which values were reduced to summary measures. †P<0,05, significantly different from pre-L-NAME infusion by paired t-test; //P<0.05, significantly different from normoxia in L-NAME group by paired t-test; ∫P<0.05, significantly different from normoxia in vehicle group by paired t-test.

6.4.2 Plasma angiotensin II and ACTH/cortisol

During normoxia L-NAME caused a fall in plasma [AII] (Figure 6.6). From regression analysis there was no relationship between the percent change in MAP and percent change [AII] after 45 min L-NAME (P=0.60, F=0.33, R^2=0.08). After 15 min hypoxia [AII] rose in the vehicle group whereas there was a significant fall in [AII] with L-NAME. Indeed plasma [AII] with L-NAME infusion remained below that with vehicle infusion for the duration of hypoxia, and after 15 min recovery (Figure 6.6).

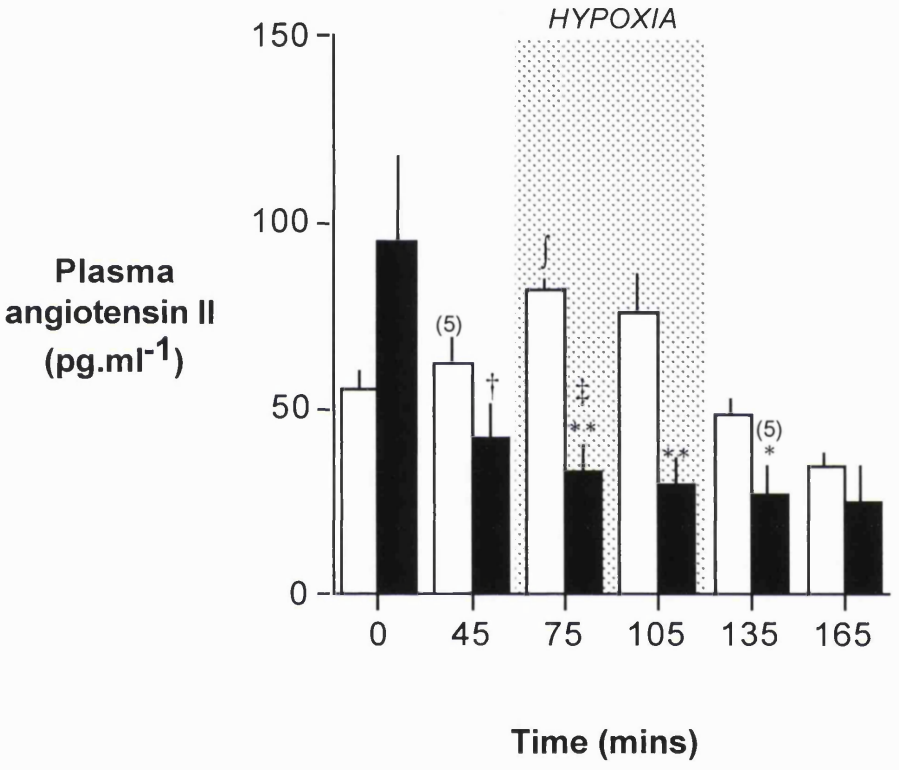


Figure 6.6 Plasma [AII] in response to 1 h hypoxia (shaded area) pre- (time 0) and during vehicle (open bars) and L-NAME (filled bars) infusion (n=6, unless otherwise indicated by bracketed number). Significantly different from 45 min (P<0.0125) by paired (‡) and unpaired (∫) t-test; †P<0.0125, significantly different from 0 min by paired t-test; *P<0.05 (unpaired t-test) and **P<0.01 (paired t-test), significantly different from vehicle group.

Plasma [ACTH] was elevated from normoxia (45 min) during hypoxia and recovery to a similar extent in vehicle and L-NAME groups (Figure 6.7). With both vehicle and L-NAME infusion plasma [cortisol] rose during hypoxia, although this only reached significance in the L-NAME group. During recovery [cortisol] remained elevated from normoxia in the L-NAME group (165 min. see Figure 6.7).

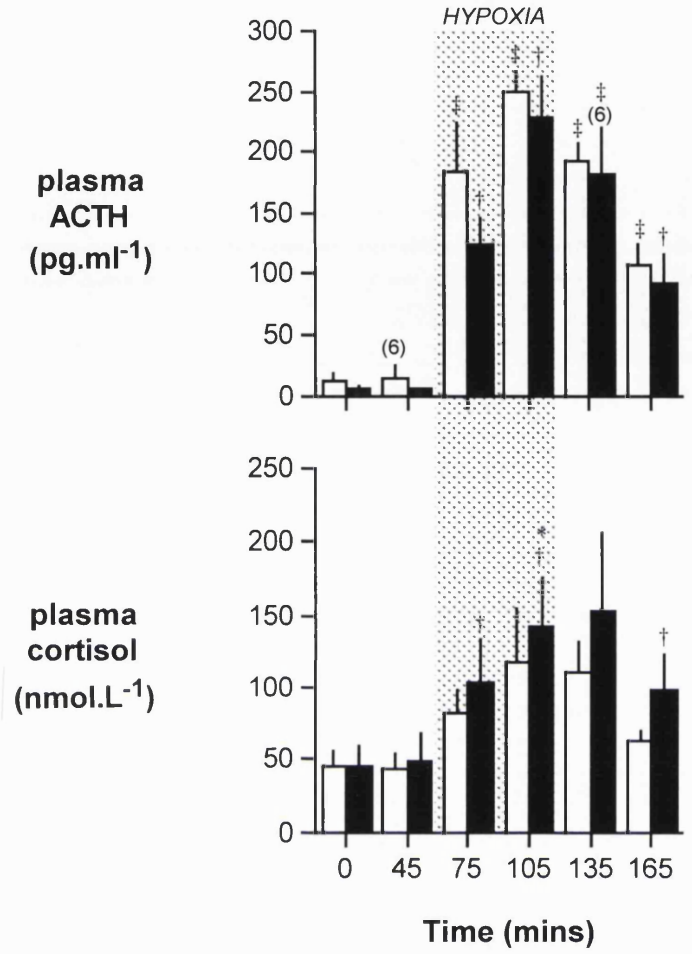


Figure 6.7 Plasma [ACTH] and [cortisol] in response to 1 h hypoxia (shaded area) pre- (time 0) and during vehicle and L-NAME infusion (n=7, unless otherwise indicated by bracketed number). † (paired t-test) and ‡ (unpaired t-test) $P<0.0125$, significantly different from 45 min (normoxia) by paired t-test; *$P<0.05$, significantly different from vehicle group by paired t-test.

6.4.3 Fetal heart rate

During normoxia FHR fell from pre-infusion levels in the vehicle and L-NAME groups although the fall was of a greater level of significance in the L-NAME group, and during normoxia FHR was lower with L-NAME than with vehicle infusion (Table 6.1 and Figure 6.8). At the onset of hypoxia there was a rapid fall in FHR in both vehicle and L-NAME groups, however the magnitude of the fall was less with L-NAME infusion (vehicle vs. L-NAME group: $P<0.05$, n=7 by paired t-test). In both groups FHR returned to pre-hypoxic levels as hypoxia proceeded. During recovery, the rebound tachycardia observed in the vehicle-infused group was absent in the L-NAME group.

6.4.4 Mean arterial pressure

There was a large rise in MAP after the infusion of L-NAME, and pressure was higher than with the infusion of vehicle throughout the normoxic hour (Table 6.1 and Figure 6.8). During hypoxia MAP rose significantly with vehicle but not L-NAME infusion.

Thus MAP was similar with L-NAME and vehicle infusion during hypoxia. During recovery pressure remained elevated compared to normoxia in the vehicle group (Figure 6.8).

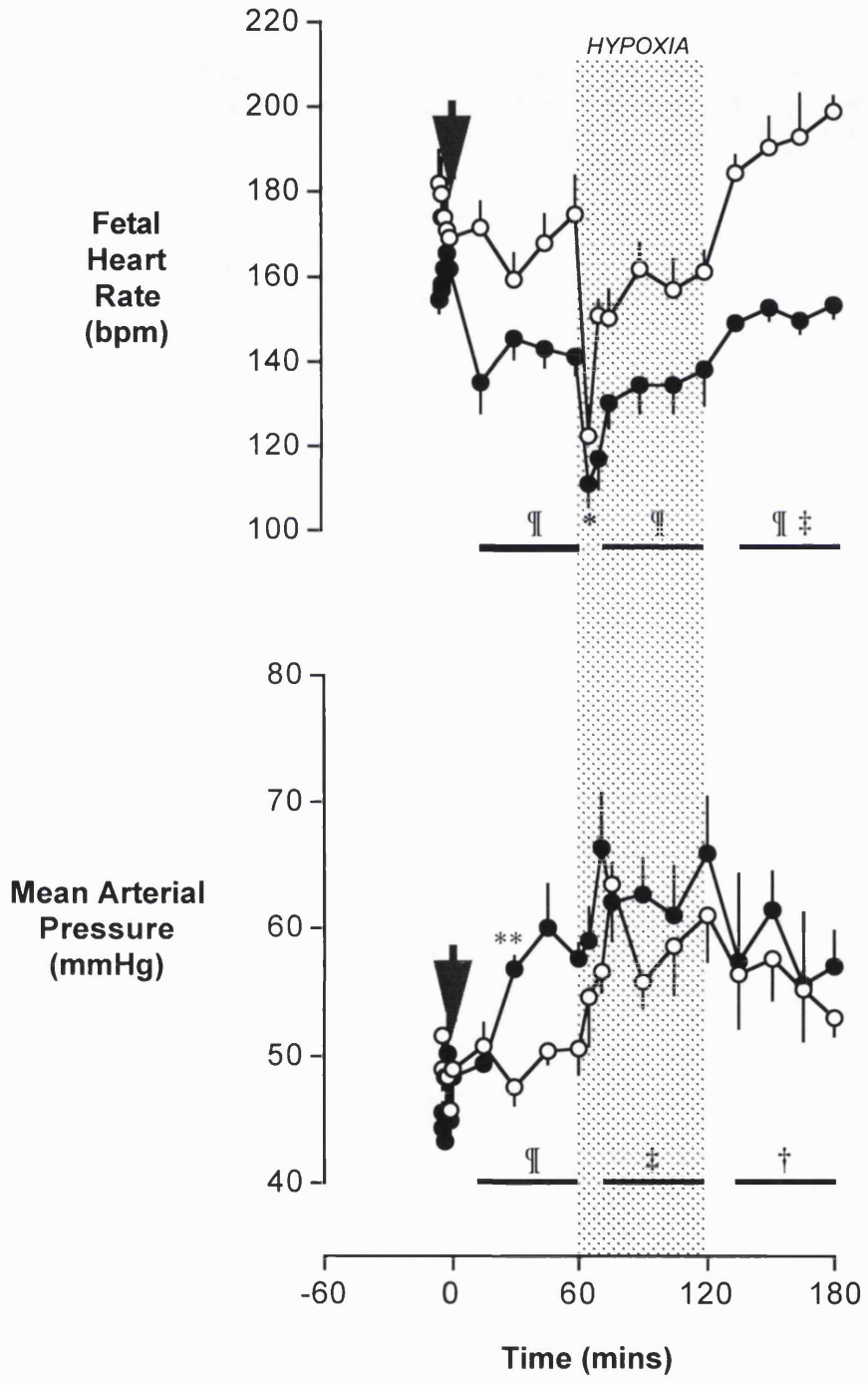


Figure 6.8 FHR (n=7) and MAP (n=6) responses to 1 h hypoxia (shaded area) during vehicle (○) and L-NAME infusion (●). Arrows show the onset of infusion at time zero. Values are shown as mean±S.E.M. †P<0.05 and ‡P<0.01, significantly different from normoxia in vehicle group, by paired t-test; ¶P<0.01, vehicle significantly different from L-NAME group by paired t-test; *P<0.05, normoxia (60 min) vs. 65 min, by paired t-test in vehicle and L-NAME groups; **P<0.01, significantly different from 0 min in L-NAME group by paired t-test. Summary measures of pre-infusion onset 1 min values are compared to post infusion values in Table 6.1.

6.4.5 Blood flow

Femoral bed

There was a tendency for FBF to fall after L-NAME infusion (Table 6.1). While this fall did not reach significance, flow was significantly lower throughout normoxia with L-NAME than with the infusion of vehicle (Figure 6.9). During hypoxia FBF fell to a similar extent in both groups. Flow returned to pre-hypoxic levels during the recovery period but was lower with the infusion of L-NAME than with the infusion of vehicle.

Carotid bed

There was a large fall in CBF after 45 min L-NAME infusion (Figure 6.9), and flow remained lower than with vehicle infusion throughout normoxia (Table 6.1 and Figure 6.9). In the vehicle infused group CBF rose during hypoxia but with the infusion of L-NAME no significant rise occurred (Figure 6.9).

	VEHICLE		L-NAME	
	PRE	POST	PRE	POST
FHR (n=7)	174±5.8	168±5.6$^{\int}$	160±3.7‡	141±4.6†§
MAP (n=6)	48.97±1.89	49.82±1.37	41.72±1.74†	57.67±1.18§‡
FBF (n=6)	49.60±3.12	52.72±4.09	45.14±5.73	41.26±6.35†
CBF (n=6)	78.48±9.86	70.50±7.30	70.88±9.87	53.69±4.85†

Table 6.1 Cardiovascular responses to vehicle and L-NAME infusion during normoxia. Values are given as mean±S.E.M. of summary measures of data before the onset (PRE: -5 to 0 min) and during the first hour of (POST: 15-60 min) infusion. $\int$P<0.05 and §P<0.01, significantly different from pre-infusion by paired t-test; †P<0.05 and ‡P<0.01, significantly different from vehicle group by paired t-test.

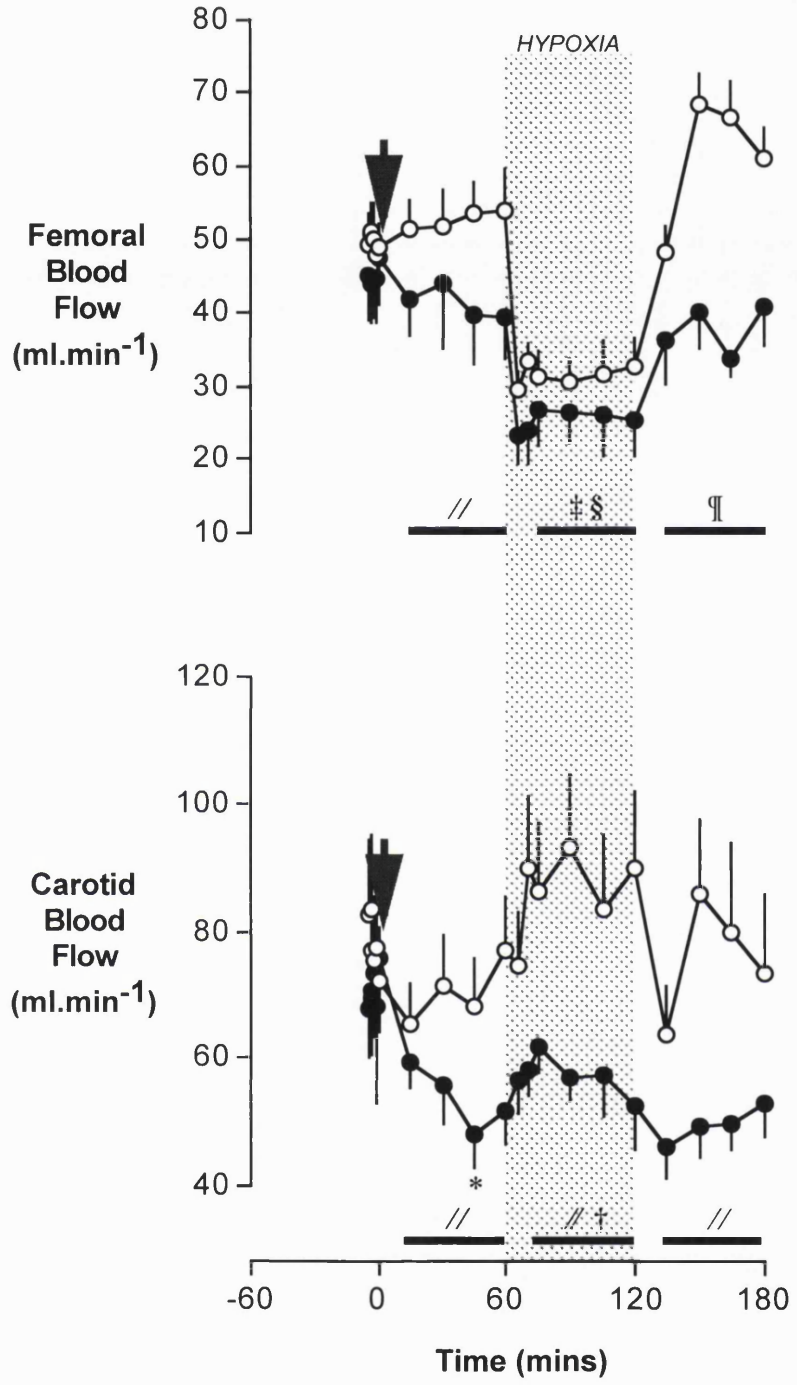


Figure 6.9 Femoral (n=6) and carotid (n=6) blood flow responses to 1 h hypoxia (shaded area) with vehicle (○) and L-NAME infusion (●). Arrows denote the onset of infusion at time zero. Values are shown as mean±S.E.M. †P<0.05 and ‡P<0.01, significantly different from normoxia in vehicle group by paired t-test; ‖P<0.05 and ¶P<0.01, vehicle significantly different from L-NAME group, by paired t-test; §P<0.01, significantly different from normoxia in L-NAME group by paired t-test; *P<0.05, significantly different from 0 min in L-NAME group. Summary measures of pre-infusion onset 1 min values are compared to post infusion values in Table 6.1.

6.4.6 Vascular Resistance

Femoral bed

During normoxia FVR was greater with the infusion of L-NAME than with the infusion of vehicle (Figure 6.10). During hypoxia resistance rose to a similar level with both vehicle and L-NAME infusion.

Carotid bed

During normoxia and hypoxia CVR was greater with L-NAME than with vehicle infusion (Figure 6.10). During hypoxia there was a small but significant fall in CVR with the infusion of vehicle but not with the infusion if L-NAME.

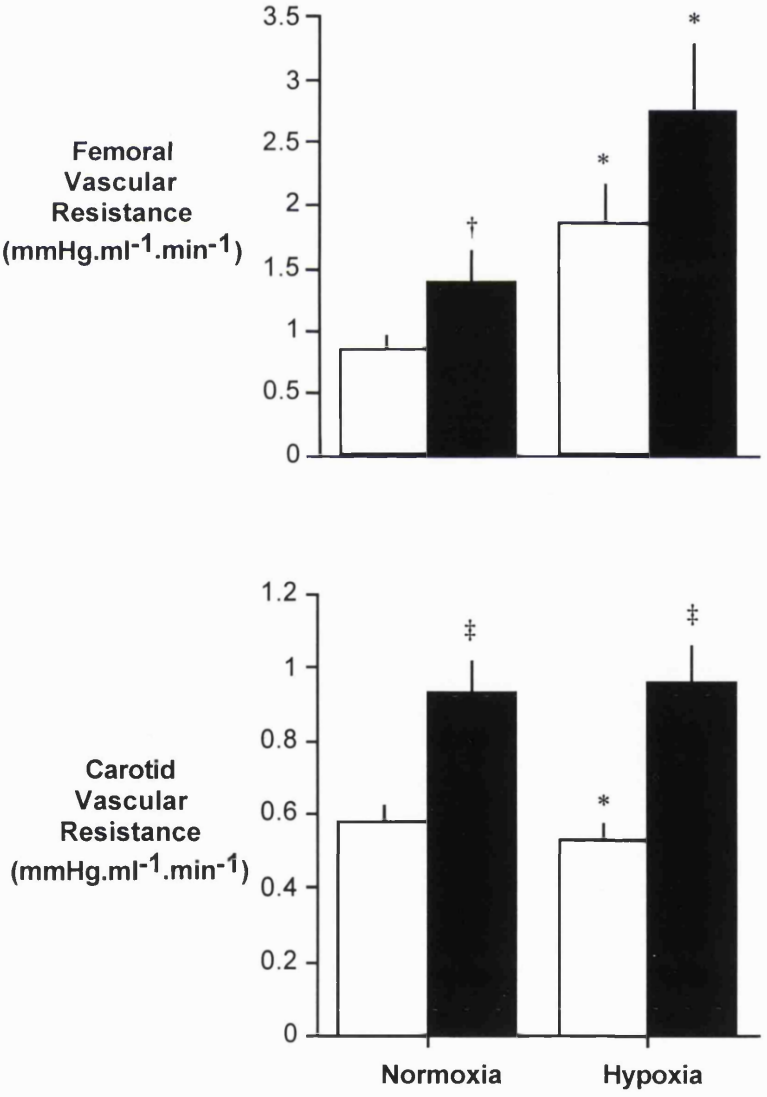


Figure 6.10 Femoral (n=5) and carotid (n=5) vascular resistance responses to 1 h acute hypoxia (shaded area) with vehicle (open bar) and L-NAME (filled bars) infusion. Vascular resistance was not calculated during recovery and pre-infusion periods due to absence of venous pressure readings. Values are shown as mean±S.E.M. †P,0.05 and ‡P<0.01, significantly different from vehicle group, by paired t-test; *P<0.05, significantly different from normoxia, by paired t-test.

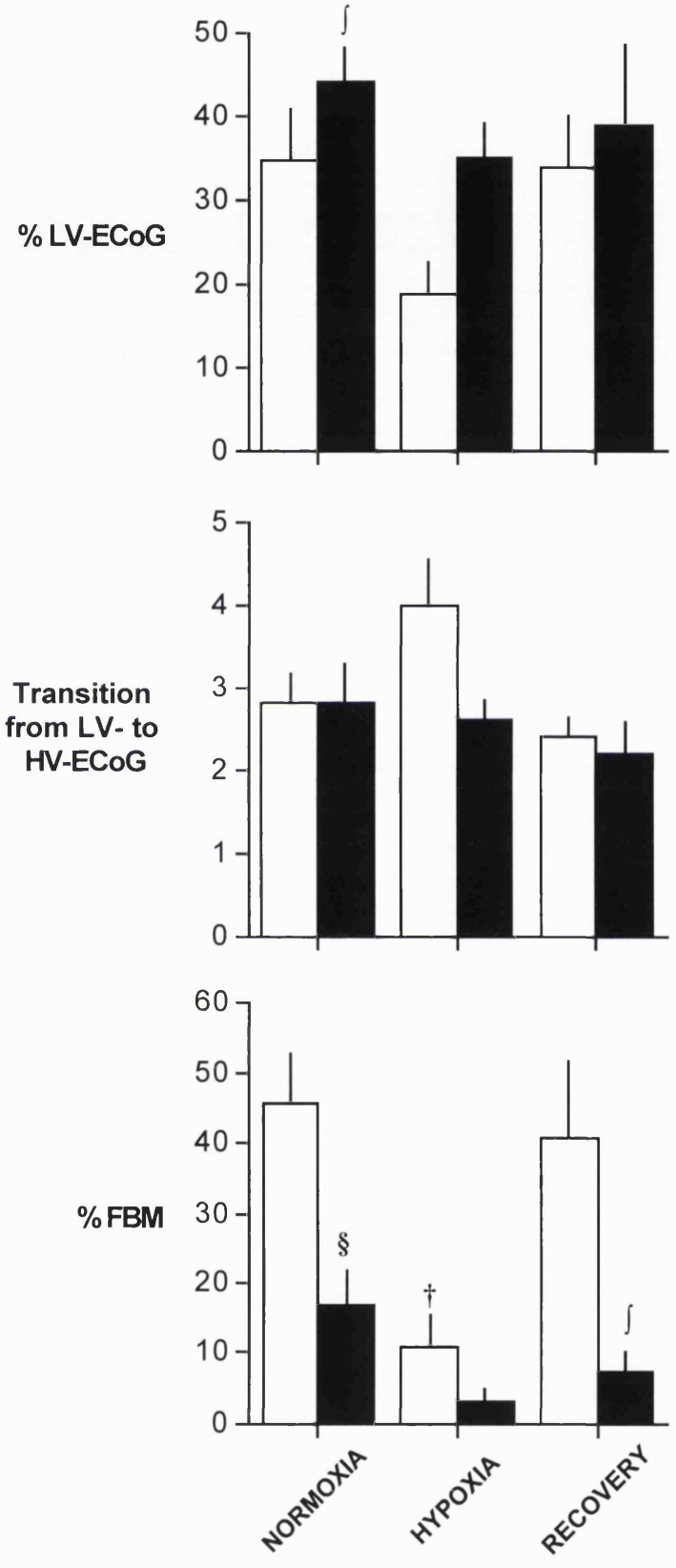


Figure 6.11 Percent LV-ECoG (n=5), number of transitions from LV- to HV-ECoG (n=5) and percent FBM's (n=4) during normoxia, hypoxia and recovery in vehicle (open bars) and L-NAME (closed bars) infused groups. Data are expressed as mean±S.E.M. †P<0.05, significantly different from normoxia by paired t-test; ∫P<0.05 and §P<0.01, L-NAME significantly different from vehicle group by paired t-test.

6.4.7 ECoG and Fetal Breathing Movements

During normoxia the percentage of LV-ECoG was greater with L-NAME than with vehicle infusion. The percentage of time spent in LV-ECoG tended to be reduced, and the number of transitions from LV- to HV-ECoG increased, during hypoxia in the vehicle infused group, although this did not reach significance (Figure 6.11). With vehicle infusion the incidence of FBMs was lower during hypoxia and returned to normoxic levels in the subsequent recovery period. In the L-NAME group the incidence of FBMs did not fall significantly during hypoxia. Furthermore during normoxia and recovery the incidence of FBMs was lower with L-NAME than with vehicle infusion, but not during hypoxia.

6.5 Discussion

The results in this Chapter show that NO-synthesis alters basal FHR, MAP, CBF, FBF, FVR, CVR, ECoG and FBMs. The modulation of FHR persists during hypoxia and recovery. In addition to this modulatory role, an increase in NO-synthesis mediates the rise in CBF and fall in CVR in the fetus during hypoxia, as this is prevented by L-NAME infusion. I have also shown that the rise in plasma [AII] during hypoxia with vehicle, is absent with L-NAME infusion.

Methodological Considerations

The use of L-NAME

To investigate the role of NO *in vivo* some studies have used exogenously administered NO, either by inhalation of NO (Roberts *et al.*, 1993) or by administration of NO-donor compounds such as sodium nitroprusside (SNP, see Chapter 7), which acts by increasing concentrations of cGMP. Other studies have exploited the fact that NO is rapidly inactivated by superoxide anions (O_2^-), by using substances such as methylene blue which generate superoxide (Iwamoto, Yoshinaga, Yang, Krasney and Krasney, 1992). The approach taken in the present study was to reduce the production of NO by inhibition of NOS activity.

A variety of NO-synthase inhibitors exist which are substituted analogues of L-arginine (Rees, Palmer, Schulz, Hodson and Moncada, 1990) and inhibit both constitutive and inducible NOS isoforms. Widely used inhibitors include L-NAME, NOLA and L-NMMA. L-NAME is easy to dissolve in saline and has been shown to produce more potent and longer lasting effects than L-NMMA (Gardiner, Compton, Bennett, Palmer and Moncada, 1990a; Rees, Palmer, Schulz, Hodson and Moncada, 1990). There have

been numerous studies in which the function of NOS *in vivo* has been investigated using L-NAME, although the doses used in these studies have been wide ranging (e.g. *ca.* $0.75mg.h^{-1}$ infusion by Gardiner, Compton, Kemp & Bennett, 1991 to conscious rats; *ca.* $3mg.min^{-1}$ infusion by White, Drew, Gurden, Penny, Roach and Watts, 1993 to anaesthetised adult dogs; $35mg.kg^{-1}$ i.v. bolus by Fernandez, Garcia, Garcia-Villalon, Monge, Gomez and Dieguez, 1993 to conscious goats). The dose of L-NAME used in the present study was similar to chronic conscious adult rabbit studies in which a rise in MAP and a fall in heart rate was observed (Goyer, Bui, Chou, Evans, Keil and Reid, 1994; Reid, Bui and Chou, 1994).

There are a number of potential approaches to assessing the degree of NOS activity *in vivo*: 1) measurement of NO produced; 2) measurement of the products of the metabolism of NO, namely nitrate levels, either in plasma or urine samples; or 3) assessment of the cardiovascular response to an endothelium-dependent vasodilator such as ACh. Of these possible approaches the first would be the ideal, however while NO-electrodes have been developed for *in vitro* use (Ichimori, Ishida, Fukahori, Nakazawa and Murakami, 1994) a number of practical problems will have to be resolved before their use becomes viable in chronic *in vivo* models. The other two options are both indirect parameters of endothelial function: Nitrate is a breakdown product of NO and, while its levels will give a global impression of NOS activity, they will not provide much insight to NOS activity at a local level; ACh binds to endothelial cell muscarinic receptors to activate the phosphoinositide cycle and generate Ca^{2+}, which in turn stimulates NOS (Bredt and Snyder, 1994). NOS-inhibition has been shown to inhibit ACh-induced depressor responses *in vivo* (Rees, Palmer, Schulz, Hodson and Moncada, 1990), others have shown that a substantial response to ACh remains following NOS-inhibition in some vascular beds (Gardiner, Compton, Kemp and Bennett, 1990; McCarthy, Woolfson, Raju and Poston, 1993). They suggest that the interpretation of ACh action may be confounded by its endothelium-independent constrictory effects (Rongen, Smits and Thien, 1994), or that its actions are mediated in part by an endothelial-derived hyperpolarizing factor (McCarthy *et al.*, 1993). Thus in this Chapter the response to ACh was not used to determine NOS activity after L-NAME because of the complexity of interpreting the results.

Animals involved in study

The seven fetuses initially involved in the present study were the same fetuses on which results were presented in Chapter 5, thus the vehicle-infused groups are identical. The L-NAME-infusion protocol was carried out on a day subsequent to the FR139317-infusion protocol [see Chapter 5].

MAP data was not available in one fetus during L-NAME infusion due to mechanical problems with the equipment. Therefore for the sake of statistical comparison this fetus was not included in grouped MAP data.

CBF data was not available in one fetus of the vehicle group due to a bad electrical connection within the flow probe, therefore measurements taken on the subsequent L-NAME infused day were not included in the analysis. FBF was considered to be below normal physiological levels (Giussani, Spencer, Moore, Bennet and Hanson, 1993) in the same fetus and it was therefore not included in the analysis. The reason for low flow was not obvious but may have been due to either partial occlusion of the vessel by the flow probe because that the signal strength of the probe itself was low.

FBMs data were not obtained in three fetuses, and ECoG in 2 fetuses, due to mechanical problems with the equipment.

Nitric oxide synthesis modulates baseline cardiovascular tone

The administration of L-NAME during normoxia in the present study caused a large rise in MAP and raised FVR. This suggests that NO-synthesis is involved in the control of resting peripheral blood flow and pressure, in accordance with numerous adult studies (Aisaka, Gross, Griffith and Levi, 1989, Rees, Palmer and Moncada, 1989; Gardiner, Compton, Bennett, Palmer and Moncada, 1990a/b; Richard, Gosgnach, Drieu la Rochelle, Giudicelli and Berdeaux, 1991; White, Drew, Gurden, Penny, Roach and Watts, 1993; Goyer, Bui, Chou, Evans, Keil and Reid, 1994). In addition previous fetal studies have implicated NOS in the maintenance of baseline systemic and pulmonary arterial pressures (Moore, Velvis, Fineman, Soifer and Heymann, 1992; Tiktinsky, Cummings and Morin, 1992), and in pulmonary (Fineman, Heymann and Soifer, 1991; Moore *et al.*, 1992; Kinsella, Ivy and Abman, 1994) and renal (Bogaert, Kogan and Mevorach, 1993) vascular resistance. Furthermore *in vitro* cGMP measurements imply a basal production of NO from the systemic vasculature (mesenteric) of the late-gestation sheep fetus (Shaul, Farrar and Zellers, 1992). It has been suggested that NO regulates blood pressure partly through a direct action in the CNS rather than through arterial baroreflex mechanisms (Jimbo, Suzuki, Ichikawa, Kumagai, Nishizawa and Saruta, 1994).

L-NAME infusion during normoxia produced a fall in heart rate in accordance with adult studies (Aisaka *et al.*, 1989; Fernandez, Garcia, Garcia-Villalon, Monge, Gomez and Dieguez, 1993; Reid, Bui and Chou, 1994) which suggests a role for NO synthesis in the regulation of resting FHR. While the mechanisms involved are unknown, one possibility is that the L-NAME induced bradycardia is a reflex response subsequent to a

rise in MAP (Rees, Palmer and Moncada, 1989). However in the present study the fall in FHR occurred before a rise in MAP suggesting that it is not a baroreflex response. Moreover NOS inhibition has been shown to reduce the sensitivity of the baroreflex (Scrogin, Veelken and Luft, 1994). Other possible explanations for the bradycardia observed in this study include inhibition of sympathetic activity, increased vagal efferent activity or a direct effect on the sino-atrial node. Of these, the first explanation seems least likely since there is evidence in the adult rat (Cunha, Cabral and Vasquez, 1993), although not in the human (Hansen, Jacobsen and Victor, 1994), to suggest that tonic NO production restrains sympathetic drive to the heart. There is evidence to suggest that the positive chronotropic effects of adrenaline are transduced, in part, by NO (Gardiner, Kemp and Bennett, 1991) and that NOS inhibition suppresses heart rate responses to β-adrenergic stimulation (Reid, Bui and Chou, 1994). In addition NOS has been located the coronary vessels and in nerve fibres projecting to pacemaker regions of the adult rat and guinea-pig (Klimaschewski *et al.*, 1992) and NO has been shown to be vital for cholinergic inhibition of calcium currents in mammalian cardiac pacemaker cells *in vitro* (Han, Shimoni and Giles, 1994). However studies on isolated atrial preparations do not support a role for basal NO release in the control of resting heart rate (Kennedy, Hicks, Brian and Seifen, 1994).

Pulmonary vascular smooth muscle is responsive to endothelium-independent stimuli such as inhaled NO from early in gestation (115 days gestation: Kinsella, Ivy and Abman, 1994) and there is a marked developmental increase in basal pulmonary NO activity after 125-135 days gestation in the fetal sheep (Shaul, Farrar and Magness, 1993). However this has not been fully investigated in the fetal systemic circulation. The present study suggests that there is basal NO activity of the systemic vascular endothelium of fetuses aged between 119 and 126 days gestation. In light of this, the effect of L-NAME on MAP, FHR and peripheral blood flow in normoxia is of particular interest in terms of vascular development in the fetus. NO may play a part in setting a basal level of, and maturational changes in, vascular tone, MAP and heart rate in the perinatal period.

The mechanisms by which NO and guanylate cyclase produce relaxation have been shown to be fully functional in cerebral arteries of the term fetus (Pearce and Longo, 1991). Indeed L-NAME has been shown to contract cerebral vessels of both pre-term (105 days gestation) and late-gestation (125-130 days gestation) ovine fetuses *in vitro* (Wagerle, Moliken and Russo, 1995). In the present study we have measured CBF as an estimate of cerebral blood flow according to data from Van Bel *et al.* (1994) [see 1.4.7]. Following the administration of L-NAME in normoxia there was a large fall in CBF which suggests a role for NO-synthesis in the regulation of fetal resting CBF in agreement with observations in the adult goat (Fernandez, Garcia, Garcia-Villalon,

Monge, Gomez and Dieguez, 1993), dog (McPherson, Koehler and Traystman, 1994) and rat (Kozniewska, Oseka and Stys, 1992; Macrae, Dawson, Norrie and McCulloch, 1993; Pelligrino, Koenig and Albrecht, 1993). The large rise in CVR, in conjunction with the rise in MAP, produced a fall in CBF. This rise in CVR could arguably be an autoregulatory response to the rise in MAP, however if this had been the case CBF would have to have remained constant, but instead it showed a large fall. On the other hand, if autoregulation were disturbed then CBF would be expected to increase and not decrease as the results in this Chapter show. Thus these data point towards a role for NO in the regulation of CBF, and probably of CVR, during normoxia in agreement with studies on rats (Kozniewska *et al.*, 1992; Prado, Watson, Kuluz and Dietrich, 1992; Meei Wei, Weiss, Sinha and Chi, 1993; Prado, Watson and Wester, 1993) and goats (Dieguez, Garcia, Fernandez, Garcia-Villalon, Monge and Gomez, 1993), but in contrast to data of Buchanan and Phillis (1993) who saw no effect of NOS inhibition on cerebral blood flow despite a rise in MAP in the adult rat. There is evidence to suggest a degree of regional heterogeneity in the response of resting cerebral blood flow and vascular resistance to NOS-inhibition in the adult, with greater reductions in flow in the hypothalamus, pituitary and cerebellum. These findings correspond to the greater NOS activity found in the cerebellum and hypothalamus (Forstermann, Gorsky, Pollock, Schmidt, Heller and Murad, 1990; Kovach, Szabo, Benyo, Csaki, Greenberg and Reivich, 1992). However a regionally *homogenous* role for NO has been observed by others (Tanaka *et al.*, 1991; for review see Iadecola, Pelligrino, Moskowitz and Lassen, 1994).

Nitric oxide is inactivated by red blood cells, with the production of nitrate and co-production of methaemoglobin, either by NO binding to the haem ring of deoxygenated haemoglobin to form nitrosylhaemoglobin (subsequently metabolised to methaemoglobin), or by direct oxidation of oxyhaemoglobin to methaemoglobin: the metabolism route would depend on the proportion of oxygenated haemoglobin in the blood (Wennmalm, Benthin and Petersson, 1992). Therefore, in the present study it might have been expected that circulating levels of methaemoglobin would be lower and oxyhaemoglobin be higher with NOS-inhibition than with the infusion of vehicle, however there was no difference between the two groups throughout the protocol.

During normoxia the incidence of FBMs was lower and percentage time spent in LV-ECoG was higher with L-NAME than with vehicle infusion. These findings suggest that NO is involved in the tonic regulation of FBMs. While it was beyond the scope of this study to elucidate the mechanism by which L-NAME exerted this effect, it is possible that NO plays a part in the regulation of FBMs at the level of the CNS [1.6.1].

Nitric Oxide Synthesis mediates the rise in CBF during Hypoxia

There is a degree of controversy as to whether NO-synthesis is stimulated (Pohl and Busse, 1989; Tenney, 1990) or inhibited (Shaul, Farrar and Zellers, 1992; McQueston, Cornfield, McMurtry and Abman, 1993) by hypoxia. This uncertainty appears to stem from the paradox that while NOS requires O_2 as a substrate, the survival of NO is enhanced in hypoxia due to reduced O_2 radical production. Other stimuli to NO release include shear stress (Smiesko and Johnson, 1993), ACh and Ca^{2+}-ATPase (Busse, Mulsch, Fleming and Hecker, 1993). While measurements of NOS activity were not made in the present study, the cardiovascular responses to hypoxia with L-NAME infusion, in particular those of the carotid vascular bed (see below), do not support an inhibitory action of hypoxia on NO production.

At rest, fetal oxidative metabolism of glucose and lactate accounts for about 50% of fetal O_2 consumption (Hay, Myers, Sparks, Wilkening, Meschia and Battaglia, 1983). Under anaerobic conditions however, the only means of cells deriving energy is by glycolysis of which lactate is the end-product. Fetal plasma lactate is already known to rise during 24 h of mild-hypoxia (PaO_2 lowered by ca. 5mmHg) (Towell, Figueroa, Markowitz, Elias and Nathanielsz, 1987) and 1 h acute hypoxia (9% O_2: Jones and Ritchie, 1983). While it is not possible to state categorically where this rise in lactate originates, one strong possibility is that the fall in blood flow to the periphery and other organs was sufficient to trigger anaerobic metabolism and thus the production of lactate. It is also possible however that placental lactate production could contribute to these raised levels. Plasma glucose is already known to rise during acute hypoxia and is likely to be caused by reduced peripheral glucose consumption as well as some fetal glucose production (Jones, Ritchie and Walker, 1983). In the present study, in light of the tendency for FBF to be lower, and FVR to be greater, in the L-NAME group than in the vehicle infused group, lactate and glucose might have been expected to rise to a greater level with L-NAME infusion. In addition NO is implicated in basal glucose uptake into skeletal muscle (Balon and Nadler, 1994). However, levels were in fact greater during hypoxia with *vehicle* infusion. The reason for this is not discernible from the present study but it was not possible to take into account the effect that L-NAME was having on placental glucose / lactate transfer or indeed on their production from other fetal organs.

Centrifugal neuronal activity arising from the CSN increases the production of NO in the carotid body *in vitro*. This NOS is likely to arise from CSN C-fibre nerve terminals in the carotid body, stimulating cGMP accumulation in type I chemoreceptor cells and modulating chemoreceptor responses to hypoxia. In addition NO released from parasympathetic nerve terminals is thought to dilate the carotid body vasculature and

modulate *steady-state* chemoreceptor activity (Wang, Stensaas, Dinger and Fidone, 1995). Furthermore in the anaesthetised adult rat, NOS inhibition augments the chemoreceptor excitatory response to hypoxia (Trzebski, Sato, Suzuki and Sato, 1995). It is therefore of interest that in the present study during hypoxia the magnitude of the inital rapid reflex bradycardia was in fact *lower* with L-NAME than with vehicle infusion. Moreover FHR fell to a similar level in both groups which therefore suggests that the fall in FHR was superimposed upon the already lowered basal FHR, rather than indicating a role for NOS in chemoreceptor discharge. FHR then increased to pre-hypoxic levels, once more lower with L-NAME than with vehicle infusion. In the vehicle-infused group a rebound tachycardia was observed during the recovery period which may be due to a positive chronotropic effect of catecholamines released in hypoxia, unmasked once the vagal suppression of FHR in hypoxia is removed. Nitric oxide is implicated in mediating heart rate responses to adrenaline in the adult (Gardiner, Kemp and Bennett, 1991) and accordingly the rebound tachycardia in the present study was attenuated by L-NAME infusion. Furthermore NO is implicated in mediating heart rate responses to β-adrenergic stimulation in the conscious adult rabbit (Reid, Bui and Chou, 1994).

A number of studies have implicated a role for NOS in the rise in pulmonary blood flow that takes place at the time of birth (Moore, Velvis, Fineman, Soifer and Heymann, 1992; Tiktinsky and Morin, 1993; Kinsella, Ivy and Abman, 1994), and NO inhalation has been used to dilate the pulmonary vascular bed in late-gestation fetuses (Kinsella, McQueston, Rosenburg and Abman, 1992) and in lambs delivered pre-term (Skimming, DeMarco and Cassin, 1994). Furthermore NOS-inhibition has been shown to augment hypoxia-induced pulmonary hypertension in the newborn (Fineman, Chang and Soifer, 1992) and chronic NOS inhibition *in utero* will simulate many of the characteristics of persistent pulmonary hypertension in the newborn lamb (Fineman, Wong, Morin, Wild and Soifer, 1994). NO has previously been reported to modulate the hypoxic pressor response in the adult (Persson, Gustafsson, Wilkund, Moncada and Hedqvist, 1990 Perrella, Edell, Krowka, Cortese and Burnett, 1992) and neonatal (Nelin and Dawson, 1993; Roberts *et al.*, 1993) pulmonary vasculature. However relatively little information exists to date on the role played by NOS in the *systemic* circulation during hypoxia. Nitric oxide is implicated in mediating hindlimb hypoxic-vasodilation is some (King, Curtis, Winn, Mewburn, Cain and Chapler, 1993) but not other (Vallet, Cutis, Winn, King, Chapler and Cain, 1994) adult dog studies. The human internal mammary artery *in vitro* responds to hypoxia with a constriction which was suggested to be due to hypoxic inhibition of NO (Pearson, Lin, Evora and Schaff, 1993). The results presented in this Chapter go some way towards implicating NOS in hypoxic changes in the periphery, since MAP and FVR tended to rise to a greater level and FBF to fall to a lower level with L-NAME than with vehicle infusion, although these changes were not significant.

There is *in vitro* evidence to suggest that NO *modulates* vascular responses to α-adrenergic stimulation (Hynes, Dang and Piper Duckles, 1988), although β-adrenoceptor mediated increases in hind-limb blood flow by adrenaline may be *mediated* by NO (Gardiner, Kemp and Bennett, 1991). It was therefore interesting to observe that the tendency for a rebound rise in blood flow during the recovery period in the vehicle group was smaller in the L-NAME group.

A number of adult studies have investigated the role of NO in hypoxic cerebrovasodilation. NO is implicated in raised cerebral blood flow during hypoxia in the adult sheep using methylene blue (Iwamoto, Yoshinaga, Yang, Krasney and Krasney, 1992), but not in anaesthetised dogs (McPherson, Koehler and Traystman, 1994). Indeed some studies found that L-NAME enhanced the cerebral vasodilatory response to hypoxia (Pelligrino, Koenig and Albrecht, 1993). Drawbacks to these studies include the non-specificity of methylene blue as an inhibitor of guanylate cyclase and the potential confounding effects of anaesthesia (see Iadecola, Pelligrino, Moskowitz and Lassen, 1994). Nitric oxide also acts as an antagonist to sympathetic cerebral vasoconstriction in late-gestation fetus *in vitro* (Wagerle, Moliken and Russo, 1995). From the data presented in this Chapter, in light of the large CVR which was established by the infusion of L-NAME during normoxia, it might have been expected that the scope for vasodilatation during hypoxia would be great. However the rise in CBF and fall in CVR during hypoxia with the infusion of vehicle was absent when NOS was inhibited. Thus, NO-synthesis appears to play a key role in mediating the vasodilatation of the carotid bed during hypoxia in the fetus. These findings tend to agree with more recent preliminary work in which a role for NOS is implicated in the rise in cerebral blood flow in the mid-gestation sheep fetus in response to 6 h hypoxia induced by reduced uterine artery blood flow (McCrabb and Harding, 1994).

The time spent in LV-ECoG activity tended to fall during hypoxia with vehicle infusion but this was less apparent in the L-NAME group. Kovach *et al.* (1992) observed an increase followed by a decease in the power of the ECoG in adult cats after NOLA administration but they suggested that this was unlikely to be via a cortical ischaemia since no change in cortical blood flow was seen. The fall in the incidence of FBMs during hypoxia was only significant in the vehicle group, but this may have been because FBMs were already depressed in the L-NAME group. The precise locus at which hypoxia acts centrally to depress FBMs is not known [1.4.9] and it will be important to relate this central inhibition of FBMs to local changes in brain stem blood flow in future studies. In adult sheep hypoxia-stimulated ventilation is not depressed by NOLA administration despite impaired cerebral hypoxic vasodilation (Iwamoto, Yang, Yoshinaga, Krasney and Krasney, 1992).

NOS inhibition blocks the rise in AII during hypoxia

A number of investigations have suggested an interaction between AII and NO. For example, in isolated rabbit afferent arterioles AII will not produce a vasoconstrictory response without prior treatment with either NOS-inhibitor or cyclooxygenase inhibitor, suggesting a role for NO in counteracting AII action (Yoshida, Tamaki, Aki, Kimura, Takenaka and Abe, 1994), and NO regulates AII receptors through a cGMP-independent mechanism (Cahill, Redmond, Foster and Sitzmann, 1995). In the present study inhibition of NOS using L-NAME caused a fall in basal plasma [AII] in agreement with adult studies (Goyer, Bui, Chou, Evans, Keil and Reid, 1994; Reid, Bui and Chou, 1994). The pre-L-NAME infusion [AII] was quite high, but not statistically different from the vehicle group. The mechanisms underlying this response is likely to be complicated. The results suggest that either L-NAME removes a tonic stimulatory action of NO on AII synthesis, or that L-NAME initiates some other physiological mechanism which in turn decreases AII-synthesis, perhaps via increased renal sympathetic nerve activity (RSNA) and RVR. Goyer *et al.* (1994) suggested that the blockade of NO-synthesis can exert a direct intrarenal action, but that changes in renal perfusion pressure and RSNA probably contribute depending on the dose of inhibitor used. Indeed sympathetic nerve activity is stimulated via a central action of NOS-inhibition (Sakuma *et al.*, 1992. see 1.6.1). If these two factors are held constant then NOLA administration has no effect on PRA (Naess, Christensen, Kirkboen and Kiil, 1993). In the adult anaesthetised rat L-NAME suppressed renin release independent of changes in renal perfusion pressure (Johnson and Freeman, 1992). On the other hand, other adult rat studies suggest that when changes in perfusion pressure and β-adrenergic activity are controlled L-NAME increases PRA, therefore implying a tonic *inhibitory* role for NO on the RAS (Sigmon, Carretero and Beierwaltes, 1992). In the present study the fall in plasma [AII] with L-NAME infusion was accompanied by a large rise in MAP. Thus while the results presented in this Chapter could support a stimulatory role for NO in the release of renin, as seen by a fall in plasma [AII] with L-NAME, the participation of factors such as renal nerve stimulation and increased renal perfusion pressure cannot be ruled out.

During hypoxia plasma [AII] rose significantly in the vehicle group but such a rise was absent in the L-NAME group. It was conceivable that the differences may be attributable to MAP differences between the two groups, however this does not seem likely since pressure rose to a similar level in both groups. I do not have any information on RSNA from these experiments, but NOS-inhibition may have augmented the hypoxia-stimulated RSNA. Fetal RVR rises during hypoxia (Robillard, Nakamura and DiBona, 1986, see Chapter 3). It is possible that NOS-inhibition augments renal vasoconstriction during

hypoxia which would in turn attenuate the rise in plasma [AII] during hypoxia. Although from the observations made in Chapter 3 on three CSD fetuses, after a large rise during normoxia, RVR did not appear to rise, and if anything decreased, during hypoxia with the infusion of L-NAME. Whether this would be the case if the CSNs were left intact remains to be investigated.

There is evidence to suggest that elevated plasma [cortisol] inhibits PRA (Wood, Keil and Rudolph, 1984). This finding led Wood *et al.* (1984) to suggest that a positive feedback loop may exist between the RAS and the hypothalamus-pituitary-adrenal axis, whereby AII stimulates ACTH, and cortisol inhibits the action of the RAS. Indeed cortisol has been shown to decrease renal and hepatic AT_1mRNA in fetal sheep (Segar, Bedell, Page, Mazursky, Nuyt and Robillard, 1995). The results presented in this thesis show a rise in plasma [ACTH] during hypoxia with both vehicle and L-NAME infusion, but that while [cortisol] tended to rise during hypoxia this only reached significance in the L-NAME group. Thus it might be that the significant rise in [cortisol] during L-NAME infusion may have contributed to the absence of a rise in plasma [AII] during hypoxia.

6.6 Conclusion

In conclusion, NO synthesis appears to alter resting peripheral and carotid vascular resistance and blood flow, MAP and FHR. This modulation of FHR persists throughout hypoxia and recovery upon which reflex and endocrine effects are superimposed. In addition results suggest that a large component of the CBF response to hypoxia is due to the increased synthesis of NO. In addition NOS-inhibition attenuates the rise in plasma [AII] during hypoxia, which could be a direct effect of NO on the kidney. It is interesting that, despite the absence of a rise in plasma [AII] during hypoxia with L-NAME, fetuses in this group still demonstrated a significant vasoconstriction in the femoral vascular bed during hypoxia. This lends further supports the concept of an interaction between a number of different mechanisms in cardiovascular control [1.5.4]. Finally, the involvement of NO in basal haemodynamics has important implications for a role for NO in vascular development. Glucocorticoids (Radomski, Palmer and Moncada, 1990) and aminoguanidine (Griffiths, Messent, MacAllister and Evans, 1993) specifically inhibit the inducible NOS isoform. In future work it will be important to assess the relative contribution of constitutive and inducible NOS to fetal hypoxic responses and to cardiovascular development.

CHAPTER 7

THE EFFECT OF LONG-TERM NITRIC OXIDE SYNTHESIS INHIBITION ON FETAL CARDIOVASCULAR CONTROL

7.1 Introduction

Results presented in the previous Chapter suggested a role for NOS in systemic cardiovascular control under normoxic conditions. These findings may therefore have important implications for NOS in fetal vascular development and thus the ontogeny of haemodynamic control.

Previously adult animal studies have shown that chronic inhibition of NOS results in a maintained systemic hypertension (Jover, Herizi, Ventre, Dupont and Mimran, 1993; Qiu, Engels and Baylis, 1994; Ribeiro, Antunes, Nucci, Lovisolo and Zatz, 1992), more profound than that observed in the pulmonary vasculature (Hampl, Archer, Nelson and Weir, 1993). It is conceivable that this sustained effect is solely attributable to the removal of tonically produced NOS, however there is mounting evidence of a contribution by other vasopressor mechanisms such as AT_1 receptor (Jover *et al.*, 1993) and α_1-adrenoceptor (Qiu *et al.*, 1994) stimulation. Furthermore NO synthesised in the CNS may fulfil a second messenger or neurotransmitter role in mediating sympathetic outflow and consequently blood pressure (Togashi *et al.*, 1992).

NOS has been implicated in the pathogenesis of preeclampsia during pregnancy since chronic inhibition of NOS, either by continuous subcutaneous administration of L-NAME (Yallampalli and Garfield, 1993) or intravenous infusion of NOLA (Molnar, Suto, Toth and Hertelendy, 1994), leads to maternal hypertension in the adult rat. This is associated with IUGR and increased mortality of pups, perhaps via reduced placental perfusion. Diket *et al.* (1994) however failed to mimic this maternal hypertensive state by chronic L-NAME administration but did show that the *fetus* was sensitive to the vasoconstrictor effects of L-NAME, as evidenced by a reduced fetal size and deformation of the hind-limb. It therefore seems possible that the fetal changes observed were mediated at least in part by a direct inhibition of fetal NOS. In light of the basal systemic haemodynamic effects of *short*-term NOS-inhibition seen in the previous Chapter, the aim of the present study was to examine the effect of prolonged NOS-inhibition, by L-NAME administration directly to the fetus (i.v.), on systemic cardiovascular control.

Some of these results have already been presented in abstract form (Green, Bennet and Hanson, 1995b, see Appendix 15).

7.2 Methods

7.2.1 Surgical preparation

Nine fetuses aged between 117 and 121 days gestation were instrumented under general anaesthesia [2.3.2]. The lower half of the fetus was exteriorised through the uterine incision. An ultrasonic flow probe (3R: Transonic Systems Inc., Ithaca NY) was placed around a femoral artery and the transducer cable secured in 2 places to the fetal skin. The lower portion of the fetus was returned to the uterus. The upper portion of the fetus was then exteriorised either through the same incision by rotating the fetus or through a second incision in the uterus following closure of the first. Two stainless steel electrodes were sewn onto the chest and an earthing electrode onto the back of the neck to monitor ECG. A catheter was sewn onto the fetal skin for drug administration into the amniotic cavity. Additional heparinised catheters were placed in a carotid artery and a jugular vein to monitor MAP and MVP and in the right brachial vein to administer antibiotics and experimental drugs. A second ultrasonic flow probe (3 or 4R) was placed around the uncatheterised carotid artery. A maternal pedal vein was catheterised to administer antibiotics.

A tracheal catheter and diaphragm EMG electrodes were implanted to record FBMs. Stainless-steel electrodes were implanted bilaterally on the parietal dura to measure ECoG [2.3.2].

Five to 6 days of recovery were allowed before experimentation.

7.2.2 Experimental procedure

Measurements

MAP, MVP, tracheal pressure, FHR, CBF, FBF, ECoG activity and diaphragm EMG were recorded continuously onto chart paper (Gould ES1000) and MacLab Chart Software (AD Instruments Pty. Ltd.) using a Macintosh LCIII computer (Apple computers Inc.) [2.4.2].

Fetal arterial blood was collected during daily recording periods for blood gas and electrolyte (0.6ml) and hormonal (2ml) analysis. In addition, blood was collected during

the hypoxia protocols: control (15 and 45 min), hypoxia (75, 90 and 105 min) and recovery (135 and 165 min). Blood was transferred immediately to chilled EDTA tubes and spun at 4°C (3000 rpm) for 10 min. Plasma was then decanted into tubes and stored at -20°C for future hormonal analysis.

Drug Preparation

On a daily basis approximately 1.8g L-NAME (Sigma Chemical Co. Ltd. UK) was dissolved in sterile-saline to give a final concentration of 60mg.ml^{-1}. Vehicle solution was 0.9% saline.

At the time of experimentation 0.5g ACh (Sigma Chemical Co. Ltd. UK) was dissolved in 20ml sterile-saline (25mg.ml^{-1}) and serial dilutions carried out to give a final concentration of 6.25x10^{-3}mg.ml^{-1}.

SNP (Sigma Chemical Co. Ltd. UK) was prepared at the time of experimentation and, as SNP is light sensitive, care was taken to protect both solid and dissolved forms from light. Approximately 100mg SNP was dissolved in sterile-saline (1mg.ml^{-1} solution) and serial dilutions were carried out to give a final solution of 100μg.ml^{-1}.

At the time of experimentation serial dilutions of phenylephrine (Boots Pharmaceuticals Ltd., UK: 10mg.ml^{-1} solution) were carried out in sterile-saline to give a final stock solution of 0.1mg.ml^{-1}.

Protocol

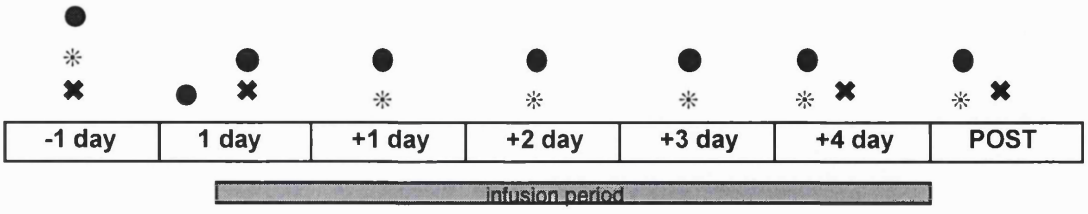


Figure 7.1 Summary of 7 day protocol. The grey bar denotes the continuous infusion period of either vehicle or L-NAME. ✻ phenylephrine, ACh and SNP challenge; ✖ hypoxic challenge; ● daily 1 h recording.

Nine fetuses were studied in the morning over a period of 7 days (see Figure 7.1 and Table 7.1). The fetuses were divided into 2 groups: those continuously-infused with vehicle (n=4) and those continuously-infused with L-NAME (n=5). On the day prior to the onset of infusion (day 1: vehicle 126±0.5 and L-NAME 125±1.0 days gestation) the

fetal baroreflex responses to phenylephrine was tested. After the cardiovascular variables had returned to control levels fetuses were challenged with ACh and SNP (✳). Cardiovascular variables were allowed to return to control levels between doses. Finally fetuses underwent a hypoxic challenge (✖, Figure 7.1).

On day 2 (vehicle: 127±0.5 and L-NAME: 126±1.0 days gestation), fetal cardiovascular variables were monitored for 1 h pre- and 1 h post-infusion onset (●), and during a subsequent 1 h hypoxic challenge (✖). The infusion regime consisted of an initial bolus (2ml: vehicle or 20mg L-NAME) followed by a continuous infusion (1.65ml.h^{-1}: vehicle or 99mg.h^{-1} L-NAME).

On day 3 (vehicle: 128±0.5 and L-NAME: 127±1.0 days gestation), day 4 (vehicle: 129±0.5 and L-NAME: 128±1.0 days gestation) and day 5 (vehicle: 130±0.5 and L-NAME: 129±1.0 days gestation) fetuses were monitored for 1 h (●) and were challenged with phenylephrine, ACh and SNP (✳) as on day 1.

On day 6 (vehicle: 131±0.5 and L-NAME: 130±1.0 days gestation) fetuses were monitored for 1 h and the fetal cardiovascular response to phenylephrine, ACh and SNP were tested prior to a 1 h hypoxic challenge. At the end of these measurements the continuous infusion of vehicle or L-NAME was stopped. On the following day (day 7: vehicle: 132±0.5 and L-NAME: 131±1.2 days gestation) the fetuses were monitored for 1 h and once more were subjected to phenylephrine, ACh, SNP and hypoxic challenges (✳✖).

In one L-NAME-infused fetus, phenylephrine and hypoxic challenges were carried out 1 day prior to infusion onset and on day 6 only, and cardiovascular responses to ACh and SNP were not investigated.

Phenylephrine challenge : Phenylephrine was administered as an i.v. (brachial vein) bolus of 75µg in 2ml saline and flushed in with saline. Chart speed was adjusted to 25mm.sec^{-1} prior to, and for ca. 1 min post-drug administration.

ACh and SNP challenge: ACh (10µg in triplicate and 15µg in triplicate) and SNP (40µg in triplicate and 100µg in triplicate) were administered via the brachial vein. All doses were given in a volume of 2ml.

Hypoxic challenge: The hypoxia protocol comprised an initial 1 h normoxic control period, followed by 1 h fetal isocapnic hypoxia. Ewes were then returned to normoxic gas mixture and measurements continued for one hour (recovery) [2.5.1].

Plasma hormone analysis

Due to the preliminary nature of this study hormonal analysis of plasma samples will be analysed after further experiments have been conducted.

7.3 Data analysis

Grouped data are expressed as mean±S.E.M. and statistical analysis was carried out where n>3.

Phenylephrine responses: Baroreflex curves were created by plotting the R-R interval of two adjacent QRS-complexes against the corresponding systolic pressure. Baroreflex sensitivity was measured as the slope of the linear portion of these curves ($sec.mmHg^{-1}$) in individual animals. The start of the linear portion was defined as the R-R interval preceding the R-R interval which showed the first increase, and end of the linear portion as the R-R interval which preceded the R-R interval which showed no further increase (Ismay, Lumbers and Stevens, 1979). Slope measurements were then used as summary measures for individual fetuses to compare baroreflex sensitivities during L-NAME infusion to pre-infusion values by paired t-test.

ACh and SNP responses: MAP was measured before and at the point of maximum response to ACh (Figure 7.17). MAP was measured prior to SNP and once the fall in MAP had reached a plateaux (Figure 7.17). ACh data were compared by Student's Paired t-test and adjusted for multiple comparisons by the Bonferroni method.

Daily cardiovascular and blood composition measurements for individual fetuses were reduced to summary measures. Student's paired t-test was used to test FHR and MAP 1 h pre and 1 h during-L-NAME infusion and 1 day pre- and 4 days during- L-NAME infusion. Regression analysis was used to describe the decline in FHR and FBF throughout the 7 day protocol.

Blood gas and cardiovascular measurements for individual fetuses were reduced to summary measures to describe normoxic, hypoxic and recovery hours. Summary measures were then tested using Student's paired t-test to compare hypoxic and recovery hours to normoxia.

Time spent making FBMs and in LV-ECoG activity was expressed as a percentage of the hours of normoxia, hypoxia and recovery. Paired t-test was then used to compare hypoxia and recovery to normoxia.

7.4 Results

	VEHICLE (n=4) *Mean time relative to infusion onset (min)*	**L-NAME (n=5)** *Mean time relative to infusion onset (min)*
-1 day	-1320±94.0	-1160±87.5
-1 hour	-22.5±0.0	-22.5±0.0
+1 hour	22.5±0.0	22.5±0.0
+1 day	1329±57.6	1543±100.6
+2 days	2736±44.0	2911±46.8
+3 days	4157±45.1	4298±37.4
+4 days	5726±104.5	5975±87.0
post	7111±61.1 *(or 1319±59.2 after infusion ceased)*	7261±20.4 *(or 1119±95.4 after infusion ceased)*

Table 7.1 Time of daily recording of cardiovascular variables before and after the onset of vehicle (n=4) and L-NAME (n=5) infusion. Values are shown as mean±S.E.M.

7.4.1 Blood gases, electrolytes, glucose and lactate

Hypoxic challenges

While statistical comparison was not possible in the vehicle group, and in some instances in the L-NAME group, during all hypoxic challenges arterial oxygenation fell and returned to normoxic levels during the subsequent recovery period, while $PaCO_2$ and pH remained unaltered throughout the protocol. Glucose and lactate rose during all hypoxic challenges and their subsequent recovery periods, although where statistical comparison was possible, the rise in lactate was only of significance 1 day pre- and 4 days post-infusion onset (Table 7.2).

		NORMOXIA		HYPOXIA		RECOVERY	
		Vehicle	*L-NAME*	*Vehicle*	*L-NAME*	*Vehicle*	*L-NAME*
Lactate	*-1 day*	1.49±0.66	0.75±0.09	1.57±0.37	2.35±0.22‡	1.83±0.36	3.47±0.44‡
(mmol.L^{-1})	*+1h*	0.75±0.04	0.66±0.07	2.02±0.39	1.89±0.43	2.89±0.27	3.13±0.99
	+4 days	0.87±0.06	0.62±0.16	1.84±0.19	1.60±0.36†	2.48±0.55	2.40±1.00
	post	1.04±0.28 (3)	0.50±0.05	1.86±0.29	1.43±0.15(3)	2.42±0.45	1.71±0.37(3)
Glucose	*-1 day*	0.90±0.10	0.87±0.08	1.54±0.48	1.42±0.33	2.14±1.08	1.22±0.18
(mmol.L^{-1})	*+1h*	0.97±0.03	0.69±0.07	1.03±0.12	1.09±0.32	1.13±0.10	1.04±0.21
	+4 days	1.03±0.07	0.84±0.08	1.11±0.09	0.97±0.07	1.19±0.15	0.99±0.16
	post	0.89±0.13	0.77±0.09(3)	1.01±0.12	1.02±0.10(3)	1.05±0.18	1.08±0.01(3)
pH	*-1 day*	7.35±0.00	7.34±0.01	7.34±0.01	7.30±0.02	7.32±0.02	7.29±0.03
	+1h	7.34±0.00	7.35±0.02	7.32±0.01	7.32±0.02	7.30±0.01	7.30±0.01
	+4 days	7.32±0.03	7.33±0.02	7.32±0.02	7.30±0.02	6.13±1.15	7.29±0.02
	post	7.35±0.02	7.35±0.01(3)	7.33±0.03	7.33±0.01(3)	7.31±0.03	7.34±0.01(3)
$PaCO_2$	*-1 day*	49.4±0.4	48.8±0.7	45.7±0.7	48.6±1.2	47.7±1.6	47.6±1.4
(mmHg)	*+1h*	50.1±0.8	47.5±1.1	46.1±0.6	46.9±1.4	47.7±2.0	45.4±1.9
	+4 days	46.8±1.8	48.9±1.4	46.2±0.8	47.9±1.0	47.3±1.1	49.0±1.0
	post	47.5±0.5	50.3±1.6(3)	44.9±1.2	46.9±2.2(3)	45.7±1.8	46.3±1.4(3)
PaO_2	*-1 day*	23(2)	23±0.7	13(2)	13±0.5‡	23.5(2)	22±0.9
(mmHg)	*+1h*	23±0.5	23±0.8	13±0.5	13±0.9‡	23±0.9	24±1.1
	+4 days	23±0.4	22±1.1	13±0.3	13±0.5‡	22±0.8	22±1.5(3)
	post	22±0.8	22±1.3(3)	13±0.1	12±1.0(3)	21±0.9	22±1.8(3)
$\%SaO_2$	*-1 day*	81.0±0.8	83.8±2.5	46.5±1.5	51.7±4.7‡	79.5±1.2	80.2±4.3
	+1h	80.7±1.1	83.4±1.7	46.0±3.3	47.0±2.6‡	80.3±1.8	81.6±2.4
	+4 days	78.9±1.2	78.8±4.1(3)	43.5±1.8	49.7±4.5(3)	78.3±1.9	77.4±2.6(3)
	post	79.6±2.3	78.7±2.3(3)	45.7±0.2	39.7±1.9(3)	76.1±2.9	77.6±2.8(3)

Table 7.2 Fetal plasma lactate, glucose, pH and blood gas measurements pre (-1day), after- (+1 h and +1day) and post- vehicle and L-NAME infusion onset, during normoxia, hypoxia and recovery. Values are shown as mean±S.E.M. Vehicle n=3 (no statistical analysis performed) and L-NAME n=4, unless indicated by bracketed numbers in the table. †P<0.05 and ‡P<0.01, significantly different from normoxia by paired t-test.

Daily measurements

There was no change in arterial oxygenation (Tables 7.4 and 7.5), acid-base status (Table 7.6) or glucose/lactate content (Table 7.3) during the protocol in vehicle or L-NAME groups.

		VEHICLE	L-NAME
Lactate	*-1 day*	0.85±0.05	0.75±0.09
(mmol.L^{-1})	*-1 hour*	0.68±0.08	0.59±0.07 (4)
	+1 hour	0.75±0.04	0.85±0.13
	+1 day	0.77±0.09	0.69±0.10
	+2 day	0.82±0.09	0.60±0.10
	+3 day	0.73±0.07	1.07±0.51
	+4 day	0.89±0.07	0.62±0.17
	POST	1.07±0.30	0.51±0.07(3)
Glucose	*-1 day*	0.85±0.10	0.84±0.07
(mmol.L^{-1})	*-1 hour*	0.86±0.11	0.60±0.04(4)
	+1 hour	0.94±0.08	0.89±0.13
	+1 day	0.90±0.09	0.81±0.15
	+2 day	0.87±0.08	0.78±0.11
	+3 day	0.88±0.12	0.88±0.15
	+4 day	1.06±0.05	0.85±0.09
	POST	0.92±0.15	0.88±0.01(3)

Table 7.3 Daily fetal plasma lactate and glucose measurements before (-1day and -1 h), during (+1 to +4 days) and after (POST) vehicle (n=3, no statistical analysis performed) and L-NAME (n=5, unless indicated otherwise by bracketed numbers in table) infusion. Values are shown as mean±S.E.M.

	VEHICLE			L-NAME		
	PaO_2	$\%SaO_2$	O_2ct	PaO_2	$\%SaO_2$	O_2ct
-1 day	24	81.8±0.6	8.5±0.2	23±0.7	84.8±2.9	15.3±6.3
-1 hour	22±0.6	79.5±1.7	7.8±0.2	22±0.9(4)	82.3±2.8(3)	6.5±1.0
+1 hour	22±0.3	79.0±0.4	7.7±0.3	25±0.7	87.6±2.4	8.1±2.0
+1 day	24±1.5	82.3±2.1	8.3±0.5	24±0.7	84.6±2.1	9.3±0.4
+2 day	23±0.9	81.9±1.8	8.3±0.5	23±0.5	83.7±1.6(4)	8.6±0.5(4)
+3 day	22±0.4	78.6±0.4	8.1±0.6	22±1.6	74.0±7.4	7.6±0.8
+4 day	23±0.7	78.4±1.8	8.0±0.3	22±0.9	78.8±4.4(4)	8.4±0.9(3)
POST	22±0.8	79.35±2.2	8.2±0.2	22±1.5(3)	78.0±2.9(3)	7.9±0.4(3)

Table 7.4 Daily fetal arterial PO_2, $\%SO_2$ and O_2ct before (-1day and -1 h), during (+1 to +4 days) and after (POST) vehicle (n=3 no statistical analysis performed) and L-NAME (n=5, unless indicated otherwise by bracketed numbers in table). Values are shown as mean±S.E.M.

	VEHICLE				L-NAME			
	Total Hb ($g.dL^{-1}$)	$\%O_2Hb$	%MetHb	%Hct	Total Hb ($g.dL^{-1}$)	$\%O_2Hb$	%MetHb	%Hct
-1day	7.9±0.2	77.5±0.7	0.5±0.0	28±1.1	7.4±0.5	81.6±1.8	1.2±0.8	25±1.4
-1hour	7.4±0.1	75.4±2.0	0.3±0.0	28±0.5	7.2±0.1(3)	78.8±2.4(3)	0.2±0.1(3)	27±0.8(4)
+1hour	7.4±0.2	74.8±1.1	0.3±0.0	28±0.4	8.5±0.2	83.4±1.8	0.2±0.1	30±0.9
+1day	7.7±0.4	77.6±2.1	0.3±0.0	26±1.5	8.2±0.2	80.9±1.9	0.0±0.2	0.6±0.3
+2day	7.7±0.5	77.4±2.0	0.3±0.1	29±2.0	7.7±0.4(4)	81.4±1.9(4)	1.0±0.7(4)	26±1.7
+3day	7.6±0.4	74.6±0.6	0.4±0.0	28±1.0	7.6±0.1(3)	71.1±7.2	0.3±0.0(3)	28±1.3
+4day	7.7±0.5	74.6±1.9	0.3±0.0	27±2.8	7.8±0.3(4)	74.9±3.6(4)	1.2±1.0(4)	27±0.7
POST	7.8±0.4	75.3±2.1	0.3±0.0	28±2.3	7.6±0.2(3)	74.9±2.6(3)	0.2±0.1(3)	27±1.2(4)

Table 7.5 Daily fetal arterial total Hb, $\%O_2Hb$, %MetHb and %Hct before (-1day and -1 h), during (+1 to +4 days) and after (POST) vehicle (n=3, no statistical analysis performed) and L-NAME (n=5, unless indicated otherwise by bracketed numbers in table) infusion. Values are shown as mean±S.E.M.

	VEHICLE				L-NAME			
	$PaCO_2$ (mmHg)	pH	HCO_3^- ($mmol.L^{-1}$)	Base Excess ($mmol.L^{-1}$)	$PaCO_2$ (mmHg) (4)	pH	HCO_3^- ($mmol.L^{-1}$) (4)	Base Excess ($mmol.L^{-1}$) (4)
-1 day	49.9±0.6	7.35±0.01	27.3±0.5	2.7±0.5	48.5±0.9	7.34±0.01	25.6±0.3	1.0±0.3
-1 hour	49.8±1.3	7.34±0.00	26.6±0.5	2.0±0.4	49.4±1.7	7.34±0.02	26.1±0.6	1.4±0.7
+1 hour	49.9±2.0	7.35±0.01	26.7±1.1	2.1±1.0	48.2±2.0	7.36±0.01	27.0±0.7	2.6±0.6
+1 day	49.8±1.2	7.31±0.01	25.5	0.7±0.6	49.6±1.9	7.33±0.02	25.9±0.5	1.2±0.7
+2 day	50.1±1.6	7.32±0.02	25.2±0.8	1.4±0.8	50.6±1.6	7.31±0.01	25.6±0.5	0.7±0.5
+3 day	48.5±1.4	7.31±0.02	24.0±1.4	-0.9±1.5	49.0±2.0	7.31±0.01	24.0±1.1	-1.1±1.1
+4 day	48.3±2.4	7.32±0.03	24.3±2.5	-0.5±2.7	49.7±1.8	7.33±0.02	25.8±0.8	0.8±1.1
POST	48.5±0.4	7.35±0.02	26.3±0.8	1.8±0.9	50.7±1.8(3)	7.35±0.01(3)	27.8±1.0	3.3±1.1

Table 7.6 Daily fetal arterial PCO_2, pH, HCO_3^- and base excess before (-1day and -1 h), during (+1 to +4 days) and after (POST) vehicle (n=3, no statistical analysis performed) and L-NAME (n=5, unless indicated otherwise by bracketed numbers in table) infusion. Values are shown as mean±S.E.M.

7.4.2 Fetal Heart Rate

With the infusion of vehicle there was a gradual fall in FHR throughout the 7-day protocol which was not apparent with the infusion of L-NAME (Figure 7.3). There was a large fall in FHR 1 h after the onset of L-NAME infusion and FHR remained lower than 1 day pre-infusion levels after 4 days of infusion. However at this time (+4 days) there was no significant difference in FHR between vehicle and L-NAME groups (Figure 7.2).

In the vehicle group, there was a rapid fall in FHR at the onset of pre- (○), 4 days after onset (Δ) and post- (✚) hypoxic challenges (Figure 7.6). The fall at the onset of hypoxia after 1 h infusion (▲) did not reach significance, however there was no significant difference between the magnitude of its fall and that of the fall in the pre-infusion challenge (Figure 7.6). During recovery in 1 h-vehicle infusion group (▲), FHR was elevated from pre-hypoxic levels.

In the L-NAME group there was a rapid fall in FHR at the onset of pre- (○), 1 h after onset (▲) and post-hypoxic (✚) challenges. The fall at the onset of hypoxia after 4 days (Δ) infusion did not reach significance although, once more, the magnitude of its fall was not different from that of the hypoxia challenge pre-L-NAME infusion (Figure 7.7).

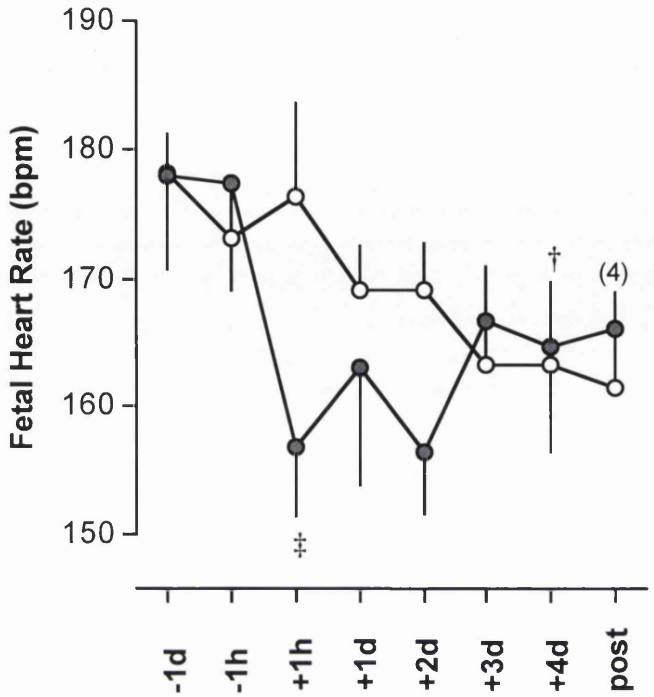


Figure 7.2 FHR before (-1d and -1 h), during (+1 h, +1d, +2d, +3d and +4d) and after (post) vehicle (○, n=4) and L-NAME (●, n=5 expect where indicated by bracketed number) infusion. For further information on time of recording refer to Table 7.1. Values are shown as mean±S.E.M. †P<0.05, significantly different from -1d with L-NAME infusion by paired t-test; ‡P<0.05, significantly different from -1 h with L-NAME infusion by paired t-test.

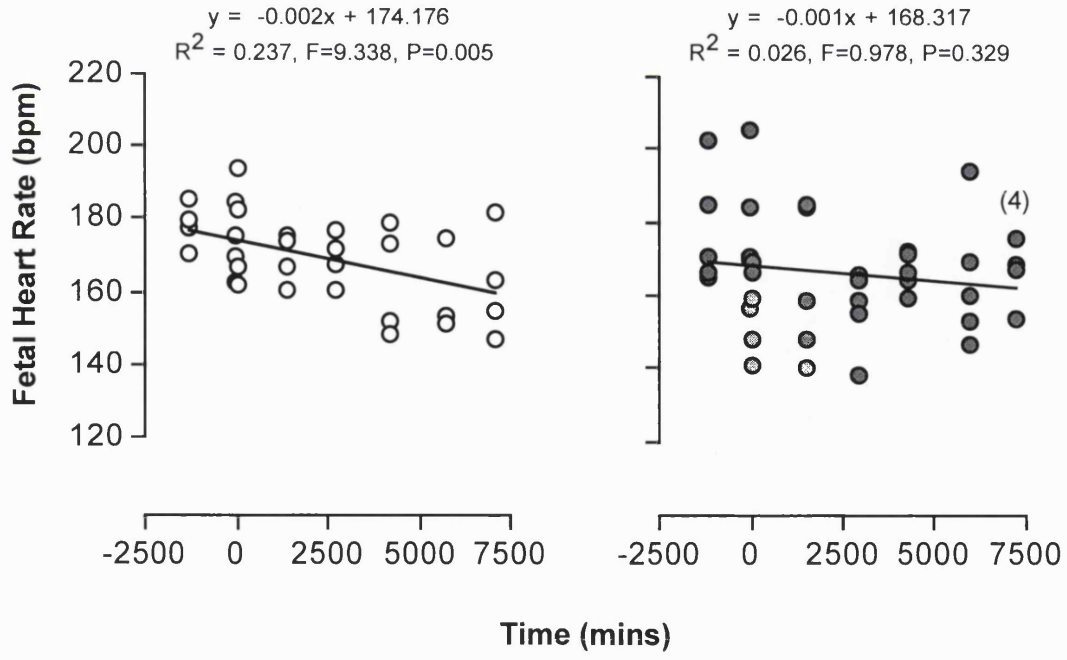


Figure 7.3 Scatter plots of FHR at mean recording times with vehicle (○, n=4) and L-NAME (●, n=5) infusion. Data has a fitted regression line and is represented by an equation in the form 'y=mx + c'. 'R2' is the square of the correlation coefficient, 'F' is the square of the test statistic and 'P' is the probability that the slope (m) is significantly different from zero. FHR showed a gradual fall throughout the duration of the vehicle infusion protocol as indicated by the slope of the regression line being significantly different from zero (P<0.01).

7.4.3 Mean Arterial and Venous Pressures

While statistical comparison was not possible, MAP did not tend to alter in 2 vehicle infused fetuses but appeared to rise after +1d in the third fetus (Figure 7.4). There was a large rise in MAP during the first hour of L-NAME infusion, however pressure returned to towards pre-infusion levels as L-NAME infusion continued. Four days after L-NAME infusion-onset MAP was in fact below pre-infusion levels (Figure 7.4). There was no apparent change in MVP throughout the course of the protocol (Figure 7.5).

In the vehicle-infusion group, the three fetuses in which measurements were obtained showed a variable tendency for pressure to rise during hypoxia (Figure 7.6B). In the L-NAME-infused group there was a tendency for MAP to rise during hypoxia pre- (○), during- (Δ) and post- (✚) L-NAME infusion although this only reached significance during the post-L-NAME infusion period. Furthermore there was no rise in MAP during the hypoxic challenge in the 1 h L-NAME-infusion period (▲. Figure 7.7).

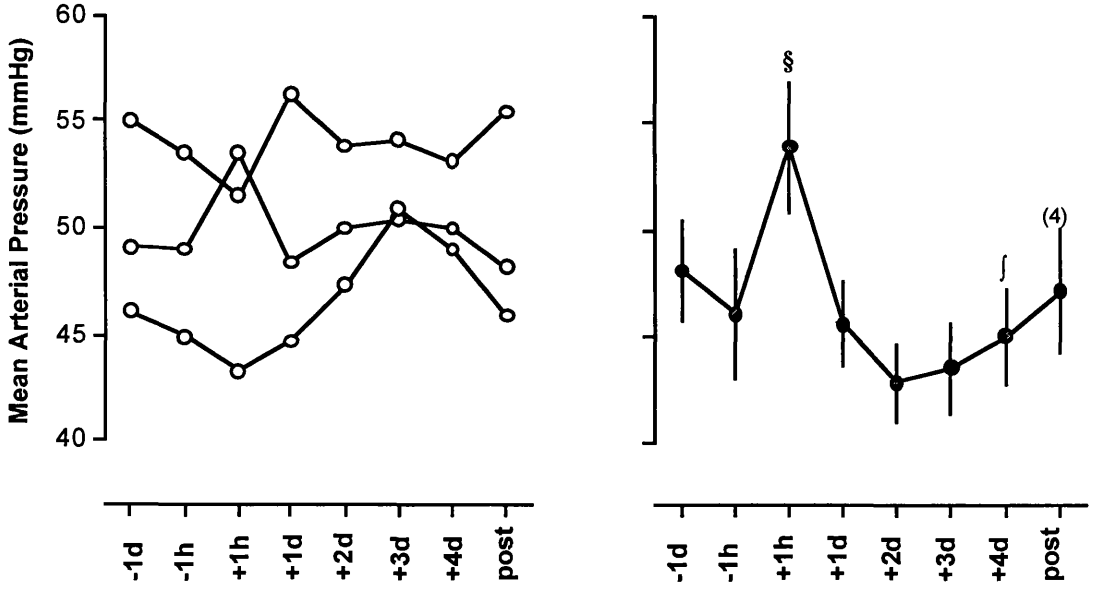


Figure 7.4 MAP before (-1d and -1 h), during (+1 h to +4d) and after (post) vehicle (○, n=3, fetuses shown separately) and L-NAME (●, mean±S.E.M., n=5 expect where indicated by bracketed number post infusion) infusion. For further information on time of recording refer to Table 7.1. ∫P<0.05, significantly different from -1d by paired t-test; §P<0.01, significantly different from -1 h by paired t-test.

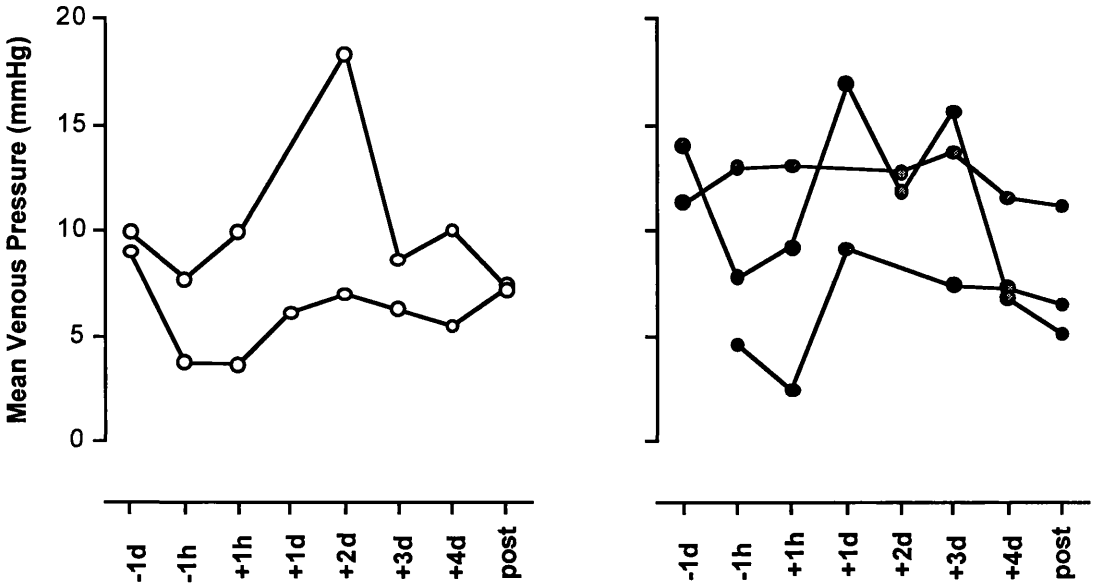


Figure 7.5 MVP before (-1d and -1 h), during (+1 h to +4d) and after (post) vehicle (○, n=2, fetuses shown separately) and L-NAME (●, n=3, fetuses shown separately) infusion. For further information on time of recording refer to Table 7.1.

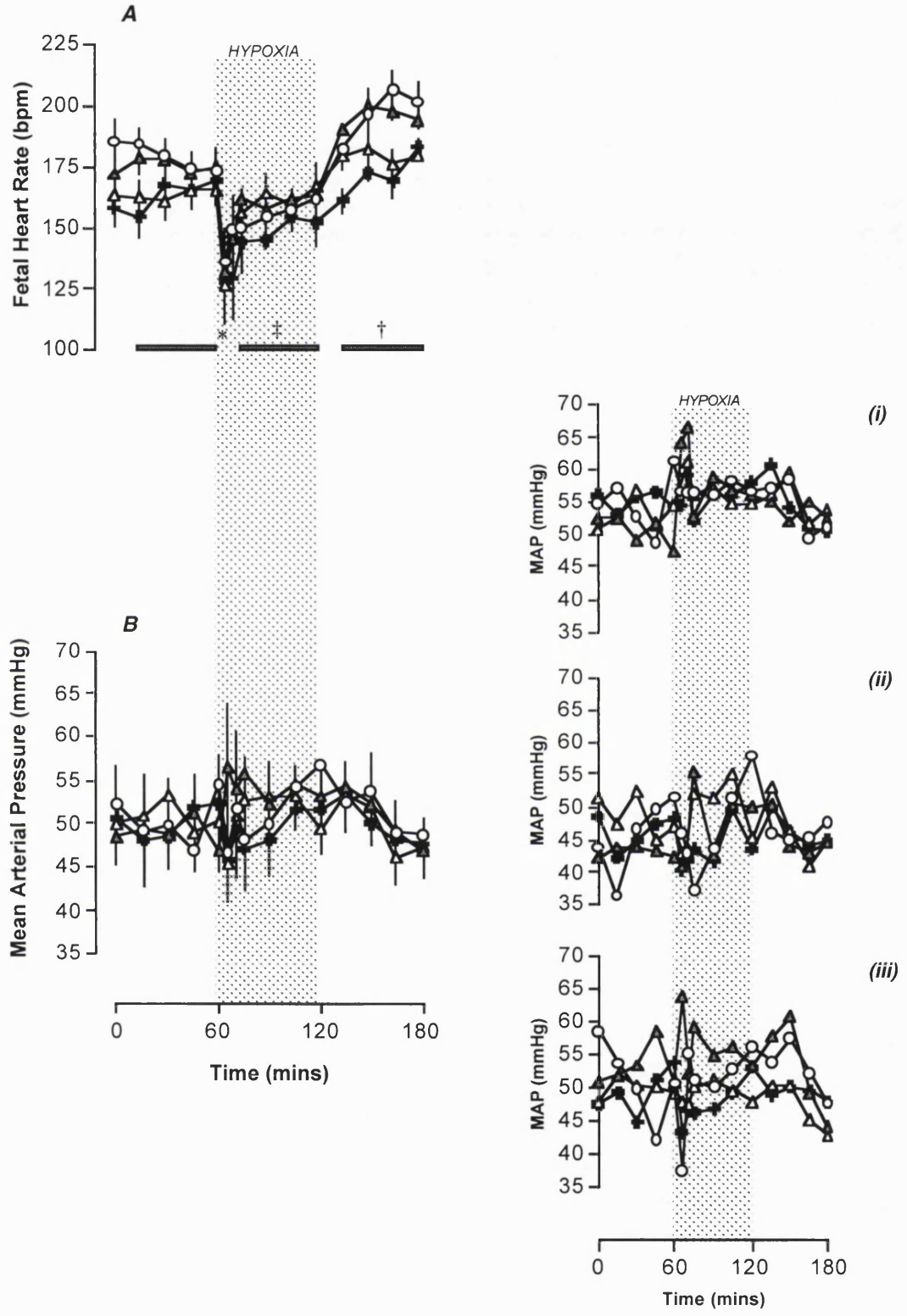


Figure 7.6 ***VEHICLE GROUP:*** A. FHR (n=4) and B. MAP (n=3, i-iii show individual fetuses) during hypoxia (shaded area) pre- (○), after 1 h (▲), after 4 days (Δ) and post (✚) vehicle infusion. Values are shown as mean±S.E.M. Horizontal bars show the time period over which measurements were reduced to summary measures. *P<0.05, significantly different from 60 min in pre-, after 4 days infusion and post-infusion groups; ‡P<0.05. significantly different from normoxia in post-infusion group; †P<0.05, significantly different from normoxia in 1 h infusion group.

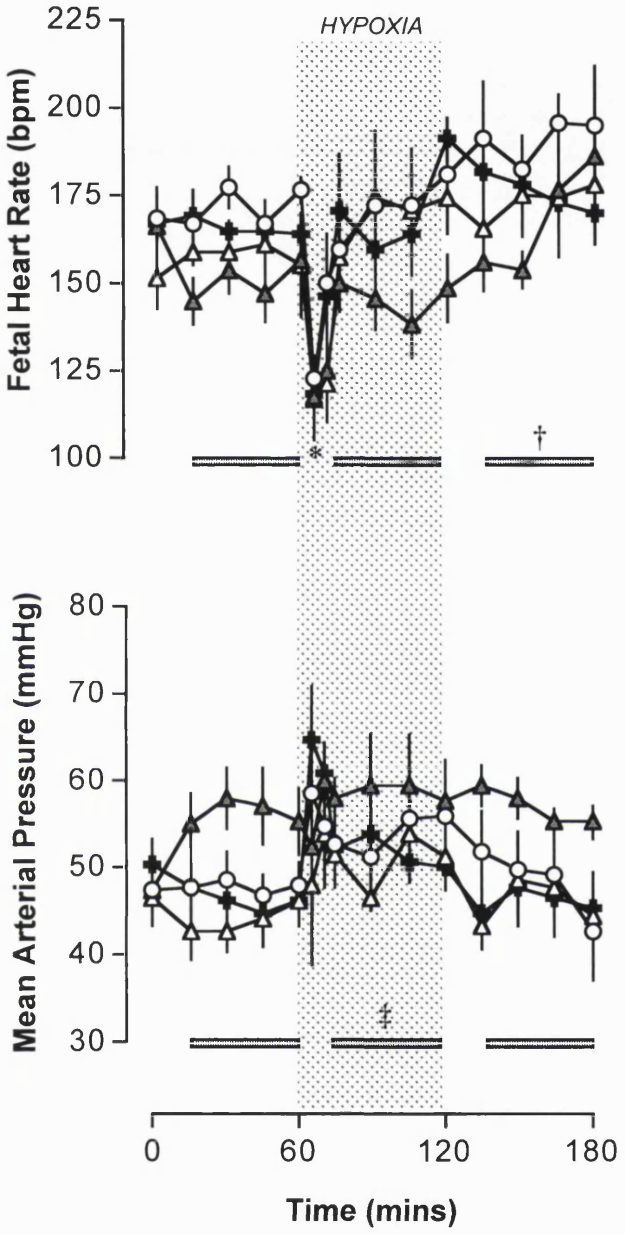


Figure 7.7 ***L-NAME GROUP:*** A. FHR (n=4) and B. MAP (n=4) during hypoxia (shaded area) pre- (○), after 1 h (▲), after 4 days (Δ) and post (✚) L-NAME infusion. Values are shown as mean±S.E.M. Horizontal bars show the time period over which measurements were reduced to summary measures. *P<0.05, significantly different from 60 min in pre-, after 1 h infusion and post-infusion groups by paired t-test; †P<0.05, significantly different from normoxia in pre-infusion group by paired t-test; ‡P<0.01, significantly different from normoxia in post-infusion group by paired t-test.

7.4.4 Blood Flow

Femoral bed

In the 2 fetuses of the L-NAME group flow significantly declined throughout the course of the protocol, while in the vehicle-infused group no such change was apparent (Figure 7.8 and 7.9).

In the vehicle group FBF tended to fall during all the hypoxic challenges however only reached significance in the pre-infusion group. Furthermore there was a rebound increase in FBF during the recovery period of the challenges during the pre- (○), 1 h- (▲) and post- (✚) infusion periods (Figure 7.11A). In the 2 L-NAME group fetuses in which FBF measurements were obtained there was a tendency for FBF to fall during all hypoxic challenges (Figure 7.12Ai and ii).

Carotid bed

CBF tended to be lower in L-NAME than in vehicle group fetuses, however in neither group did CBF appear to alter throughout the infusion protocol (Figure 7.10).

CBF tended to rise during the majority of hypoxic challenges in the 3 vehicle-group (Figure 7.11B), and 2 L-NAME-group (Figure 7.12Bi and ii) fetuses studied.

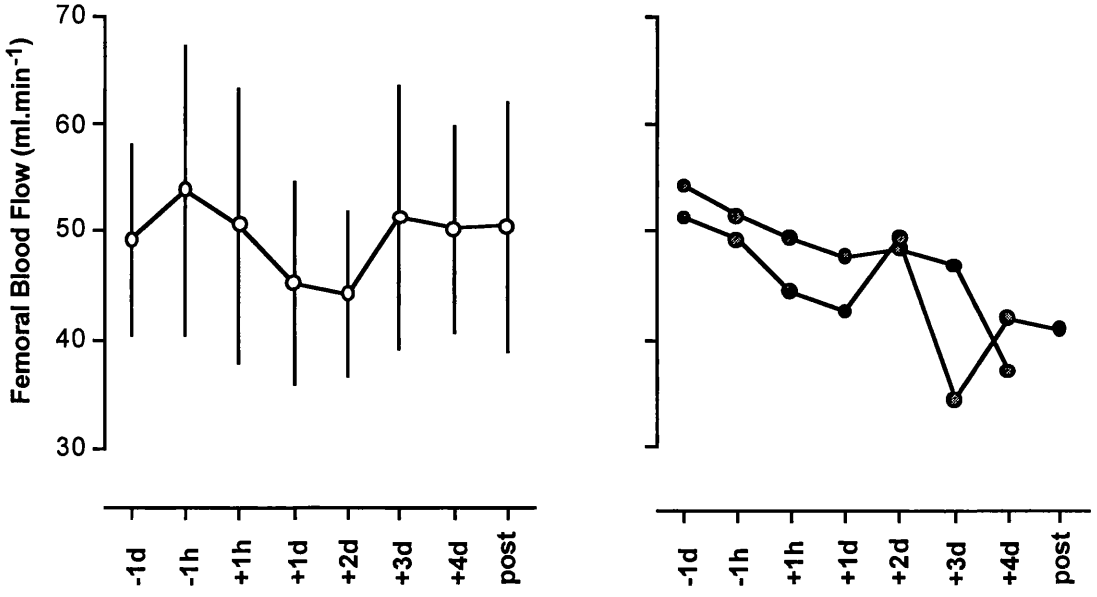


Figure 7.8 FBF before (-1d and -1 h), during (+1 h to +4d) and after (post) vehicle (○, n=4, mean±S.E.M.) and L-NAME (●, n=2, fetuses shown separately) infusion. For further information on time of recording refer to Table 7.1.

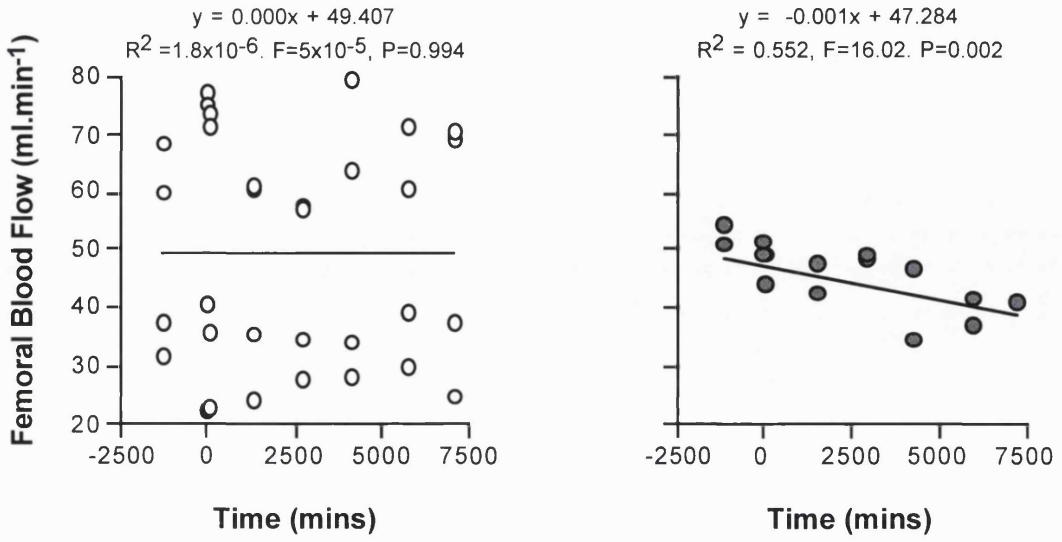


Figure 7.9 Scatter plots of FBF at mean recording times with vehicle (○, n=4) and L-NAME (●, n=2) infusion. Data has a fitted regression line and is represented by an equation in the form 'y=mx + c'. 'R2' is the square of the correlation coefficient, 'F' is the square of the test statistic and 'P' is the probability that the slope (m) is significantly different from zero. FBF showed a gradual fall throughout the duration of the L-NAME infusion protocol as indicated by the slope of the regression line, fitted to 2 fetuses, being significantly different from zero (P<0.01).

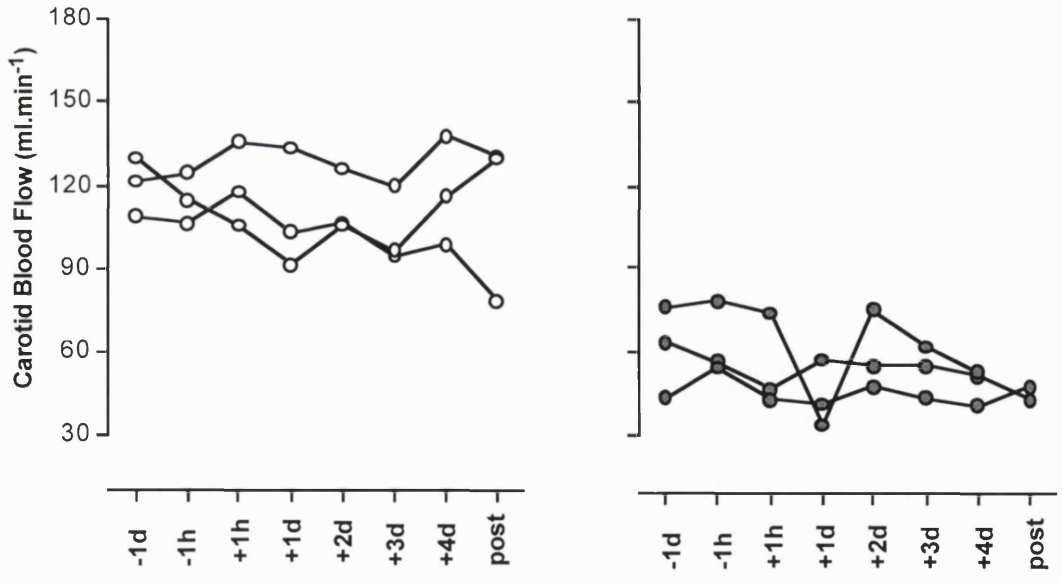


Figure 7.10 CBF before (-1d and -1 h), during (+1 h to +4d) and after (post) vehicle (○, n=3, fetuses shown separately) and L-NAME (●, n=3, fetuses shown separately) infusion. For further information on time of recording refer to Table 7.1.

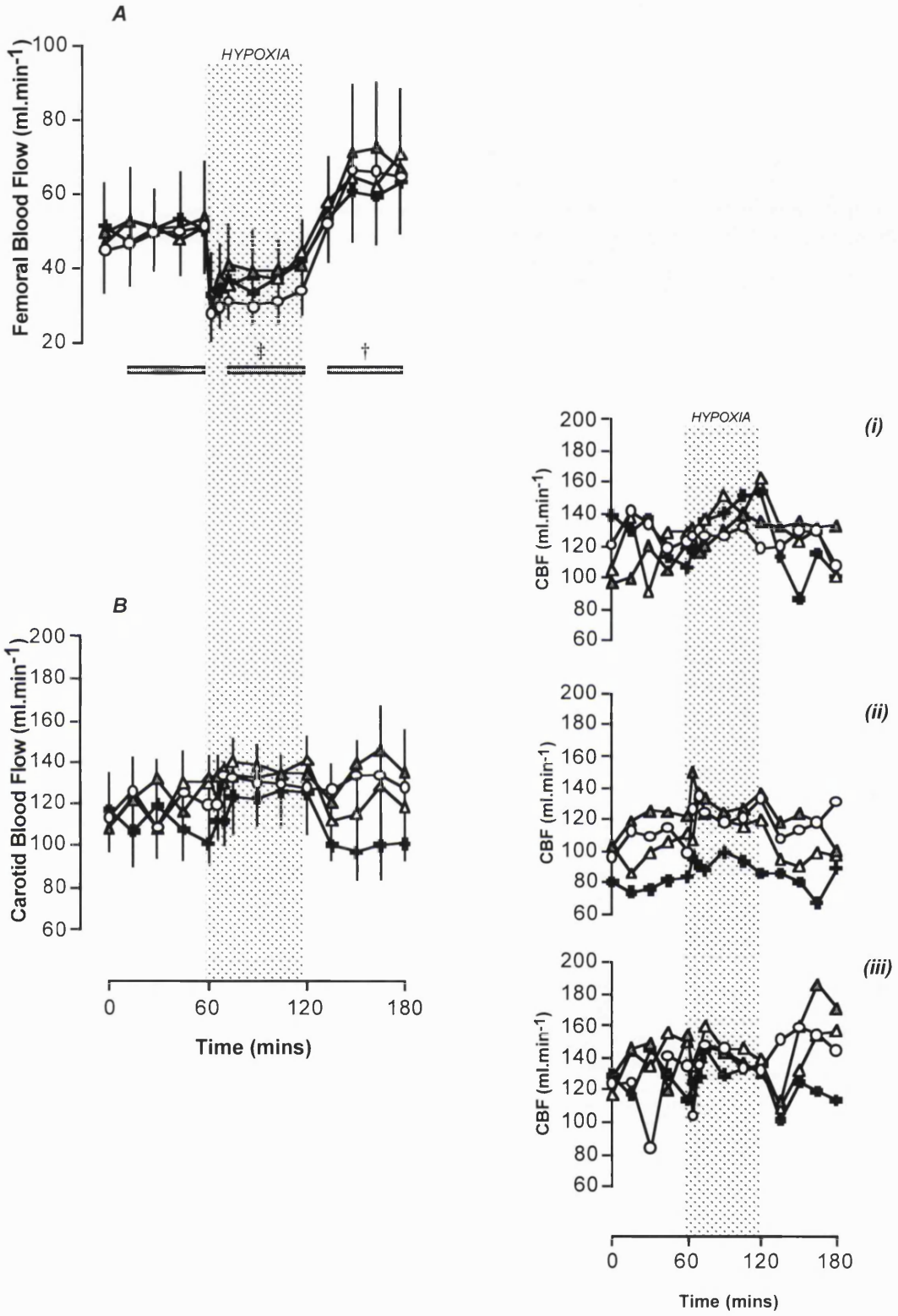


Figure 7.11 ***VEHICLE GROUP:*** A. FBF (n=4) and B. CBF (n=3, i-iii show individual fetuses) during hypoxia (shaded area) pre- (❍), after 1 h (▲), after 4 days- (Δ) and post- (✚) vehicle infusion. Values are shown as mean±S.E.M. Horizontal bars show the time period over which measurements were reduced to summary measures. ‡P<0.05. significantly different from normoxia in pre-infusion group; †P<0.05, significantly different from normoxia in pre-infusion, 1 h- and post-infusion group.

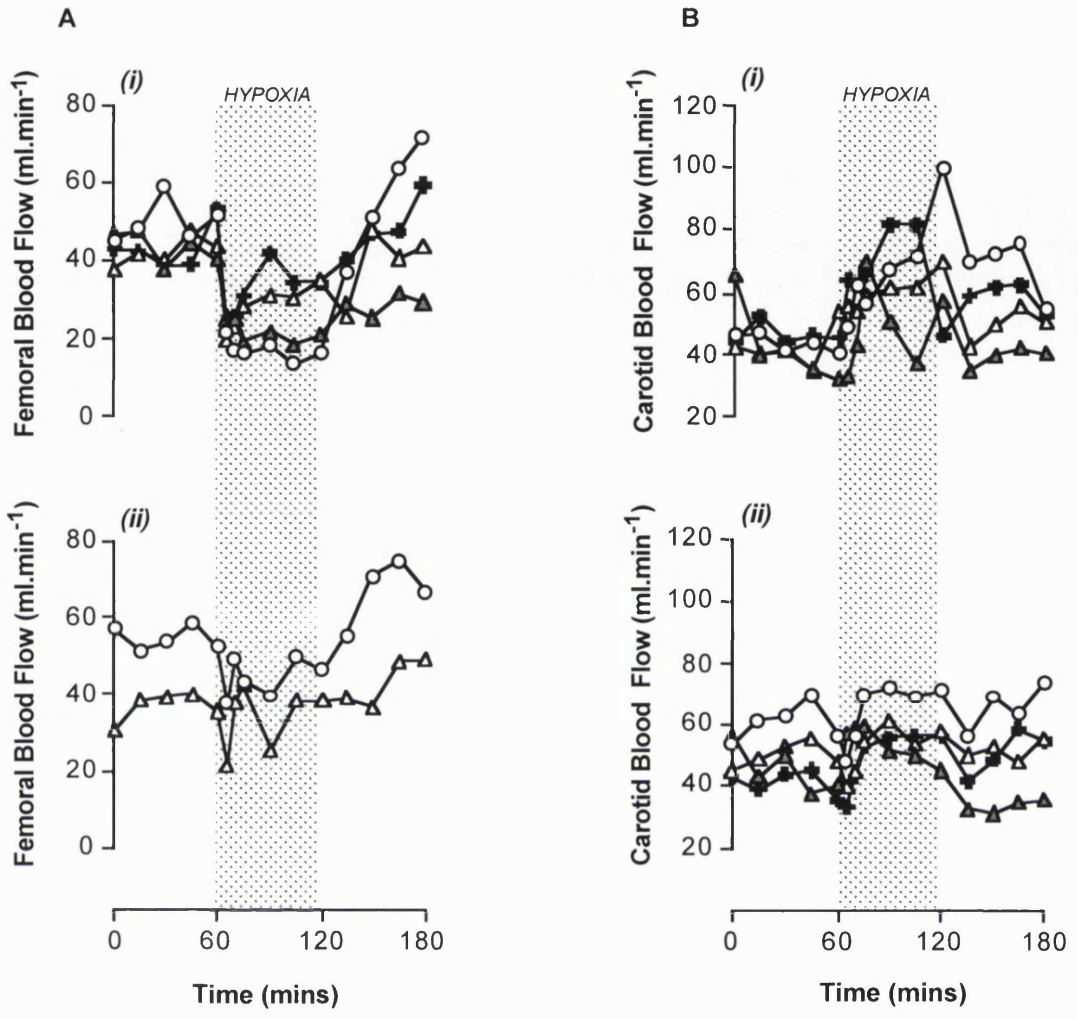


Figure 7.12 A. FBF (n=2, fetuses i and ii) and B. CBF (n=2, fetuses i and ii) during hypoxia (shaded area) pre- (○), after 1 h- (▲), after 4 days- (Δ) and post- (✚) L-NAME infusion. Values are shown as mean±S.E.M.

7.4.5 Baroreflex responses to phenylephrine

Baroreflex responses were obtained in 2 vehicle- (Figure 7.13, 7.16 and Table 7.7) and 4 L-NAME- (Figures 7.14 to 7.16 and Table 7.7) group fetuses. The relationship between systolic pressure and R-R interval (baroreflex sensitivity), as indicated by slope measurements, did not change throughout the L-NAME infusion period (Figures 7.14 to 7.16, and Table 7.7).

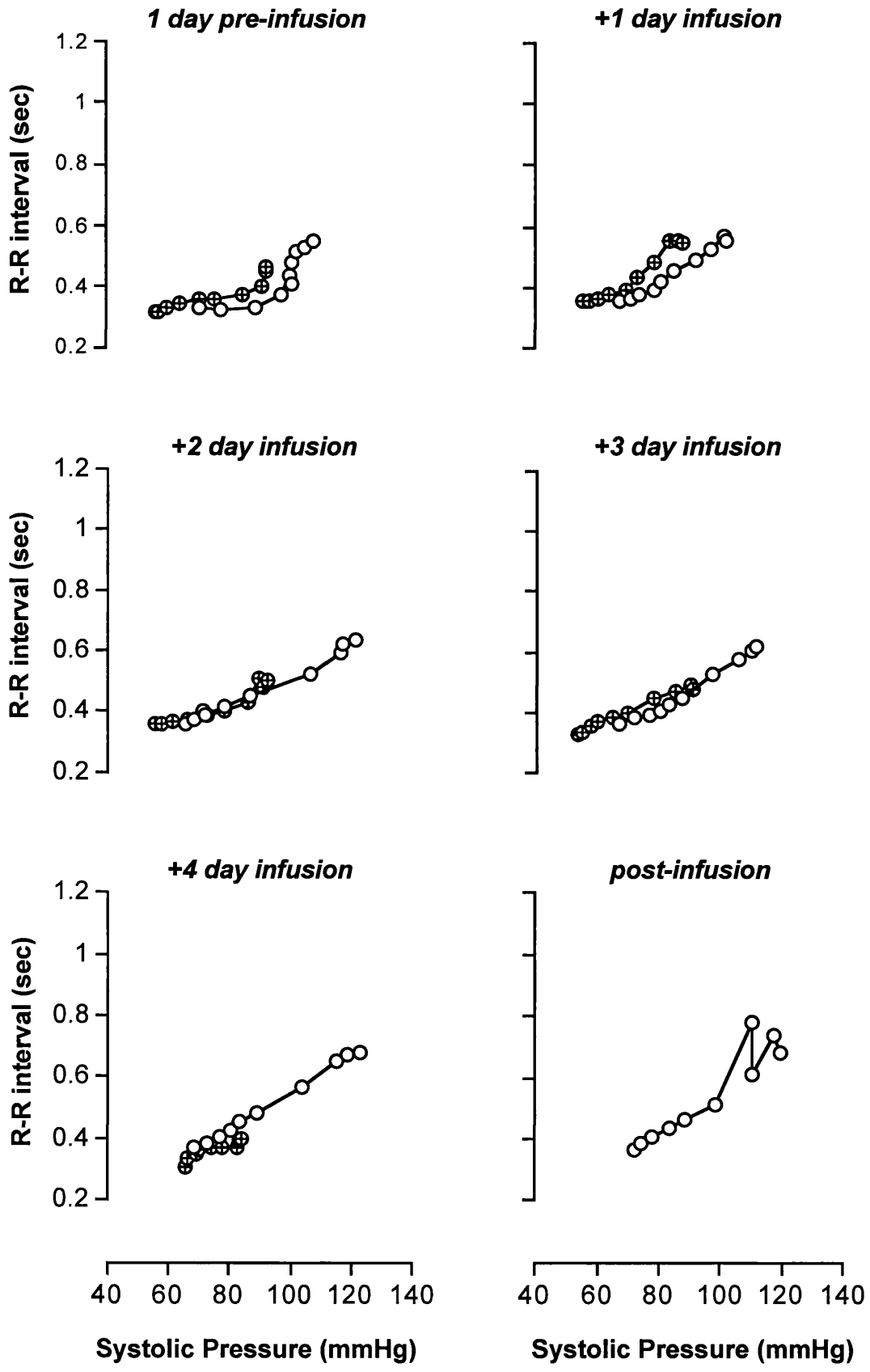


Figure 7.13 ***VEHICLE GROUP:*** The relationship between systolic pressure and R-R interval before (-1d), during (+1d to +4d) and after (post) vehicle infusion in 2 fetuses (⊕ and ○) shown individually.

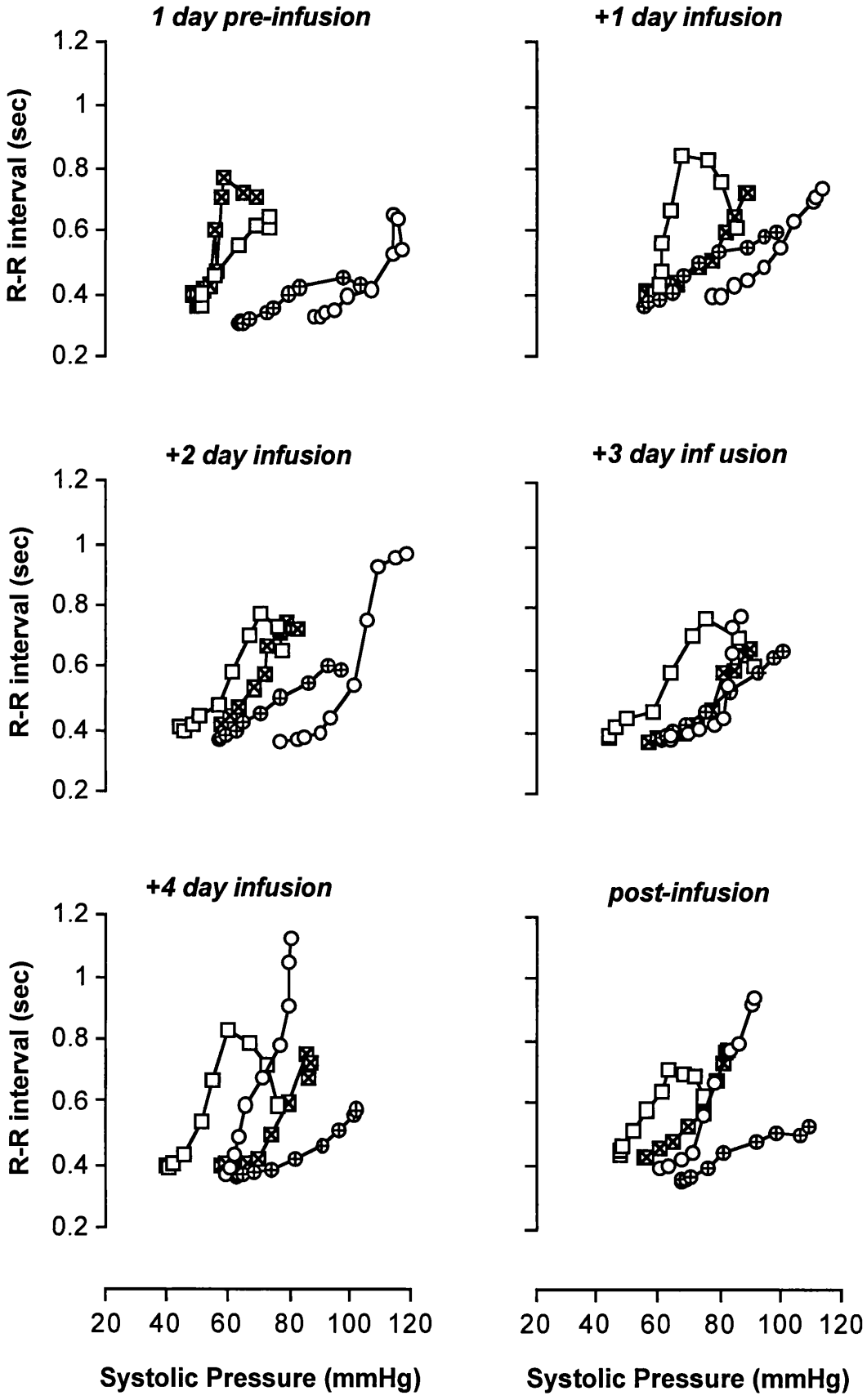


Figure 7.14 ***L-NAME GROUP:*** The relationship between systolic pressure and R-R interval before (-1d), during (+1d to +4d) and after (post) L-NAME infusion in 4 fetuses (⊕ ,○,□,⊠) shown individually.

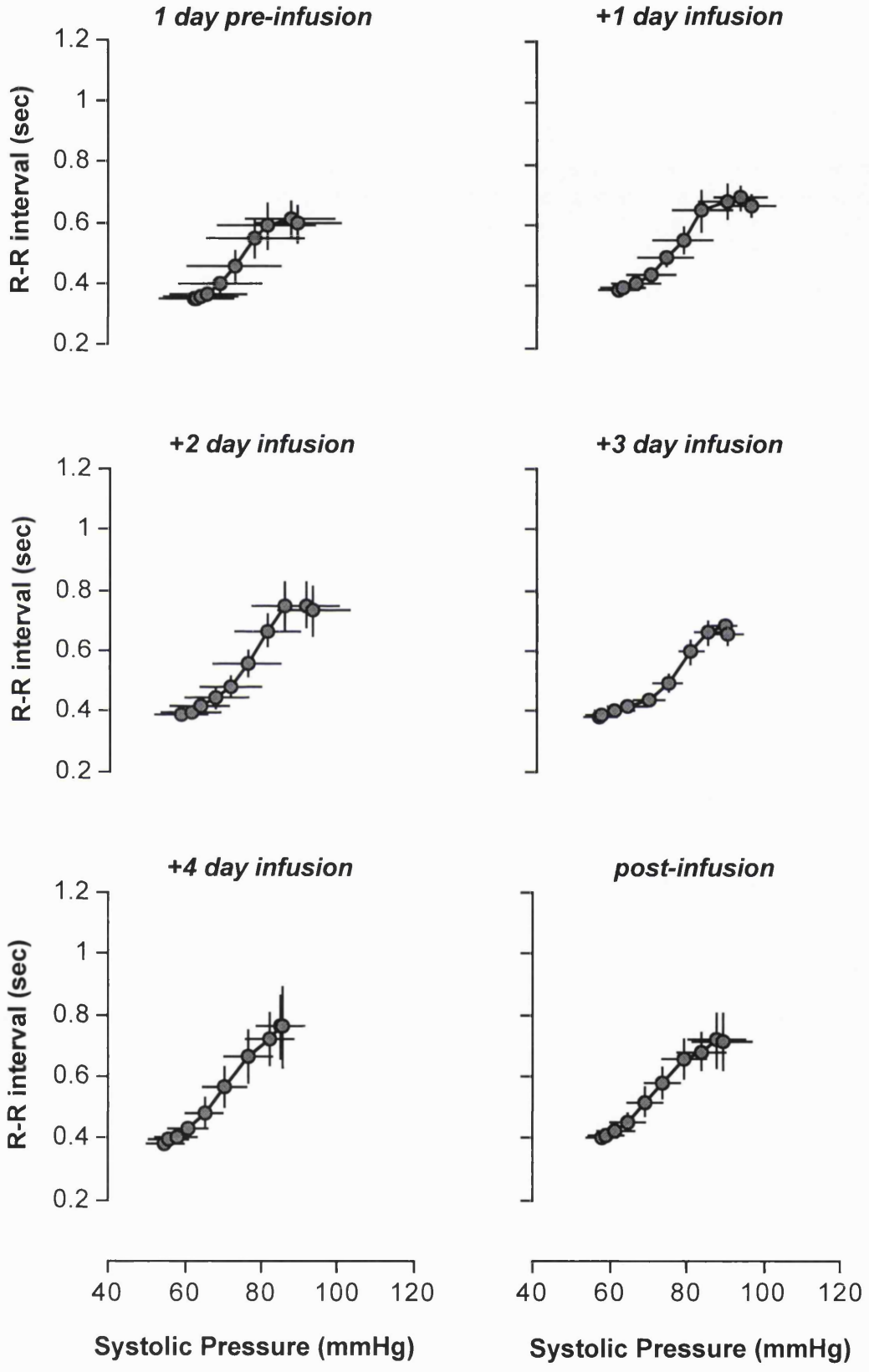


Figure 7.15 ***L-NAME GROUP:*** The relationship between systolic pressure and R-R interval before (-1d), during (+1d to +4d) and after (post) L-NAME infusion (n=4). Values are given as mean±S.E.M. of R-R interval, and ±S.E.M. of systolic pressure.

	VEHICLE	L-NAME
-1 day	0.013	0.01
	0.005	0.005
		0.013
		0.041
+1 day	0.006	0.011
	0.007	0.006
		0.053
		0.009
+2 day	0.005	0.022
	0.004	0.006
		0.015
		0.016
+3 day	0.006	0.049
	0.004	0.007
		0.012
		0.012
+4 day	0.006	0.031
	0.003	0.005
		0.021
		0.017
Post infusion	0.01	0.021
	-	0.005
		0.015
		0.013

Table 7.7 Baroreflex slopes before (-1d), during (+1d to +4d) and after (post) vehicle (○, n=2, fetuses shown separately) and L-NAME (●, n=4, fetuses shown separately) infusion.

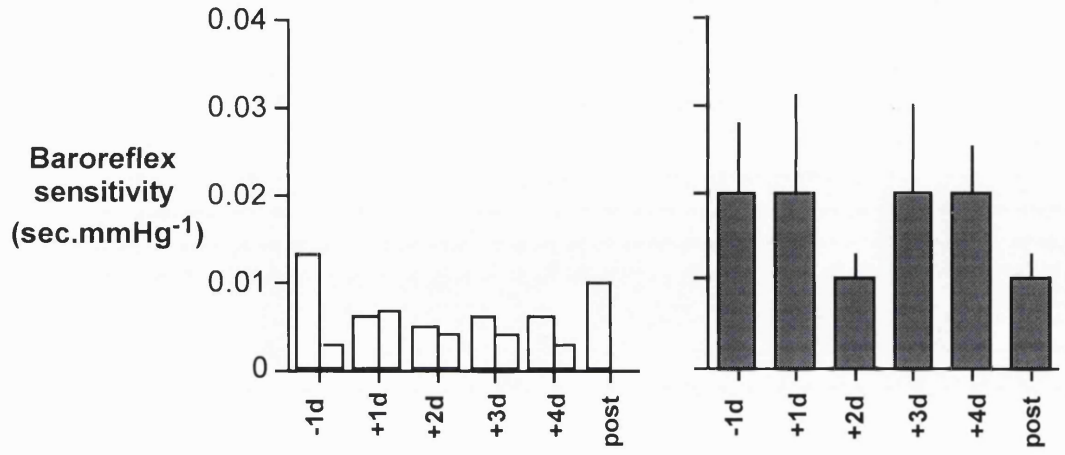


Figure 7.16 Baroreflex sensitivity before (-1d), during (+1d to +4d) and after (post) vehicle (shaded bars, n=2, fetuses shown separately) and L-NAME (shaded bars, n=4 Mean±S.E.M.) infusion.

7.4.6 Mean arterial pressure responses to ACh and SNP

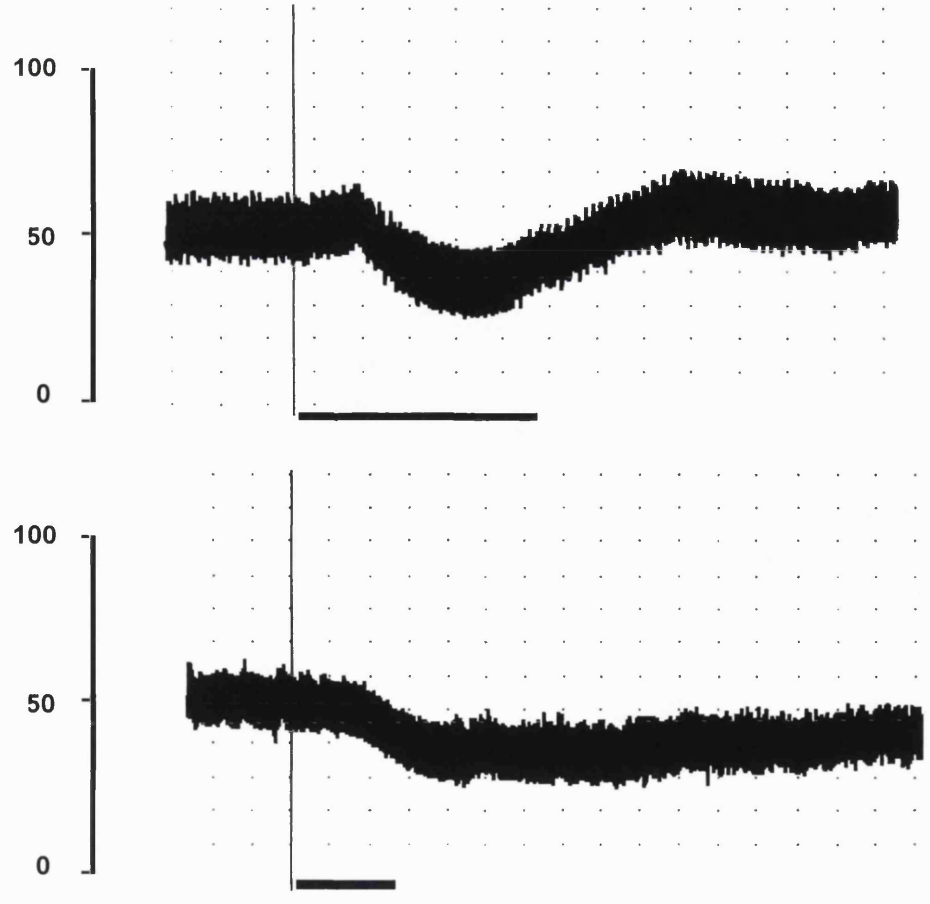


Figure 7.17 Raw trace from one fetus showing the arterial pressure (ordinate: mmHg) response to 10μg ACh (top trace) and 40μg SNP (bottom trace). The horizontal bars represent a 20 sec interval.

In the L-NAME group there was no difference in the magnitude of the MAP response between the two doses of either ACh or SNP studied throughout the 7-day protocol (Tables 7.8 and 7.9). However on -1day, in the 2 vehicle and 3 out of 4 L-NAME infused fetuses, the response to 15μg ACh was less than with the lower dose of 10μg (Table 7.8 and Figure 7.18). Furthermore, at -1 day the magnitude of the MAP response to a given dose of ACh appeared to decline over the course of three replicate doses, particularly in the vehicle group, suggesting a tachyphylaxis (Figure 7.18). A tachyphylaxis was not as apparent with repeated SNP doses (Figure 7.18).

In neither vehicle nor L-NAME groups was there any obvious trend in the MAP response to SNP over the course of the protocol (Table 7.9). The response to ACh tended to be reduced after the infusion of L-NAME however this only reached significance on +1 day (10μg ACh: Table 7.8). In contrast, the MAP response to ACh of the two vehicle-infused fetuses appeared to remain unaltered throughout the protocol (Table 7.8).

		VEHICLE (mmHg)		**L-NAME (mmHg)**				
	ACh dose	*fetus 1*	*fetus 2*	*fetus 1*	*fetus 2*	*fetus 3*	*fetus 4*	*MEAN*
-1 day	*10μg*	16.1	15.6	16.2	10.9	14.7	12.3	13.5±1.2
	15μg	13	13.4	11.7	8	10.9	12.7	10.8±1.0
+1 day	*10μg*	15.6	14.1	9.56	3.11	8.44	4.44	6.4±1.6†
	15μg	20.7	15.2	5.44	5.44	9.33	6.11	6.6±0.9
+2 day	*10μg*	15.3	13.3	6.33	6.44	3.56	10.6	6. 7±1.4
	15μg	14.7	16.6	7.78	7.33	8.89	7.56	7.9±0.4
+3 day	*10μg*	18.4	14.6	6.78	6.33	10.2	7.22	7.6±0.9
	15μg	15.6	14.1	6.56	8.56	8.89	8.78	8.2±0.6
+4 day	*10μg*	17.6	15.8	9.89	10.6	10.6	9.11	10.0±0.3
	15μg	18.9	19.5	10.4	11.8	4.67	8	8.7±1.6
post	*10μg*	20.1	12.7	10	9.67	9.33	7.22	9.1±0.6
	15μg	18.6	15.6	7.67	7.11	11.2	7.89	8.5±0.9

Table 7.8 Magnitude of MAP responses to 10 and 15μg ACh pre- (-1day), during- (+1 day to +4 day) and post- vehicle (n=2, fetuses shown individually) and L-NAME (n=4, fetuses shown individually) infusion. Values at any given dose are shown as summary measures of 3 replicate administrations. †P<0.01, significantly different from -1day, by paired t-test.

		VEHICLE		L-NAME			
	SNP dose	*fetus 1*	*fetus 2*	*fetus 1*	*fetus 2*	*fetus 3*	*fetus 4*
-1 day	*40μg*	16.4	14.7	18.3	14.1	16.9	13.8
	100μg	14.2	19.2	16.2	14	16.6	17.3
+1 day	*40μg*	20.7	15	14.3	18.3	17.2	14.9
	100μg	21.6	19.1	17.9	14.4	18.6	16.9
+2 day	*40μg*	19.3	13.8	15.3	12.8	21.8	15
	100μg	17.3	17.1	17.3	15.4	18	16.9
+3 day	*40μg*	19	17.9	11.8	16	22.8	16.8
	100μg	23.1	19	12.6	12.8	21.3	20.2
+4 day	*40μg*	17.4	17	18.3	15.6	14.3	16.1
	100μg	19.7	17.9	13.8	17.7	19.4	17.7
post	*40μg*	-	10.5	11.8	17.6	20.6	18
	100μg	-	18.4	18.4	18.2	24.4	19.8

Table 7.9 Magnitude of MAP responses to 40 and 100μg SNP pre- (-1day), during- (+1 day to +4 day) and post- vehicle (n=2, fetuses shown individually) and L-NAME (n=4, fetuses shown individually) infusion. Values at any given dose are shown as summary measures of 3 replicate administrations.

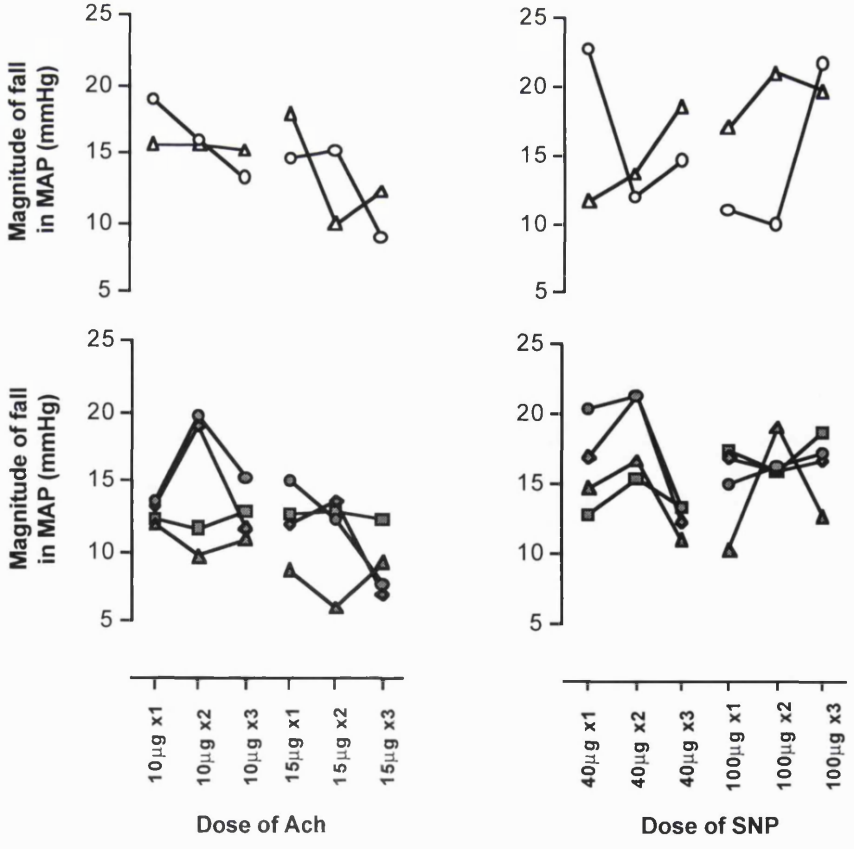


Figure 7.18 Magnitude of fall in MAP in response to 3 replicate doses of ACh (10 and 15μg) and SNP (40 and 100μg) 1 day prior to the onset of vehicle (n=2, individual fetuses ○ and Δ) and L-NAME (n=4, individual fetuses ■, ▲, ◆ and ●) infusion.

7.4.7 ECoG and fetal breathing movements

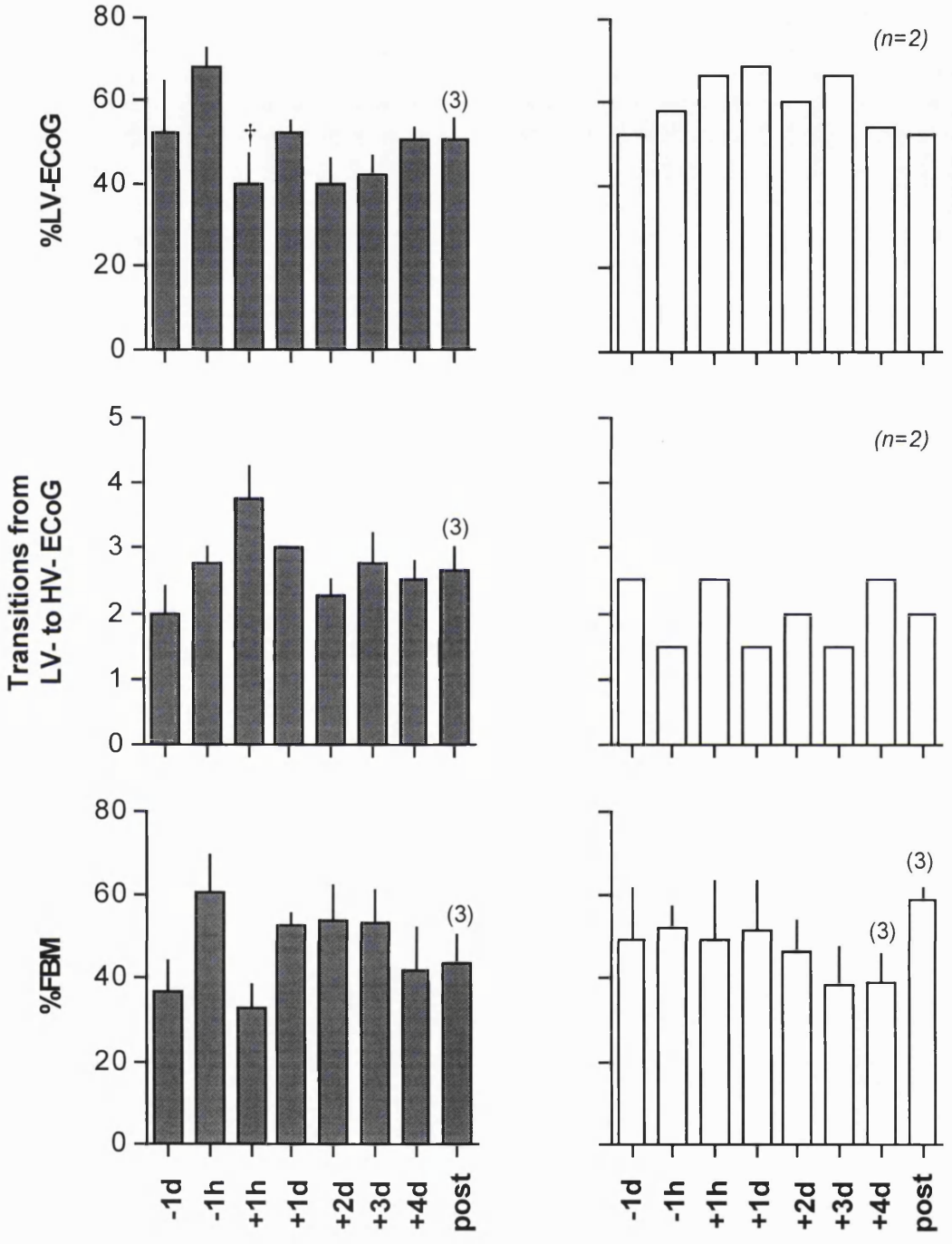


Figure 7.19 %LV-ECoG, number of transitions from LV- to HV-ECoG and %FBMs before (-1d and -1h), during (+1h, +1d, +2d, +3d, +4d) and after (post) vehicle (open bars) and L-NAME (filled bars) infusion. Values are shown as mean±S.E.M. n=4 except where indicated by bracketed numbers. †P<0.05, significantly different from -1 h, by paired t-test.

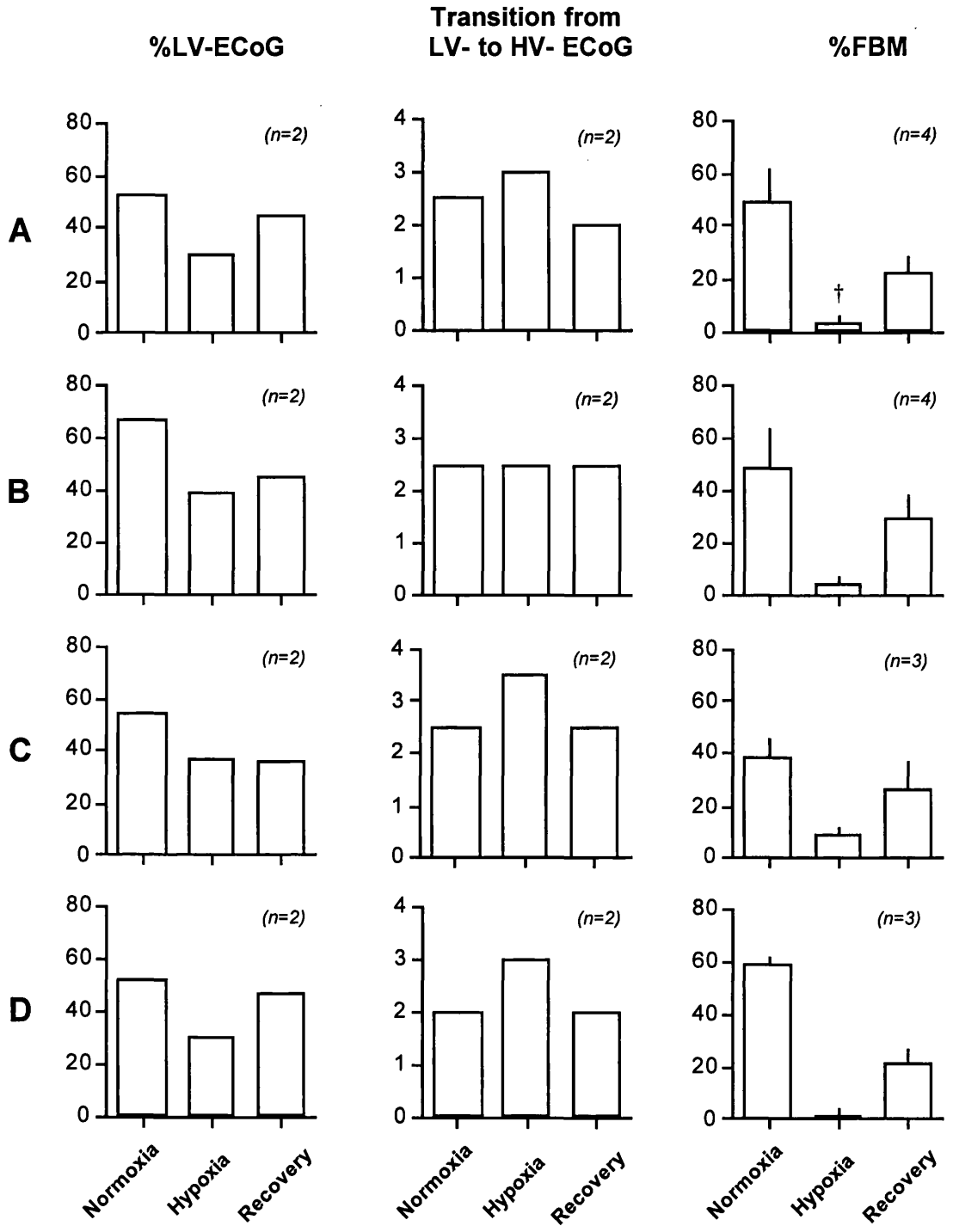


Figure 7.20 ***VEHICLE GROUP:*** %LV-ECoG, number of transitions form LV- to HV-ECoG and %FBMs (A) pre-, (B) after 1 h, (C) after 4 days and (D) post- continuous vehicle infusion, during normoxia, hypoxia and recovery. Values are shown as mean±S.E.M. The number of fetuses is indicated by the bracketed numbers. †P<0.05, significantly different from normoxia by paired t-test.

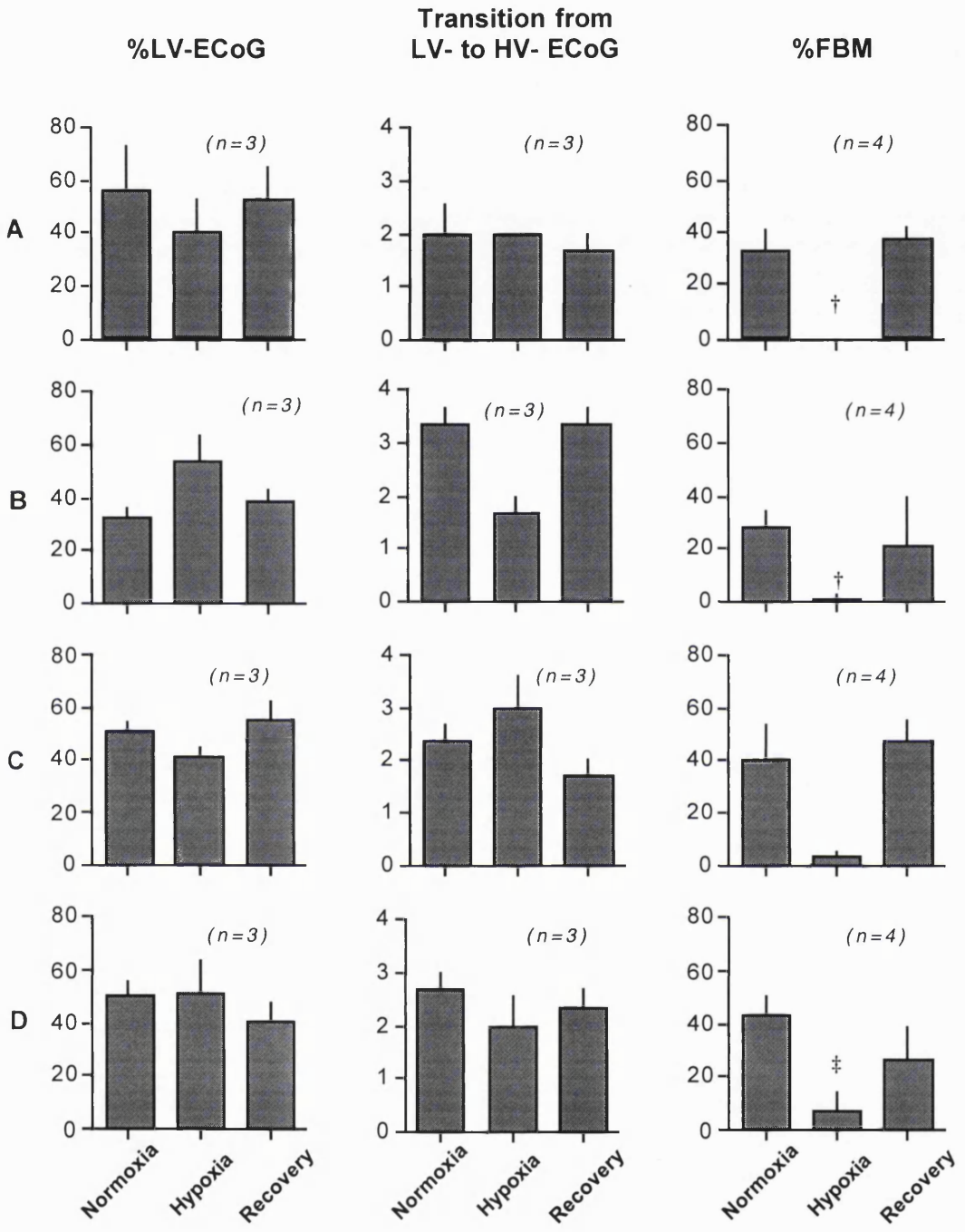


Figure 7.21 ***L-NAME GROUP:*** %LV-ECoG, number of transitions form LV- to HV-ECoG and %FBMs (A) pre-, (B) after 1 h, (C) after 4 days and (D) post- continuous L-NAME infusion, during normoxia, hypoxia and recovery. Values are shown as mean±S.E.M. The number of fetuses is indicated by the bracketed numbers. †P<0.05 and ‡P<0.01, significantly different from normoxia by paired t-test.

From daily measurements, the time spent in LV-ECoG was lower 1 h post-, compared to 1 h pre-L-NAME infusion onset and correspondingly the incidence of FBMs was lower, but overall there was no obvious trend for FBMs or ECoG activity to increase or decrease throughout either the vehicle or L-NAME infusion period (Figure 7.19).

In the 2 vehicle group fetuses in which measurements were obtained, the time spent in LV-ECoG tended to decrease, and number of transitions from LV- to HV-ECoG to either rise or stay the same, during the hypoxic challenges. The incidence of FBMs tended to fall during hypoxia in all groups although this only reached significance in the pre-infusion period (A (n=4), Figure 7.20). Statistical analysis was not carried out in groups C and D due to the small sample size. In the L-NAME group FBMs were lower during all the hypoxic challenges although this did not reach significance during the challenge after 4 days of infusion (C. Figure 7.21). In pre- (A) and 4 days post- (C) infusion periods LV-ECoG tended to decline during hypoxia.

7.5 Discussion

The results presented in this Chapter confirm that L-NAME administration produces an acute rise in MAP and fall in FHR. As this infusion continued, while FHR remained lower, MAP returned towards pre-infusion levels. In addition preliminary data suggests that continuous chronic NOS-inhibition causes a gradual fall in FBF.

Methodological considerations

The use of L-NAME

Previously, long-term NOS-inhibition studies in the adult rat have used L-NAME, which has the advantage in that it can be dissolved in drinking water (Ribeiro, Antunes, deNucci, Lovisolo and Zatz, 1992; Qiu, Engels and Baylis, 1994) or administered by gavage (Jover, Herizi, Ventre, Dupont and Mimran, 1993). The dose per hour of L-NAME used in the present study was the same as that of the previous Chapter and therefore allowed a degree of comparison to be made between the two studies [6.2.2].

The problems associated with assessing NOS activity *in vivo* were discussed in Chapter 6 [section 6.5]. Notwithstanding the potential difficulties of interpretation, an attempt to gain a degree of information on NOS activity was made in the present study by investigating the MAP response to ACh on a daily basis. There was no difference between the magnitude of the MAP response to the high (15μg) and low (10μg) doses of ACh throughout the protocol. Furthermore it was interesting that prior to the onset of infusion (-1 day) the depressor response was in fact less with 15μg than it was with 10μg ACh in all but one of the fetuses. Thus while pressure had been allowed to return to baseline levels between doses (ca. 10 min), a tachyphylaxis over the course of the 2 doses (in triplicate) may have occurred. On the other hand it is possible that the doses of ACh chosen were too similar and that any increase in response to the dose-increase was masked by overlapping variation to the individual doses themselves. The results showed that the response to 10μg ACh tended to be smaller following L-NAME but not vehicle

infusion. This indicates that the mechanisms which mediate ACh depressor responses had been partially inhibited, but that a substantial response remains in agreement with previous adult studies (Gardiner, Compton, Kemp and Bennett, 1990; Richard, Gosgnach, Drieu la Rochelle, Giudicelli and Berdeaux, 1991; Tresham, Dusting, Coghlan and Whitworth, 1991; Jover *et al.*, 1993) and suggests that other factors, such as prostanoids (Woolfson and Poston, 1990) or an endothelial-derived hyperpolarising factor (see McCarthy, Woolfson, Raju and Poston, 1993) may contribute to mediating the relaxant response to ACh.

Previously it has been shown that treatment with NOS-inhibitors causes an up-regulation of the 'receptor' for NO, guanylate cyclase, *in vivo*. Consequently there is a greater response to nitrovasodilators, such as SNP, that work by activation of guanylate cyclase (see Moncada, Palmer and Higgs, 1991). It was suggested that longer-term inhibition of NOS could result in a chronic type of supersensitivity as a result of *de novo* guanylate cyclase synthesis. However the results of the present study, while preliminary, do not support an increase in sensitivity after acute or chronic L-NAME infusion.

Animals involved in study

Five L-NAME-group and 4 vehicle-group fetuses were originally involved in this study.

In 1 fetus of the L-NAME group hypoxic challenges were only made on 2 occasions.

Daily FHR data were obtained in all fetuses. Hypoxic and daily MAP data were not available in one vehicle-group fetus due to a blocked arterial catheter. Daily and hypoxic FBF data was available in all vehicle- and 2 out of 5 of the L-NAME-group fetuses. Daily and hypoxic CBF data was available in 3 out of 4 of the vehicle group. In the L-NAME group CBF measurements were made daily in 3, and hypoxic measurements in only 2 fetuses. Where blood flow measurements were unavailable this was due to total absence of flow signal probably due to insufficient peritoneal fluid surrounding the vessel.

Baroreflex responses to phenylephrine, and ACh / SNP trials were only carried out in 2 out of 4 vehicle-group and 4 out of 5 L-NAME group fetuses.

During daily and hypoxic recording periods ECoG activity was not measurable in 2 vehicle-, and 1 to 2 L-NAME-group, fetuses due to mechanical problems with the equipment. Daily FBMs measurements were obtained in all vehicle-group fetuses except for the last 2 days of the protocol when data from 1 fetus was missing. Consequently FBMs data was not available for this fetus during hypoxic challenges on these 2 days.

Statistical analysis was carried out only when the number of fetuses with any given variable was > 3. It was beyond the time constraints of this thesis to conduct more experiments, however it is obvious that more experiments will have to be carried out before any firm conclusions can be made on the effect of chronic L-NAME on fetal cardiovascular control.

NOS and basal cardiovascular control

With the infusion of vehicle, FHR gradually declined over the time course of the protocol in agreement with other fetal sheep studies (Kitanaka, Alonso, Gilbert, Siu, Clemons and Longo, 1989). This may be due to increasing tone from the maturing parasympathetic nervous system (Thornburg and Morton, 1993) or to changes at the level of the SA-node (Hanson, 1993). The initial fall in FHR with acute L-NAME infusion confirmed the findings presented in Chapter 6. As L-NAME infusion continued FHR remained lower, and then increased again so that after 3-4 days of L-NAME infusion FHR was below pre-infusion levels but no different from FHR in the vehicle group (see Figure 7.2). NOS has been localised in cardiac ganglion cells and nerve fibres innervating the sinoatrial and atrioventricular nodes (Klimaschewski *et al.*, 1992). One possible explanation of the present results is that NO-synthesis contributes to the SA node-mediated decline in FHR with gestation, so that the gestational age (seen in vehicle group) and chronic L-NAME infusion effects end up being the same. The increase in FHR after 3-4 days L-NAME may suggest that NOS-inhibition by L-NAME was losing its potency perhaps due to an up-regulation of NOS, or alternatively an up-regulation of guanylate cyclase could increase the effect of other vasodilators that act by stimulating guanylate cyclase (Moncada, Rees, Schulz and Palmer, 1991).

Previous adult rat studies have shown a sustained rise in MAP with chronic L-NAME infusion (Jover, Herizi, Ventre, Dupont and Mimran, 1993; Baylis, Mitruka and Deng, 1992; Ribeiro, Antunes, Nucci, Lovisolo and Zatz, 1992; Qiu, Engels and Baylis, 1994) which is thought to be mediated by vasopressor mechanisms such as AT_1 receptor stimulation (Jover *et al.*, 1993) and/or α_1-adrenergic receptor stimulation (Qiu *et al.*, 1994). Arginine vasopressin is another vasoactive hormone which could contribute to a sustained hypertension since its plasma levels are increased by acute L-NAME administration (Goyer, Bui, Chou, Evans, Keil and Reid, 1994). The results of the present study however show that pressure returned towards pre-infusion levels after the initial hypertensive response to L-NAME. The absence of a sustained hypertension may simply reflect a pressure 'adaptation' from an up-regulation of NOS. However, it is possible that some differences in the involvement of vasopressor mechanisms, such as AT_1 receptor stimulation, may exist between the adult studies and the present fetal study. Angiotensin II assay results presented in Chapter 5 show that acute L-NAME infusion

produces a fall in plasma [AII] which is either pressure driven or via a direct intrarenal action. In the adult rat plasma [AII] has been shown to be identical with and without chronic L-NAME (Jover, Herizi, Ventre, Dupont and Mimran, 1993), although others have shown PRA to fall after 3 weeks L-NAME administration (Navaro *et al.*, 1994). Measurement of plasma [AII] will be warranted in the present study once additional fetuses have been recruited, although it may be that the use of an AT_1 receptor antagonist would be more effective in determining any role for the RAS. A decline in FHR over gestational age range is accompanied by a rise in MAP (see Hanson, 1993). In the vehicle-infused group because of the small number of fetuses in which MAP measurements were obtained it was not possible to determine whether such a rise occurred over the time course of the present study. Determination of such a rise in MAP will be necessary in order to fully interpret the significance of the MAP adaptation to chronic L-NAME infusion.

NO-synthesis has previously been suggested to be involved in fetal development, in particular of the peripheral vasculature (Diket *et al.*, 1994). Recent work by Voelker *et al.* (1995) suggests that constitutive rather than inducible NOS is likely to be crucial in fetal growth. The results presented in this Chapter show a gradual fall in FBF in two L-NAME-infused fetuses which lends support to the idea of NO involvement in peripheral vascular development. However it was not possible to determine the relative contribution of the NOS isoforms in the present study.

Acute L-NAME infusion tended to decrease the incidence of FBMs suggesting, as in Chapter 6, a role for NO in the tonic regulation of FBMs. However unlike Chapter 6, this decrease in FBMs occurred with a *decrease* in the time spent in LV-ECoG activity. The reason for this discrepancy between the studies is not clear. Chronic L-NAME inhibition does not appear to affect the incidence of LV-ECoG activity or the incidence of FBMs, however further experimentation will be required.

NOS and cardiovascular function

As well as monitoring the effect of chronic L-NAME infusion on basal cardiovascular control, it was the aim of this study to gain information on its effects on cardiovascular function by testing the cardiovascular responses to 1 h isocapnic hypoxia and the baroreflex response to a phenylephrine pressor response. The hypoxic challenges were identical to those presented in the previous Chapters [2.5.1]. The rapid initial bradycardia at the onset of hypoxia was similar in all the hypoxic challenges of the vehicle and L-NAME groups. The data differs from that of Chapter 6 in that the magnitude of the fall in FHR was not significantly lower 1 h after L-NAME infusion-onset than the fall before infusion started, but it is possible that the smaller group size of the present study may account for this difference. The hypoxic challenge given 1 h post L-NAME infusion

onset, a time at which a large rise in pressure had been observed, elicited no further rise in MAP. This is in agreement with the results of the previous acute L-NAME study [6.4.4]. In accordance with previous studies (Chapter 6, and Giussani, Spencer, Moore, Bennet and Hanson, 1993) MAP tended to rise during the other hypoxic challenges to the L-NAME group, although this only reached significance in the post-infusion period. The lack of significance is likely to be attributed to the small group size (n=4) since there were no methodological differences. From the numbers obtained in the study to date, it was not possible to determine the effect of chronic L-NAME infusion on carotid and peripheral blood flow responses to hypoxia. There was no apparent effect of chronic NOS-inhibition on ECoG and FBMs during hypoxia, however further experiments will be required before full statistical analysis can be carried out.

Baroreceptor sensitivity is known to fall (shift of baroreflex curve to the right) over ca. 100 to 135 (Blanco, Dawes, Hanson and McCooke, 1988) and ca. 114 to 129 days gestation (Crowe, Bennet and Hanson, 1995) in the sheep fetus. In the present study there was no apparent shift in the baroreflex curve to the right despite the fall in FHR which was observed over this period in the two vehicle-group fetuses, although additional fetuses will be required to confirm this. Chronic L-NAME administration has been shown to reduce arterial baroreceptor gain in some adult rat studies (Scrogin, Veelken and Luft, 1994), but not in others (Vasquez, Cunha and Cabral, 1994). Indeed some portion of the L-arginine-NO-cGMP pathway is thought to attenuate the acute (rapid) resetting that occurs after a sustained increase in pressure in the anaesthetised adult rat (Vargas da Silva, Dias da Silva, Ballejo, Salgado and Salgado, 1994). It has been suggested that endogenous NO acts directly on CNS, rather than on the arterial baroreceptor reflex, to inhibit sympathetic nerve activity ([1.6.1] Jimbo, Suzuki, Ichikawa, Kumagai, Nishizawa and Saruta, 1994). Two levels at which NO-synthesis can act in the regulation of blood pressure therefore seem possible: first, in an autocrine manner at the vascular level and second, by interaction at some other point in the baroreflex pathway. The NTS is the site of first synapse of arterial baroreceptor afferents. Nitric oxide is known to be produced from adult rat NTS neurones and acts to increase the activity of adjacent neurones in the NTS by an increase in cGMP (Tagawa *et al.*, 1994). However in the present study there was no obvious difference between the baroreflex sensitivity of vehicle and L-NAME groups, nor was there any apparent change in the baroreflex gain with chronic L-NAME infusion. Thus these results do not support a role for endogenous NO in the regulation the fetal baroreflex response to a rise in arterial pressure in agreement with adult rat studies (Gardiner, Compton, Kemp and Bennett, 1991), although firm conclusions cannot be drawn until more animals have been included in this study. Any previously observed effect of L-NAME on baroreflex function is thought to result from a direct action of L-NAME, perhaps at the NTS, rather than via an alteration of vascular distensibility (Scrogin, Veelken and Luft, 1994). In this

study the baroreflex response was not tested after acute (ca. 1 h) L-NAME since its response would have been confounded by the hypoxic challenge that was given at this time. However in light of the potential direct effects of L-NAME on the CNS it may be important to address its acute actions in future studies.

7.6 Conclusion

In conclusion, the acute effects of L-NAME on FHR and MAP confirm the results presented in Chapter 6. After *chronic* L-NAME infusion FHR remained lower and then increased to the vehicle group level, while MAP returned towards pre-infusion levels. A degree of adaptation is indicated which could indicate a decline in the efficacy of L-NAME over time, an 'up-regulation' of other mechanisms, or in the case of FHR, a role for NOS in the SA node mediated decline in FHR during gestation. The gradual decline in FBF during the infusion of L-NAME in 2 fetuses lends support to a role for NOS in peripheral cardiovascular control, but undoubtedly further experiments will be necessary to confirm this. In the future therefore, it will be important to extend the preliminary findings of this study but also to put these systemic cardiovascular measurements in the context of fetal growth. To do this it will be vital to utilise molecular techniques of fetal growth assessment such as plasma and tissue [IGF] and [IGFBP], and DNA synthesis measurement.

CHAPTER 8
GENERAL DISCUSSION

8.1 Overview of thesis

This thesis has investigated mechanisms, reflex neuronal, endocrine and local hormonal, underlying fetal cardiovascular control during normoxia and hypoxia. The key findings from these studies are:

CHAPTER 3: A component of the initial fall in RBF during acute hypoxia is reflexly mediated with a CSN afferent- and an α-adrenergic nerve efferent- limb. In both intact and CSD fetuses the fall in RBF after 30 min hypoxia is likely to be contributed to by catecholamines, and there appears to be no difference between the two groups in the rise in plasma [adrenaline] and [noradrenaline] during hypoxia. It seems likely that during hypoxia a balance exists between vasoconstrictor and vasodilator factors in the control of RBF, and I have presented preliminary data which suggests that NOS-inhibition in CSD fetuses alters RBF responses to hypoxia. Lastly, the change in RBF during hypoxia does not appear to be a major determinant of UO.

CHAPTER 4: The rise in plasma [AII] during hypoxia is not altered by section of the CSNs, thus it seems likely that vasoconstrictor hormones other than AII account for the proposed non α-adrenergic chemoreflexly released vasoconstrictor factor (see [1.5.2-catecholamines] and Figure 8.1). The removal of chemoreflex mechanisms by CSD uncovers a role for AII in the control of peripheral (i.e. FBF) blood flow during hypoxia.

CHAPTER 5: Endogenous (probably endothelial-derived) ET-1 is implicated in the regulation of basal CVR and FHR. The modulation of FHR appears to persist throughout hypoxia and provide a background upon which the chemoreflex and endocrine effects are superimposed. Endothelin-1 modulates CVF during hypoxia, but does not alter the fall in FBF or rise in FVR during hypoxia.

CHAPTER 6: Endogenous NO-synthesis (once again probably derived from the endothelium) affects peripheral and carotid vascular resistance and blood flow, MAP and FHR during normoxia. As in Chapter 5, NOS modulation of FHR persists throughout hypoxia with the effects of other control mechanisms superimposed. A stiking feature of the results is that a large proportion of the rise in CBF during hypoxia appears to be due to increased synthesis of NO. The rise in plasma [AII] seen during hypoxia is absent during NOS-inhibition. It was beyond the scope of this thesis to determine the

mechanisms underlying this effect, but it is interesting to note that in spite of the absence of a rise in circulating [AII], the peripheral vascular responses to hypoxia remain unaltered.

CHAPTER 7: After the onset of NOS-inhibition there was an acute fall in FHR and rise in MAP in accordance with the observations made in Chapter 6. As the infusion continued FHR remained lower and then rose again so that after 3 days of L-NAME infusion, FHR was no different from vehicle-treated group. After the acute rise, MAP returned towards pre-infusion levels. A gradual decline in FBF was observed in 2 fetuses during the infusion of L-NAME which may implicate NOS in peripheral vascular development.

The results of the investigations in this thesis, as outlined above, can be categorised according to three levels of cardiovascular control: neuronal, endocrine and local, as discussed in Chapter 1 [1.5.4]. It is too simplistic to suppose that one factor holds the complete answer to cardiovascular control, indeed there is an ever-increasing body of literature on the relationship between the control systems, whether it be: additive, e.g. that MAP can be maintained as long as one of the AII, AVP or α-adrenergic pressor systems is kept intact (Paller and Linas, 1984); or interactive, where either one substance *mediates* the effect of another, for example there is evidence to suggest that NO mediates in part the vasodilator action of bradykinin, or a substance *modulates* the action of other *in vivo*, a role that has been suggested for NO [see 1.5.4]. In this Chapter I will attempt to summarise the results contained in this thesis in the context of the interaction between cardiovascular control mechanisms.

8.2 No single pressor system provides the complete answer to cardiovascular control

Adult work has already suggested that under basal conditions blood pressure is maintained as long as one of AVP, AII and α-adrenergic systems remains intact (Paller and Linas, 1984). In Chapter 4 I presented results from experiments which combined the removal of carotid chemoreflex mechanisms and ACE-inhibition. Under basal conditions these manipulations, combined or alone, did not alter FHR, MAP or blood flow to the carotid or femoral vascular beds which either suggests that the RAS is not the pivotal pressor factor in the fetus under normoxic conditions or that other pressor systems are upregulated to compensate for the removal of AII.

As described in Chapter 1 [1.5.2-catecholamines], it has been proposed that the CSNs may mediate the release of vasoconstrictor hormones to complement the efferent α-adrenergic limb, since while intact fetuses survive following α-adrenergic blockade, α-

adrenergic blockade combined with CSD is deleterious to fetal survival in hypoxia. Arginine vasopressin does not appear to fulfil this role since CSD does not attenuate the rise in plasma [AVP] during hypoxia (Giussani, McGarrigle, Spencer, Moore, Bennet and Hanson, 1994b). Angiotensin II is an alternative candidate [1.5.2-angiotensin II]. I examined this possibility by measuring plasma [AII] in response to hypoxia in intact and CSD fetuses. During hypoxia I observed a rise in plasma [AII] in accordance with previous fetal sheep studies (Broughton-Pipkin, Lumbers and Mott, 1974). However this increase was not attenuated in CSD fetuses. Previously no information has been available as to whether the rise in plasma [catecholamines] is chemoreflexly mediated. In Chapter 3 I obtained preliminary measurements in intact and CSD fetuses which suggest that plasma [adrenaline] and [noradrenaline] rise to similar levels during hypoxia in intact and CSD fetuses. Therefore I suggest that another vasoconstrictor may account for the survival of the intact fetus following α-adrenergic blockade (Figure 8.1).

Angiotensin II may not contribute to the rapid chemoreflex components of the fetal cardiovascular responses to hypoxia, but it does still rise during hypoxia and therefore may contribute to the sustained components of the cardiovascular responses to hypoxia, such as the maintained peripheral vasoconstriction and the slower rise in MAP. In Chapter 4 I observed that in intact fetuses neither ACE inhibition nor CSD alone altered the MAP or peripheral blood flow responses to hypoxia. However when carotid reflex mechanisms were removed a role for AII in the fall in peripheral blood flow and rise in MAP during hypoxia was revealed. This finding is consistent with the concept that no single mechanism is responsible for cardiovascular control at rest (Paller and Linas, 1984) or during stress such as haemorrhage (Scroop, Stankewytsch-Janusch and Marker, 1992). This idea is reinforced further by the AII assay results presented in Chapter 6 which show that the rise in plasma [AII] during hypoxia is inhibited by the infusion of L-NAME: despite the absence of a rise in [AII], MAP rises and FBF falls during hypoxia to a similar level as in the vehicle-infused group where [AII] did rise.

In previous studies the CSNs have been shown to provide the afferent limb of rapid chemoreflex FHR and systemic and pulmonary vascular responses to hypoxia (Moore and Hanson, 1991; Giussani, Spencer, Moore, Bennet and Hanson, 1993). In Chapter 3 I reported that in CSD fetuses during hypoxia there was an initial rise in RBF which was followed by a gradual fall in RBF to levels similar to those seen in the intact fetus. This initial rise in flow was also observed by Robillard *et al.* (1986) after renal denervation. Thus it seems likely that the initial hypoxic-fall in RBF is mediated by a carotid chemoreflex with a CSN afferent- and a renal nerve efferent-limb (Figure 8.1).

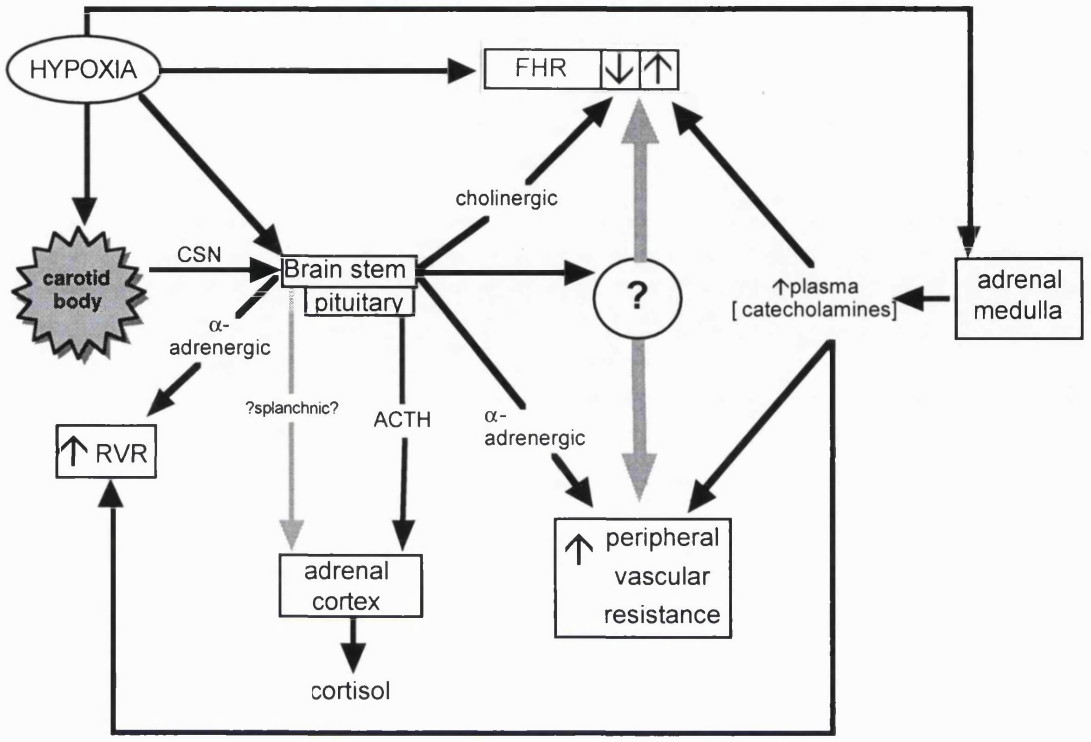


Figure 8.1 Flow diagram to show the carotid chemoreflex contribution to the initial fall in RBF and the question of the unidentified reflexly mediated vasoconstrictor factor (?). The black arrows represent known pathways whereas the grey arrows denote those that have yet to be identified.

As hypoxia proceeds a rise in plasma [catecholamines] is likely to contribute to the fall in RBF in both groups especially since there is no apparent difference between intact and CSD fetuses in the [adrenaline] and [noradrenaline] attained during hypoxia.

8.3 Do local mechanisms of vasodilatation and vasoconstriction modulate / mediate cardiovascular control?

The vascular endothelium has already been identified in Chapter 1 as a source of vasoactive substances [1.5.3]. In Chapter 5, endogenous ET-1 was implicated in the regulation of basal CVR and FHR. In Chapter 6 and 7 I produced evidence to suggest that during normoxia endogenous NO release alters basal FHR, MAP and peripheral and carotid vascular resistance and blood flow. The anatomical location of endothelial cells, adjacent to the blood stream, means that not only are they in a prime position to interact with circulating hormonal factors but are also susceptible to the continual stimuli provided by pulsatile blood flow, and thus are able to modulate or mediate the action of other vasoconstrictor mechanisms. The modulation of FHR by these two endothelial-derived factors appears to persist throughout hypoxia with the reflex neuronal (i.e. rapid vagally-mediated bradycardia) and hormonal (i.e. the catecholamine-mediated positive chronotropic effect) effects superimposed. Contrary to the findings of others, who have

suggested that NO attenuates the vasoconstrictor response to nerve stimulation and noradrenaline in the rat tail artery *in vitro* (Vo, Reid and Rand, 1991), I have found no evidence to suggest that either NO or ET-1 modulate or mediate the fall in peripheral blood flow during hypoxia. Other studies however have suggested that the extent to which NO serves to counteract vasoconstrictor mechanisms is dependent on the vascular bed (Granstam, Wang and Bill, 1993. [see 1.5.4]). This idea of vascular bed specificity would seem to be consistent with the results presented in Chapter 6, where despite finding no role for NOS in modulating FBF changes during hypoxia, I found that a large proportion of the rise in CBF during hypoxia is mediated by an increased synthesis of NO.

In Chapter 5 and 6 I also attempted to address the interaction between circulating endocrine hormones and local endothelial-derived factors by measuring plasma [AII] during ET_A receptor blockade and NOS-inhibition. ET_A receptor blockade did not alter circulating [AII] during normoxia or hypoxia whereas, as mentioned above [8.2], blockade of NO-synthesis produced a fall in plasma [AII] during normoxia and inhibited the rise in [AII] during hypoxia. The possible mechanisms underlying the effect of NOS-inhibition on plasma [AII] are discussed in Chapter 6. My results cannot address the question of interaction between circulating AII and endothelial-derived factors at the blood-endothelium interface, since plasma [hormone] is unlikely to reflect local levels of production accurately [Chapter 5], and therefore a method of measuring the local production of ET-1 and NO is needed. This is not currently feasible for *in vivo* study.

Robillard *et al.* (1986) found the initial rise in RBF to be indomethacin-sensitive. In addition I have presented preliminary results in CSD fetuses [Figure 3.5] to suggest that NOS mediates a tonic renal vasoconstriction. Surprisingly, during hypoxia with NOS-inhibition RBF *increases*. It is possible that the action of another vasodilator substance is being unmasked. Thus a picture is emerging whereby reflex- and catecholamine-mediated renal vasoconstriction appear to operate against a background of opposing vasodilatation. There is disagreement as to whether PGs could account for the vasodilator opposition to the fall in RBF during hypoxia since blockade of PG-synthesis does not always attenuate the fall in RBF (Millard, Baig and Vatner, 1979; Arnold-Aldea, Auslender and Parer, 1991).

8.5 Future work

Following the work contained in this thesis a number of issues remain unresolved and a number of questions have been highlighted which will need to be addressed in future research.

8.5.1 Immediate questions arising from this thesis

1) In the context of the findings of other studies, the results presented in this thesis highlight the importance of pursuing the idea that no single mechanism is likely to account fully for cardiovascular control. In this vein, I suggest that one possible approach is to manipulate specific cardiovascular control mechanisms, alone and in combination with other mechanisms, since this is likely to provide more information both on their interactions and on the up-regulation of one mechanism in the absence of others. For example:

a) One of the findings in Chapter 4 was that AII was unlikely to be the unidentified chemoreflexly-released factor, as had been the finding from investigations on AVP by Giussani *et al.* (1994b). Angiotensin II, like AVP, production is controlled by a number of mechanisms. Thus it seems quite possible that even if a component of their release was chemoreflexly mediated, the effect of CSD may have been masked by the up-regulation of another control mechanism. In future studies these multifactorial mechanisms of release may need to be taken into account, possibly by challenging CSD fetuses with acute hypoxia as before but while holding other known release mechanisms constant. One possible experiment would be to measure circulating [AVP] in intact and CSD fetuses during hypoxia combined with ACE-inhibition.

b) Another issue that has arisen from the results presented in Chapter 4 in particular, is the need to pursue the idea that removal of a single pressor system does not have much effect on blood pressure regulation. To date the effect of inhibiting more than one pressor system on fetal cardiovascular control has not been investigated. This might be accomplished by combining AII receptor blockade (e.g. using DUP 753, see Chiu *et al.*, 1990), AVP V_1 cardiovascular receptor blockade (e.g. using $d(CH_2)_5$-Tyr(Me) arginine vasopressin, see Piacquadro, Brace and Cheung, 1990), and α-adrenergic blockade (e.g. using phentolamine, see Giussani, Spencer, Moore, Bennet and Hanson, 1993) in accordance with previous adult studies (Paller and Linas, 1984).

c) In Chapter 6 NOS-inhibition blocked the rise in plasma [AII] during hypoxia. Whether this effect reflected the endogenous action of NO on AII production was difficult to determine due to confounding factors such as increased MAP seen at this time. Furthermore the absence of a rise in plasma [AII] did not appear to alter the cardiovascular responses to hypoxia. Thus a future study could involve examining the effect of NOS-inhibition on the RAS during normoxia and hypoxia while controlling for the effect of changes in MAP on renal perfusion pressure. This might be achieved by occlusion of the fetal aorta (see Johnson and Freeman, 1992) during systemic L-NAME

infusion. Depending on the outcome of this experiment, the use of a systemically administered AII receptor blocker, such as DUP753, could help to delineate the relative contribution of renal and local RAS to cardiovascular control during normoxia and hypoxia.

2) I have suggested that the major control of UO during normoxia and hypoxia in the fetus is probably via the direct action of hormonal factors on the kidney. If this is to be addressed in the fetus it might be done by administering specific hormone receptor antagonists or hormone synthesis blockers intrarenally, thus avoiding the confounding effects of systemic manipulations on renal perfusion pressure and RBF. In future UO measurement studies it will be necessary to take into account indices of behavioural state such as ECoG and FBMs because, since the outset of the work contained in this thesis, evidence has emerged to suggest that UO is greater as the fetus switches between behavioural states (Stigter, Visser and Mulder, 1995).

3) In Chapter 3 I presented data on the effect of CSD on plasma [catecholamines]. Due to the variability in [catecholamines] observed between fetuses, additional fetuses will need to be studied before the contribution of carotid chemoreflex mechanisms to the rise in [adrenaline] and [noradrenaline] during hypoxia can be fully elucidated.

4) Given the key role for NOS in the rise in CBF during hypoxia seen in Chapter 6, it will be important to pursue the role of NO-synthesis in fetal cerebrovascular control. Use of radioactive microspheres or NIRS (Wyatt, Edwards and Reynolds, 1993) would allow the regional cerebral blood flow changes to be determined following either systemic or intracerebral administration of a NOS-inhibitor. Some preliminary data of this type has already been obtained in the fetus which confirms a major role for NOS in increasing cerebral blood after 6 h hypoxia (McCrabb and Harding, 1994). NO-selective measuring electrodes have been developed (Ichimori, Ishida, Fukahori, Nakazawa and Murakami, 1994), although they will need to be refined before their chronic *in vivo* use is feasible.

5) In Appendix 6 I present preliminary data on the use of ^{1}H NMR in the measurement of fetal urinary organic acids. It is possible that this technique will be of great importance in future work, either on urine, plasma or indeed whole organs, in relating blood flow changes of the type reported in this thesis to tissue metabolism.

8.5.2 Long-term focus for research

Undoubtedly great advances have been made in the field of fetal physiology since techniques of chronic fetal instrumentation have been refined. Indeed this *in vivo* technique still yields vast amounts of information on the *integrated* responses to wide-ranging perturbations of the system. It is however becoming increasingly obvious that for the mechanisms underlying cardiovascular control to be resolved, *in vivo* studies will have to go hand-in-hand with the use of molecular and other *in vitro* techniques.

1) One exciting avenue for long-term future research stems from the school of thought that the growth of individual organs of the fetus is dependent on the blood flow to them. During prolonged fetal hypoxia (24-48 h) the sheep fetus is able to maintain the redistribution of CVO, as shown by increased blood flow to the heart, brain and adrenal glands (Bocking, Gagnon, White, Homan, Milne and Richardson, 1988), and fetal oxygen consumption as long as acidaemia does not develop (Bocking, White, Homan and Richardson, 1992). However during prolonged hypoxia, after an acute fall in blood flow to skeletal muscle, a degree of adaptation of skeletal muscle blood flow is observed, so that systemic oxygen delivery becomes proportional to the worsening arterial oxygen content (Rurak, Richardson, Patrick, Carmichel and Homan, 1990). The sustained redistribution of CVO to the heart, brain and adrenal glands is thought to be vital in sustaining the growth and metabolism of these organs, while on the other hand DNA synthesis, an index of increasing cell number, is reduced in the skeletal muscle (see Bocking, 1993). Whether changes in the growth of different organs during hypoxia is governed primarily by altered blood flow or altered oxygen delivery will require further investigation.

2) The effect of acute NOS-inhibition on basal fetal cardiovascular parameters in Chapter 5 led me to suggest that NOS may play an important role in fetal cardiovascular development and the ontogeny of haemodynamic control. As a consequence of these findings I then presented preliminary data in Chapter 7 on the effect of long-term inhibition of NOS on fetal cardiovascular control and have shown a degree of adaptation in the FHR and MAP responses following the acute rise in MAP and fall in FHR. I suggest that it will be important to pursue the role of NOS in fetal cardiovascular development in the context of fetal growth, particularly since a number of recent studies implicate NOS in this control. Prolonged inhibition of NOS by L-NAME administration to the pregnant rat produced substantial IUGR (Yallampalli and Garfield, 1993; Molnar *et al.*, 1994) with pronounced effects on the hindlimb (Diket *et al.*, 1994). This is not always associated with maternal hypertension and therefore may represent a direct action on the fetus. One possible technique for the chronic administration of NOS inhibitor to the fetus in future studies would be the implantation of a mini-pump (Birnbaum *et al.*,

1994). In order to address adequately the role of NOS in fetal systemic cardiovascular control in the context of fetal growth it will be vital to be able to measure fetal growth in more accurate terms than simply relying on organ and body weights and lengths. Molecular techniques for measuring the rate of DNA synthesis in relation to protein synthesis, and tissue IGFs and IGFBPs, provide the means of assessing tissue growth and the mechanisms by which growth is affected, respectively (Hill and Han, 1991).

3) In Chapter 5 circulating plasma [ET-1] was measured in CSD fetuses, as well as in intact fetuses administered with vehicle or FR139317. It does not seem likely that plasma levels of endothelial-derived factors reflect accurately the production at the vascular level, therefore a means of continuously monitoring their *in vivo* local production is needed. Chronically implanted measuring devices, such as the NO-electrode which is currently being refined, may be of future use, however until then the only option is to remove tissue for analysis *in vitro*. This could involve measuring the expression of, for example, ET-1 and its receptor, or measurement of the tissue activity *in vitro*, for example vessels (McCarthy, Woolfson, Raju and Poston, 1993), in response to stimulation. This information could then be related to *in vivo* cardiovascular measurements made near to the time of tissue removal. From the literature, unlike the cerebral (Pearce and Longo, 1991) and pulmonary vasculature (Shaul, Farrar and Magness, 1993; North *et al.*, 1994), there appears to be relatively little information on ET receptor populations or indeed on the expression of ET or NO in the systemic vasculature of the fetus. This will need to be addressed in future work to increase the knowledge of the role of local endothelial-derived factors in the development of cardiovascular control.

REFERENCES

ABRAMS, R.M., GERHARDT, K.J. & BURCHFIELD, D.J. (1991) Behavioural state transition and local cerebral blood flow in fetal sheep. *Journal of Developmental Physiology* **15**, 283-288.

ADAMSON, S.L., MORROW, R.J., BULL, S.B. & LANGILLE, B.L. (1989) Vasomotor responses of the umbilical circulation in fetal sheep. *American Journal of Physiology* **256**, R1056-R1062.

AGUILERA, G., SCHIRAR, A., BAUKAL, A. & CATT, K.J. (1981) Circulating angiotensin II and adrenal receptors after nephrectomy. *Nature* **289**, 507-509.

AISAKA, K., GROSS, S.S., GRIFFITH, O.W. & LEVI, R. (1989) N^G-methylarginine, an inhibitor of endothelium-derived nitric oxide synthesis, is a potent pressor agent in the guinea pig: does nitric oxide regulate blood pressure in vivo? *Biochemical and Biophysical Research Communications* **160**, 881-886.

AITKIN, M. (1981) Regression models for repeated measurements. *Biometrics* **37**, 831-832.

AKAGI, K., BERDUSCO, E.T.M. & CHALLIS, J.R.G. (1990) Cortisol inhibits ACTH but not the AVP response to hypoxaemia in fetal lambs at days 123-128 of gestation. *Journal of Developmental Physiology* **14**, 319-324.

AL-OBAIDI, M. & KARIM, F. (1992) Primary effects of carotid chemoreceptor stimulation on gracilis muscle and renal blood flow and renal function in dogs. *Journal of Physiology* **455**, 73-88

ALTMAN, D.G. (1991) Practical Statistics for Medical Research. *Chapman & Hall.*

ANDERSON, D.F. & BINDER, N.D. (1989) Suprarenal aortic flow reduction versus angiotensin I infusion in fetal sheep. *Journal of Developmental Physiology* **11**, 317-321.

ANDO, J., OHTSUKA, A., KORENAGA, R. & KIMIYA, A. (1991) Effect of extracellular ATP level on flow-induced Ca_2^+ response in cultured vascular endothelial cells. *Biochemical and Biophysical Research Communications* **179**, 1192-1199.

ANDO, K., HIRATA, Y., SHICHIRI, M., EMORI, T. & MARUMO, F. (1989) Presence of immunoreactive endothelin in human plasma. *FEBS Letters* **245**, 164-166.

ANDRAWIS, N.S., GILLIGAN, J. & ABERNETHY, D.R. (1992) Endothelin-1-mediated vasoconstriction: Specific blockade by verapamil. *Clinical Pharmacology and Therapeutics* **52**, 583-589.

ARDUINI, D. & RIZZO, G. (1991) Fetal renal artery velocity waveforms and amniotic fluid volumes in growth-retarded and post-term fetuses. *Obstetrics and Gynecology* **77**, 370-373.

ARNOLD-ALDEA, S.A., AUSLENDER, R.A. & PARER, J.T. (1991) The effect of the inhibition of prostaglandin synthesis on renal blood flow in fetal sheep. *American Journal of Obstetrics and Gynecology* **165**, 185-190.

ASSALI, N.S., HOLM, L.W. & SEHGAL, N. (1962) Hemodynamic changes in fetal lamb in utero in response to asphyxia, hypoxia and hypercapnia. *Circulation Research* **11**, 423-430.

BALDWIN, B.A. & BELL, F.R. (1963) The anatomy of the cerebral circulation of the sheep and ox. The dynamic distribution of the blood supplied by the carotid and vertebral arteries to cranial regions. *Journal of Anatomy* **97**, 203-215.

BALON, T.W. & NADLER, J.L. (1994) Nitric oxide release is present from incubated skeletal muscle preparations. *Journal of Applied Physiology* **77**, 2519-2521.

BALTRONS, M.A., AGULLO, L. & GARCIA, A. (1995) Dexamethasone up-regulates a constitutive nitric oxide synthase in cerebellar astrocytes but not in granule cells in culture. *Journal of Neurochemistry* **64**, 447-450.

BANERJEE, A., ROMAN, C. & HEYMANN, M.A. (1994) Bradykinin receptor blockade does not affect oxygen-mediated pulmonary vasodilation in fetal lambs. *Pediatric Research* **36**, 474-480.

BANSAL, V., TOGA, H. & RAJ, J.U. (1993) Tone dependent nitric oxide production in ovine vessels in vitro. *Respiration Physiology* **93**, 249-260.

BARER, G., EMERY, C., STEWART, A., BEE, D. & HOWARD, P. (1993) Endothelial control of the pulmonary circulation in normal and chronically hypoxic rats. *Journal of Physiology* **463**, 1-16.

BARNES, P.J. (1994) Endothelins and pulmonary diseases. *Journal of Applied Physiology* **77**, 1051-1059.

BARTELDS, B., VAN BEL, F., TEITEL, D.F. & RUDOLPH, A.M. (1993) Carotid, not aortic, chemoreceptors mediate the fetal cardiovascular response to acute hypoxemia in lambs. *Pediatric research* **34(1)**, 51-55.

BASSENGE, E., BUSSE, R. & POHL, U. (1987) Abluminal release and asymmetrical response of the rabbit arterial wall to endothelium-derived relaxing factor. *Circulation Research* **61**, II69-73.

BAYLIS, C., MITRUKA, B. & DENG, A. (1992) Chronic blockade of nitric oxide synthesis in the rat produces systemic hypertension and glomerular damage. *Journal of Clinical Investigation* **90**, 278-281.

BECKMAN, J.S. (1991) The double-edged role of nitric oxide in brain function and superoxide-mediated injury. *Journal of Developmental Physiology* **15**, 53-59.

BEGUIN, F., DUNIHOO, D.R. & QUILLIGAN, E.J. (1974) Effect of carbon dioxide elevation on renal blood flow in the fetal lamb in utero. *American Journal of Obstetrics and Gynecology* **119**, 630-637.

BEHM, R., MEWES, H., DEMUINCK KEIZER, W.H., UNGER, T. & RETTIG, R. (1993) Cardiovascular and renal effects of hypoxia in conscious carotid body-denervated rats. *Journal of Applied Physiology* **74**, 2795-2800.

BELIK, J. (1994) Myogenic response in large pulmonary arteries and its ontogenesis. *Pediatric Research* **36**, 34-40.

BELIK, J. (1995) The myogenic response of arterial vessels is increased in fetal pulmonary hypertension. *Pediatric Research* **37**, 196-210.

BENYO, Z., KISS, G., SZABO, C., CSAKI, C. & KOVACH, A.G.B. (1991) Importance of basal nitric oxide synthesis in regulation of myocardial blood flow. *Cardiovascular Research* **25**, 700-703.

BERRY, D.D., JAEKLE, R.K. & ROSE, J.C. (1994) Effect of atrial natriuretic factor on release of arginine vasopressin and renin in fetal lambs. *American Journal of Physiology* **267**, R984-R989.

BEVAN, J.A. & HENRION, D. (1994) Pharmacological implications of the flow-dependence of vascular smooth muscle tone. *Annual Review of Pharmacology and Toxicology* **34**, 173-190.

BEVAN, J.A. & JOYCE, E.H. (1990) Flow-induced resistance artery tone: balance between constrictor and dilator mechanisms. *American Journal of Physiology* **258**, H663-H668.

BIRNBAUM, S.C., KIEN, N., MARTUCCI, R.W., GELZLEICHTER, T.R., WITSCHI, H., HENDRICKX, A.G. & LAST, J.A. (1994) Nicotine- or epinephrine-induced vasoconstriction and fetal growth in the rat. *Toxicology* **94**, 69-80.

BISSONNETTE, J.M., HOHIMER, A.R., RICHARDSON, B.S. & MACHIDA, C.M. (1984) Regulation of cerebral blood flow in the fetus. *Journal of Developmental Physiology* **6**, 275-280.

BLANCO, C.E., DAWES, G.S., HANSON, M.A. & MCCOOKE, H.B. (1982) The arterial chemoreceptors in fetal sheep and newborn lambs. *Journal of Physiology* **330**, 38P.

BLANCO, C.E., DAWES, G.S., HANSON, M.A. & MCCOOKE, H.B. (1984) The response to hypoxia of arterial chemoreceptors in fetal sheep and new-born lambs. *Journal of Physiology* **351**, 25-37.

BLANCO, C.E., DAWES, G.S., HANSON, M.A. & MCCOOKE, H.B. (1988) Carotid baroreceptors in fetal and newborn sheep. *Pediatric Research* **24**, 342-346.

BLANCO, C.E., DAWES, G.S. & WALKER, D.W. (1983) Effect of hypoxia on polysynaptic hind-limb reflexes of unanaesthetized fetal and new-born lambs. *Journal of Physiology* **339**, 453-466.

BLANK, M.A., FUORTES, M., NYREN, O. & JAFFE, B.M. (1991) Effect of endothelin-1 and vasoactive intestinal contractor on blood flow and output of vasoactive intestinal polypeptide in the feline colon. *Life Sciences* **48**, 1937-1944.

BLOCK, B.S., SCHLAFER, D.H., WENTWORTH, R.A., KREITZER, L.A. & NATHANIELSZ, P.W. (1990) Intrauterine asphyxia and the breakdown of physiologic circulatory compensation in fetal sheep. *American Journal of Obstetrics and Gynecology* **162**, 1325-1331.

BOCKING, A.D. (1993) Effects of chronic hypoxaemia on circulatory control. In: Fetus and Neonate, Physiology and Clinical Applications. The Circulation. M.A. Hanson, J.A.D. Spencer and C.H. Rodeck (eds.). *Cambridge University press* pp. 215-224.

BOCKING, A.D., GAGNON, R., WHITE, S.E., HOMAN, J., MILNE, K.M. & RICHARDSON, B.S. (1988) Circulatory responses to prolonged hypoxemia in fetal sheep. American Journal of Physiology 159, 1418-1424.

BOCKING, A.D., WHITE, S.E., HOMAN, J. & RICHARDSON, B.S. (1992) Oxygen consumption is maintained in fetal sheep during prolonged hypoxaemia. *Journal of Developmental Physiology* **17**, 169-174.

BODDY, K., DAWES, G.S., FISHER, R., PINTER, S. & ROBINSON, J.S. (1974) Foetal respiratory movements, electrocortical and cardiovascular responses to hypoxaemia and hypercapnia in sheep. *Journal of physiology* **243**, 599-618.

BOGAERT, G.A., KOGAN, B.A. & MEVORACH, R.A. (1993) Effects of endothelium-derived nitric oxide on renal hemodynamics and function in the sheep fetus. *Pediatric Research* **34**, 755-761.

BOUTROY, M-J. (1989) Fetal effects of maternally administered clonidine and angiotensin-converting enzyme inhibitors. *Developmental Pharmacology and Therapeutics* **13**, 199-204.

BOYLE, D.W., HIRST, K., ZERBE, G.O., MESCHIA, G. & WILKENING, R.B. (1990) Fetal hind limb oxygen consumption and blood flow during acute graded hypoxia. *Pediatric Research* **28**, 94-100.

BOYLE, D.W., MESCHIA, G. & WILKENING, R.B. (1992) Metabolic adaptation of fetal hindlimb to severe, nonlethal hypoxia. *American Journal of Physiology* **263**, R1130-R1135.

BRACE, R.A. (1994) Fetal fluid balance. In: Textbook of fetal physiology. Eds. G.D. Thorburn and R. Harding. *Oxford Medical Publications* pp. 205-218.

BRACE, R.A., BAYER, L.A. & CHEUNG, C.Y. (1989) Fetal cardiovascular, endocrine, and fluid responses to atrial natriuretic factor infusion. *American Journal of Physiology* **257**, R580-R587.

BRACE, R.A. & CHEUNG, C.Y. (1987) Cardiovascular and fluid responses to atrial natriuretic factor in sheep fetus. *American Journal of Physiology* **253**, R561-R567.

BRACE, R.A., MINER, L.K., SIDEROWF, A.D. & CHEUNG, C.Y. (1988) Fetal and adult urine flow and ANF responses to vascular volume expansion. *American Journal of Physiology* **255**, R846-R850.

BRACE, R.A. & MOORE, T.R. (1991) Diurnal rhythms in fetal urine flow, vascular pressure and heart rate in sheep. *American Journal of Physiology* **261**, R1015-R1021.

BRACE, R.A., WLODEK, M.E., MCCRABB, G.J. & HARDING, R. (1994) Swallowing and urine flow responses of ovine fetuses to 24h of hypoxia. *American Journal of Physiology* **266**, R1345-R1352.

BRADLEY, L.M., CZAJA, J.F. & GOLDSTEIN, R.E. (1990) Circulatory effects of endothelin in newborn piglets. *American Journal of Physiology* **259**, H1613-H1617.

BREDT, D.S., HWANG, P.M. & SNYDER, S.H. (1990) Localization of nitric oxide synthase indicating a neural role for nitric oxide. *Nature* **347**, 768-770.

BREDT, D.S. & SNYDER, S.H. (1994) Nitric oxide: A Physiologic messenger molecule. *Annual Review in Biochemistry* **63**, 175-195.

BROOKS, A.N. & CHALLIS, J.R.G. (1992) Adrenocorticotrophin responses to hypoxaemia in fetal sheep are sustained in the presence of naloxone. *Journal of Developmental Physiology* **17**, 221-225.

BROUGHTON PIPKIN, F., KIRKPATRICK, S.M.L., LUMBERS, E.R. & MOTT, J.C. (1974) Renin and angiotensin-like levels in foetal, new-born and adult sheep. *Journal of Physiology* **241**, 575-588.

BROUGHTON PIPKIN, F. LUMBERS, E.R. & MOTT, J.C. (1974) Factors influencing plasma renin and angiotensin II in the conscious pregnant ewe and its foetus. *Journal of Physiology* **243**, 619-636.

BROUGHTON PIPKIN, F. & SYMONDS, E.M. (1977) Factors affecting angiotensin II concentrations in the human infant at birth. *Clinical Science and Molecular Medicine* **52**, 449-456.

BROUGHTON PIPKIN, F., SYMONDS, E.M. & TURNER, S.R. (1982) The effect of captopril (SQ14,225) upon mother and fetus in the chronically cannulated ewe and in the pregnant rabbit. *Journal of Physiology* **323**, 415-422.

BUCHANAN, J.E. & PHILLIS, J.W. (1993) The role of nitric oxide in the regulation of cerebral blood flow. *Brain Research* **610**, 248-255.

BUDDINGH, F., PARKER, H.R., ISHIZAKI, G. & TYLER, W.S. (1971) Long-term studies of the functional development of the fetal kidney in sheep. *American Journal of Veterinary Medicine* **32**, 1993-1998.

BUSIJA, D.W. & LEFFLER, C.W. (1989) Dilator effects of amino acid neurotransmitters on piglet pial arterioles. *American Journal of Physiology* **257**, H1200-H1203.

BUSSE, R., HECKER, M. & FLEMING, I. (1994) Control of nitric oxide and prostacyclin synthesis in endothelial cells. *Arzneimittelforschung* **44**, 392-396.

BUSSE, R., MULSCH, A., FLEMING, I. & HECKER, M. (1993) Mechanisms of nitric oxide release from the vascular endothelium. *Circulation* **87**, V18-V25.

CACHOFEIRO, V., SAKAKIBARA, T. & NASJLETTI, A. (1992) Kinins, nitric oxide, and the hypotensive effect of captopril and ramiprilat in hypertension. *Hypertension* **19**, 138-145.

CAHILL, P.A., REDMOND, E.M., FOSTER, C. & SITZMANN, J.V. (1995) Nitric oxide regulates angiotensin II receptors in vascular smooth muscle cells. *European Journal of Pharmacology* **288**, 219-229.

CALDWELL, P.R.B., SEEGAL, B.C. & HSU, K.C. (1976) Angiotensin-converting enzyme: Vascular endothelial localization. *Science* **191**, 1050-1051.

CARTER, A.M. (1993) Fetal placental circulation. In: Fetus and Neonate, Physiology and Clinical Applications. The Circulation. M.A. Hanson, J.A.D. Spencer and C.H. Rodeck (eds.). *Cambridge University press* pp. 116-136.

CARTER, A.M. & GU, W. (1988) Cerebral blood flow in the fetal guinea-pig. *Journal of Developmental Physiology* **10**, 123-129.

CARTER, A.M., HOMAN, J., FRASER, M., RICHARDSON, B.S. & CHALLIS, J.R.G. (1995) Inhibition of ACTH secretion blocks hypoxia-induced increase of adrenal cortical blood flow in fetal sheep. *American Journal of Physiology* **269**, E598-E604.

CASSIN, S., KRISTOVA, V., DAVIS, T., KADOWITZ, P. & GAUSE, G. (1991) Tone-dependent responses to endothelin in the isolated perfused fetal sheep pulmonary circulation in situ. *Journal of Applied Physiology* **70**, 1228-1234.

CASTRO, M.I., VALEGO, N.K., ZEHNDER, T.J. & ROSE, J.C. (1993) Bioactive-to-immunoreactive ACTH activity changes with severity of stress in late-gestation ovine fetus. *American Journal of Physiology* **265**, E68-E73.

CHANG, J-K., MOORE, P., FINEMAN, J.R., SOIFER, S.J. & HEYMANN, M.A. (1992) K^+ channel pulmonary vasodilation in fetal lambs: role of endothelium-derived nitric oxide. *Journal of Applied Physiology* **73**, 188-194.

CHATFIELD, B.A., MCMURTRY, I.F., HALL, S.L. & ABMAN, S.H. (1991) Hemodynamic effects of endothelin-1 on ovine fetal pulmonary circulation. *American Journal of Physiology* **261**, R182-R187.

CHEN, H-C. & WOOD, C.E. (1993) The adrenocorticotropic hormone and arginine vasopressin responses to hypercapnia in fetal and maternal sheep. *American Journal of Physiology* **264**, R324-R330.

CHEUNG, C.Y. (1991) Role of endogenous atrial natriuretic factor in the regulation of fetal cardiovascular and renal function. *American Journal of Obstetrics and Gynecology* **165**, 1558-1567.

CHEUNG, C.Y. (1992) Autonomic and arginine vasopressin modulation of the hypoxia-induced atrial natriuretic factor release in immature and mature ovine fetuses. American *Journal of Obstetrics and Gynecology* **167**, 1443-1453.

CHEUNG, C.Y. (1994) Regulation of atrial natriuretic factor release by endothelin in ovine fetuses. *American Journal of Physiology* **267**, R380-R386.

CHEUNG, C.Y. & BRACE, R.A. (1988) Fetal hypoxia elevates plasma atrial natriuretic factor concentration. *American Journal of Obstetrics and Gynecology* **159**,1263-1268.

CHEUNG, C.Y., GIBBS, D.M. & BRACE, R.A. (1987) Atrial natriuretic factor in maternal and fetal sheep. *American Journal of Physiology* **252**, E279-E282.

CHIU, A.T., HERBI, W.F., MCCALL, D.E., ARDECKY, R.J., CARINI, D.J., DUNCIA, J.V., PEASE, L.J., WONG, P.C., WEXLER, R.R., JOHNSON, A.L. & TIMMERMANS, P.B.M.W.M. (1989) Identification of angiotensin II receptor subtypes. *Biochemical and Biophysical Research Communications* **165**, 196-203.

CHIU, A.T., MCCALL, D.E., PRICE, W.A., WONG, P.C., CARINI, D.J., DUNCIA, J.V., WEXLER, R.R., YOO, S.E., JOHNSON, A.L. & TIMMERMANS, P.B.M.W.M. (1990) Nonpeptide angiotensin II receptor antagonists. VII. Cellular and biochemical pharmacology of DUP 753, an orally active antihypertensive agent. *The Journal of Pharmacology and Experimental Therapeutics* **252**, 711-725.

CLAPPISON, B.H., ANDERSON, W.P. & JOHNSTON, C.I. (1981) Renal hemodynamics and renal kinins after angiotensin-converting enzyme inhibition. *Kidney International* **20**, 615-620.

CLEWLOW, F., DAWES, G.S., JOHNSTON, B.M. & WALKER, D.W. (1983) Changes in breathing, electrocortical and muscle activity in unanaesthetised fetal lambs with age. *Journal of Physiology* **341**, 463-476.

CLOZEL, M., BREU, V., BURRI, K., CASSAL, J-M., FISCHLI, W., GRAY, G.A., HIRTH, G., LOFFLER, B-M., MULLER, NEIDHART, W. & RAMUZ, H. (1993) Pathophysiological role of endothelin revealed by the first orally active endothelin receptor antagonist. *Nature* **365**, 759-761.

COCEANI, F., ARMSTRONG, C. & KELSEY, L. (1989) Endothelin is a potent constrictor of the lamb ductus arteriosus. *Canadian Journal of Physiology and Pharmacology* **67**, 902-904.

COCEANI, F., KELSEY, L. & SEIDLITZ, E. (1992) Evidence for an effector role of endothelin in closure of the ductus arteriosus at birth. *Canadian Journal of Physiology and Pharmacology* **70**, 1061-1064.

COCEANI, F., KELSEY, L. & SEIDLITZ, E. (1994) Occurrence of endothelium-derived relaxing factor - nitric oxide in the lamb ductus arteriosus. *Canadian Journal of Physiology and Pharmacology* **72**, 82-88.

COCK, M.L., WLODEK, M.E., HOOPER, S.B., MCCRABB, G.J. & HARDING, R. (1994) The effects of twenty-four hours of reduced uterine blood flow on fetal fluid balance in sheep. *American Journal of Obstetrics and Gynecology* **170**, 1442-1451.

COHEN, W.R., PIASECKI, G.J., COHN, H.E., YOUNG, J.B. & JACKSON, B.T. (1984) Adrenal secretion of catecholamines during hypoxemia in fetal lambs. *Endocrinology* **114**, 383-390.

COHEN, W.R., PIASECKI, G.J., & JACKSON, B.T. (1982) Plasma catecholamines during hypoxemia in fetal lamb. *American Journal of Physiology* **243**, R520-R525.

COHN, H.E., PIASECKI, G.J. & JACKSON, B.T. (1980) The effect of fetal heart rate on cardiovascular function during hypoxemia. *American Journal of Obstetrics and Gynecology* **138**, 1190-1199.

COHN, H.E., SACKS, E.J., HEYMANN, M.A. & RUDOLPH, A.M. (1974) Cardiovascular responses to hypoxemia and acidemia in fetal lambs. *American Journal of Obstetrics and Gynecology* **120**, 817-824.

COMLINE, R.S., SILVER & SILVER, M. (1965) Factors responsible for the stimulation of the adrenal medulla during asphyxia in the foetal lamb. *Journal of physiology* **178**, 211-238.

COOTE, J.H., HILTON, S.M. & PEREZ-GONZALEZ, J.F. (1979) Inhibition of the baroreceptor reflex on stimulation in the brain stem defence centre. *Journal of Physiology* **288**, 549-560.

CORNFIELD, D.N., CHATFIELD, B.A., MCQUESTON, J.A., MCMURTRY, I.F. & ABMAN, S.H. (1992) Effects of birth-related stimuli on L-arginine-dependent pulmonary vasodilation in ovine fetus. *American Journal of physiology* **262**, H1474-H1481.

CORNFIELD, D.N., MCQUESTON, J.A., MCMURTRY, I.F., RODMAN, D.M. & ABMAN, S.H. (1992) Role of ATP-sensitive potassium channels in ovine fetal pulmonary vascular tone. *American Journal of physiology* **263**, H1363-H1368.

COSTEROUSSE, O., ALLEGRINI, J., HUANG, H., BOUNHIK, J. & ALHENC-GELAS, F. (1994) Regulation of ACE gene expression and plasma levels during rat postnatal development. *American Journal of Physiology* **267**, E745-E753.

COURT, D.J., PARER, J.T., BLOCK, B.S.B & LLANOS, A.J. (1984) Effects of beta-adrenergic blockade on blood flow distribution during hypoxemia in fetal sheep. *Journal of Developmental Physiology* **6**, 349-358.

CREMONA, G., WOOD, A.M., HALL, L.W., BOWER, E.A. & HIGENBOTTAM, T. (1994) Effect of inhibitors of nitric oxide release and action on vascular tone in isolated lungs of pig, sheep, dog and man. *Journal of physiology* **481**, 185-195.

CROWE, C., BENNET, L. & HANSON, M.A. (1995) Blood pressure and cardiovascular reflex development in fetal sheep. Relation to hypoxaemia, weight, and blood glucose. *Reproduction Fertility and Development* **7**, 1-10.

CUNHA, R.S., CABRAL, A.M. & VASQUEZ, E.C. (1993) Evidence that the autonomic nervous system plays a major role in the L-NAME-induced hypertension in conscious rats. *American Journal of Hypertension* **6**, 806-809.

CURRIE. I.S. & BROOKS, A.N. (1992) Corticotrophin-releasing factors in the hypothalamus of the developing fetal sheep. *Journal of Developmental Physiology* **17**, 241-246.

DANIEL, S.S., STARK, R.I., HUSAIN M.K., SANOCKA, U.M. & JAMES, L.S. (1984) Excretion of vasopressin in the hypoxic lamb: comparison between fetus and newborn. *Pediatric Research* **18**, 227-231.

DANIEL, S.S., STARK, R.I., ZUBROW, A.B., FOX, H.E., HUSAIN, M.K. & JAMES, L.S. (1983) Factors in the release of vasopressin by the hypoxic fetus. *Endocrinology* **113**, 1623-1628.

DANIEL, S.S., YEH, M-N., BOWE, E.T., FUKUNAGA, A. & JAMES, L.S. (1975) Renal response of the lamb fetus to partial occlusion of the umbilical cord. *Journal of Pediatrics* **87**, 788-794.

DASHWOOD, M.R., ALLEN, S.P., LUU, T.N. & MUDDLE, J.R. (1994) The effect of the ET_A receptor antagonist, FR139317, on [^{125}I]-ET-1 binding to the artherosclerotic human coronary artery. *British Journal of Pharmacology* **112**, 386-389.

DASHWOOD, M.R., MCQUEEN, D.S., SYKES, R.M., MUDDLE, J.R., DALY, M. DE B., EVRARD, Y. & SPYER, K.M. (1993) The effects of almitrine on (^{3}H)5HT and (^{125}I) endothelin binding to central and peripheral receptors: An in vitro autoradiographic study in the cat. *Advances in Experimental Medicine and Biology* **337**, 17-23.

DAVIDGE, S.T., MCLAUGHLIN, M.K. & ROBERTS, J.M. (1994) Nitric oxide stimulates prostacyclin and thromboxane production by endothelial cells. *Proceedings of the Society for Gynecologic Investigation, Chicago IL, USA* , 0145.

DAVIDSON, D. (1987) Circulating vasoactive substances and hemodynamic adjustments at birth in lambs. *Journal of Applied Physiology* **63(2)**, 676-684.

DAVIS, J.O. AND FREEMAN, R.H. (1976) Mechanisms regulating renin release. *Physiological Reviews* **56**, 1-56.

DAWES, G.S., GARDNER, W.N., JOHNSTON, B.M. & WALKER, D.W. (1980) Activity of intercostal muscles in relation to breathing movements, electrocortical activity, and gestational age in fetal lambs. *Journal of Physiology* **307**, 47P.

DAWES, G.S., GARDNER, W.N., JOHNSTON, B.M. & WALKER, D.W. (1983) Breathing in fetal lambs: the effect of brain stem section. *Journal of Physiology* **335**, 535-553.

DAWES, G.S., LEWIS, B.V., MILLIGAN, J.E., ROACH, M.R. & TALNER, N.S. (1968) The hind limbs of foetal and new-born lambs to asphyxia and aortic chemoreceptor stimulation. *Journal of Physiology* **195**, 55-81.

DENTON, K.M. & ANDERSON, W.P. (1994) Intrarenal haemodynamic and glomerular responses to inhibition of nitric oxide formation in rabbits. *Journal of Physiology* **475**, 159-167.

DIBONA, G.F. (1989) Neural control of renal function: cardiovascular implications. *Hypertension* **13**, 539-548.

DIEGUEZ, G., GARCIA, J.L., FERNANDEZ, N., GARCIA-VILLALON, A.L., MONGE, L. & GOMEZ, B. (1993) Role of NO in goat basal cerebral circulation and after vasodilatation to hypercapnia or brief ischemias. *American Journal of Physiology* **265**, R1410-R1415.

DIKET, A.L., PIERCE, M.R., MUNSHI, U.P., VOELKER, C.A., ELOBY-CHILDRESS, S., GREENBURG, S.S., ZHANG, X-J., CLARK, D.A. & MILLER, J.S. (1994) Nitric oxide inhibition causes intrauterine growth retardation and hind-limb disruptions in rats. *American Journal of Obstetrics and Gynaecology* **171**, 1243-1250.

DOHI, Y., HAHN, A.W.A., BOULANGER, C.M., BUHLER, F.R. & LUSCHER, T.F. (1992) Endothelin stimulated by angiotensin II augments contractility of spontaneously hypertensive rat resistance arteries. *Hypertension* **19**, 131-137.

DONCKIER, J.E., HANET, C., BERBINSCHI, A., GALATI, L., ROBERT, A., VAN MECHELEN, H., POULEUR, H. & KETELSLEGERS, J-M. (1991) Cardiovascular and endocrine effects of endothelin-1 at pathophysiological and pharmacological plasma concentrations in conscious dogs. *Circulation* **84**, 2476-2484.

DONOGHUE, S., FELDER, R.B., JORDAN, D. & SPYER, K.M. (1984) The central projections of carotid baroreceptors in the cat: A neurophysiological study. *Journal of Physiology* **347**, 397-409.

DORLAND'S Medical Dictionary (1989) W.B. Saunders Company 24th Edition.

DZAU, V.J. (1984a) Vascular wall renin-angiotensin pathway in control of the circulation. *The American Journal of Medicine* **October 5**, 31-36.

DZAU, V.J. (1984b) Vascular renin-angiotensin: a possible autocrine or paracrine system in control of vascular function. *Journal of Cardiovascular Pharmacology* **6**, S377-S382.

EDELSTONE, D.I., RUDOLPH, A.M. & HEYMANN, M.A. (1980) Effects of hypoxemia and decreasing umbilical flow on liver and ductus blood flows in fetal lambs. *American Journal of Physiology* **238**, H656-H663.

EDWARDS, A.V. & JONES, C.T. (1987) The effect of splanchnic nerve stimulation on adrenocortical activity in conscious calves. *Journal of Physiology* **328**, 385-396.

EL-DAHR, S.S., DIPP, S. & CHAO, J. (1992) Differential developmental expression of the rat kininogen genes. *Pediatric Research* **32**, 649-653.

EMMELUTH, C. & BIE, P. (1992) Effects, release and disposal of endothelin-1 in conscious dogs. *Acta Physiologica Scandinavica* **146**, 197-204.

ERVIN, M.G., CASTRO, R., SHERMAN, D.J., ROSS, M.G., PADBURY, J.F., LEAKE, R.D. & FISHER, D.A. (1991) Ovine fetal renal and hormonal responses to changes in plasma epinephrine. *American Journal of Physiology* **260**, R82-R89.

ERVIN, M.G., ROSS, M.G., CASTRO, R., SHERMAN, D., LAM, R.W., CASTRO, L., LEAKE, R.D. & FISHER, D.A. (1988) Ovine fetal and adult atrial natriuretic factor metabolism. *American Journal of Physiology* **254**, R40-R46.

ERVIN, M.G., ROSS, M.G., LEAKE, R.D. & FISHER, D.A. (1992) V_1- and V_2-receptor contributions to ovine fetal renal and cardiovascular responses to vasopressin. *American Journal of Physiology* **262**, R636-R643.

ERVIN, M.G., TERRY, K.A., CALVARIO, G.C., CASTRO, R., ROSS, M.G., LEAKE, R.D. & FISHER, D.A. (1994) Vascular effects alter early-gestation fetal renal responses to vasopressin. *American Journal of Physiology* **266**, R722-R729.

ESPINOZA, M., RIQUELME, R., GERMAIN, A.M., TEVAH, J., PARER, J.T. & LLANOS. A.J. (1989) Role of endogenous opioids in the cardiovascular responses to asphyxia in fetal sheep. *American Journal of Physiology* **256**, R1063-R1068.

FARACI, F.M. & HEISTAD, D.D. (1991) Regulation of cerebral blood vessels by humoral and endothelium-dependent mechanisms. Update on humoral regulation of vascular tone. *Hypertension* **17**, 917-922.

FELDER, R.B. & MIFFLIN, S.W. (1988) Modulation of carotid sinus afferent input to nucleus tractus solitarius by parabrachial nucleus stimulation. *Circulation Research* **63**, 35-49.

FENOY, F.I., SCICLI, G., CARRETERO, O. & ROMAN, R.J. (1991) Effect of an angiotensin II and a kinin receptor antagonist on the renal hemodynamic response to captopril. *Hypertension* **17**, 1038-1044.

FERGUSON, A.V. & SMITH, P. (1990) Cardiovascular responses induced by endothelin microinjection into area postrema. *Regulatory Peptides* **27**, 75-85.

FERNANDEZ, N., GARCIA, J.L., GARCIA-VILLALON, A.L., MONGE, L., GOMEZ, B. & DIEGUEZ, G. (1993) Cerebral blood flow and cerebrovascular reactivity after inhibition of nitric oxide synthesis in conscious goats. *British Journal of Pharmacology* **110**, 428-434.

FERRER, M., ENCABO, A., MARIN, J. & BALFAGON, G. (1992) Vasoconstrictive effects of angiotensin I and II in cat femoral arteries. Role of endothelium. *General Pharmacology* **23**, 1171-1175.

FINEMAN, J.R., CHANG, R. & SOIFER, S.J. (1992) EDRF inhibition augments pulmonary hypertension in intact newborn lambs. *American Journal of Physiology* **262**, H1365-H1371.

FINEMAN, J.R., HEYMANN, M.A. & SOIFER, S.J. (1991) N^{ω}-nitro-L-arginine attenuates endothelium-dependent pulmonary vasodilation in lambs. *American Journal of Physiology* **260**, H1299-H1306.

FINEMAN, J.R., WONG, J., MORIN, F.C., WILD, L.M. & SOIFER, S.J. (1994) Chronic nitric oxide inhibition in utero produces persistent pulmonary hypertension in newborn lambs. *Journal of Clinical Investigation* **93**, 2675-2683.

FINEMAN, J.R., WONG, J. & SOIFER, S.J. (1993) Hyperoxia and alkalosis produce pulmonary vasodilation independent of endothelium-derived nitric oxide in newborn lambs. *Pediatric Research* **33**, 341-346.

FINNEY, D.J. (1990) Repeated measurements: What is measured and what repeats? *Statistics in Medicine* **9**, 639-644.

FISCHLI, W., CLOZEL, M. & GUILLY, C. (1989) Specific receptors for endothelin on membranes from human placenta. Characterization and use in a binding assay. *Life Sciences* **44**, 1429-1436.

FISHER, D.J. (1986) Comparative effects of metabolic acidemia and hypoxemia on cardiac output and regional blood flows in unanaesthetized newborn lambs. *Pediatric Research* **20**, 756-760.

FISHER, D.J., HEYMANN, M.A. & RUDOLPH, A.M. (1982) Fetal myocardial oxygen and carbohydrate consumption during acutely induced hypoxemia. *American Journal of Physiology* **242**, H657-H661.

FLAHERTY, J.T., PIERCE, J.E., FERRANS, V.J., PATEL, D.J., TUCKER, W.K. & FRY, D.L. (1972) Endothelial nuclear patterns in the canine arterial tree with particular reference to hemodynamic events. *Circulation Research* **XXX**, 23-33.

FLEMING, I., GRAY, G.A., SCHOTT, C. & STOLET, J-C. (1991) Inducible but not constitutive production of nitric oxide by vascular smooth muscle cells. European *Journal of Pharmacology* **200**, 375-376.

FORSTERMANN, U. (1994) Biochemistry and molecular biology of nitric oxide synthases. *Arzneimittelforschung* **44**, 392-396.

FORSTERMANN, U., GORSKY, L.D., POLLOCK, J.S., SCHMIDT, H.H.H.W., HELLER, M. & MURAD, F. (1990) Regional distribution of EDRF/NO-synthesizing enzyme(s) in rat brain. *Biochemical and Biophysical Research Communications* **168**, 727-732.

FORSTERMANN, U., SCHMIDT, H.H.H.W., POLLOCK, J.S., SHENG, H., MITCHELL, J.A., WARNER, T.D., NAKANE, M. & MURAD, F. (1991) Isoforms of nitric oxide synthase. Characterization and purification form different cell types. *Biochemical Pharmacology* **42**, 1849-1857.

FOXALL, P.J.D., BEWLEY, S., NEILD, G.H., RODECK, C.H. & NICHOLSON, J.K. (1995) Analysis of neonatal and fetal urine using proton nuclear magnetic resonance spectroscopy. *Archives of Disease in Childhood* **73**, F153-F157.

GARDINER, S.M., COMPTON, A.M., BENNETT, T., PALMER, R.M.J. & MONCADA, S. (1990a) Regional haemodynamic changes during oral ingestion of N^G-monomethyl-L-arginine methyl ester in conscious Brattleboro rats. *British Journal of Pharmacology* **101**, 10-12.

GARDINER, S.M., COMPTON, A.M., BENNETT, T., PALMER, R.M.J. & MONCADA, S. (1990b) Control of regional blood flow by endothelium-derived nitric oxide. *Hypertension* **15**, 486-492.

GARDINER, S.M., COMPTON, A.M., KEMP, P.A. & BENNETT, T. (1990) Regional and cardiac haemodynamic responses to glyceryl trinitrate, acetylcholine, bradykinin and endothelin-1 in conscious rats: effects of N^G-nitro-L-arginine methyl ester. *British Journal of Pharmacology* **101**, 632-639.

GARDINER, S.M., COMPTON, A.M., KEMP, P.A. & BENNETT, T. (1991) Effects of N^G-nitro-L-arginine methyl ester or indomethacin on differential regional and cardiac haemodynamic actions of arginine vasopressin and lysine vasopressin in conscious rats. *British Journal of Pharmacology* **102**, 65-72.

GARDINER, S.M., KEMP, P.A. & BENNETT, T. (1991) Effects of N^G-nitro-L-arginine methyl ester on vasodilator responses to adrenaline or BRL 38227 in conscious rats. *British Journal of Pharmacology* **104**, 731-737.

GARDINER, S.M., KEMP, P.A., MARCH, J.E., BENNETT, T., DAVENPORT, A.P. & EDVINSSON, L. (1994) Effects of an ET_1-receptor antagonist, FR139317, on regional haemodynamic responses to endothelin-1 and [$ala^{11,15}$]Ac-endothelin-1 (6-21) in conscious rats. *British Journal of Pharmacology* **112**, 477-486

GIAID, A., YANAGISAWA, M., LANGLEBEN, D., MICHEL, R.P., LEVY, R., SHENNIB, H., KIMURA, S., MASAKI, T., DUGUID, W.P. & STEWART, D.J. (1993) Expression of endothelin-1 in the lungs of patients with pulmonary hypertension. *The New Engalnd Journal of Medicine* **328**, 1732-1739.

GILES, W.B. & TRUDINGER, B.J. (1986) Umbilical cord whole blood viscosity and the umbilical artery flow velocity time waveforms: a correlation. *British Journal of Obstetrics and Gynaecology* **93**, 466-470.

GIUSSANI, D.A. (August 1992) The role of the carotid chemoreceptors in the control of the fetal cardiovascular system during hypoxaemia. *Ph.D. Thesis, University College London.*

GIUSSANI, D.A., MCGARRIGLE, H.H.G., MOORE, P.J., BENNET, L., SPENCER, J.A.D. & HANSON, M.A. (1994a) Carotid sinus nerve section and the increase in plasma cortisol during acute hypoxia in fetal sheep. *Journal of Physiology* **477**, 75-80.

GIUSSANI, D.A., MCGARRIGLE, H.H.G., SPENCER, J.A.D., MOORE, P.J., BENNET, L. & HANSON, M.A. (1994b) Effect of carotid denervation on plasma vasopressin levels during acute hypoxia in the late-gestation sheep fetus. *Journal of Physiology* **477(1)**, 81-87.

GIUSSANI, D.A., SPENCER, J.A.D., MOORE, P.J., BENNET, L. & HANSON, M.A. (1993) Afferent and efferent components of the cardiovascular reflex responses to acute hypoxia in term fetal sheep. *Journal of Physiology* **461**, 431-449.

GIUSSANI, D.A., SPENCER, J.A.D. & HANSON, M.A. (1994c) Fetal cardiovascular reflex responses to hypoxaemia. *Fetal and Maternal Medicine Reviews* **6**, 17-37.

GLUCKMAN, P.D. & JOHNSTON, B.M. (1987) Lesions in the upper lateral pons abolish the hypoxic depression of breathing in unanaesthetized fetal lambs in utero. *Journal of Physiology* **382**, 373-383.

GOLDSCHMIDT, J.E. & TALLARIDA, R.J. (1991) Pharmacological evidence that captopril possesses an endothelium-mediated component of vasodilation: effect sulfhydryl groups on endothelium-derived relaxing factor. *The Journal of Pharmocology and Experimental Therapeutics* **257**, 1136-1145.

GOLFMAN, L.S., HATA, T., BEAMISH, R.E. & DHALLA, N.S. (1993) Role of endothelin in heart function in health and disease. *Canadian Journal of Cardiology* **9**, 635-653.

GOMEZ R.A. & ROBILLARD, J.E. (1984) Developmental aspects of the renal responses to hemorrhage during converting-enzyme inhibition in fetal lambs. *Circulation Research* **54**, 301-312.

GOMEZ-NINO, A., ALMARAZ, L. & GONZALEZ, C. (1994) In vitro activation of cyclo-oxygenase in the rabbit carotid body: effect of its blockade on[^{3}H]catecholamine release. *Journal of Physiology* **476**, 257-267.

GONZALEZ-HERNANDEZ, T., GONZALEZ-GONZALEZ, B., MANTOLAN-SARMIENTO, B., MENDEZ-MEDINA, R., FERRES-TORRES, R. & MEYER, G. (1994) Transition NADPH-diaphorase activity in motor nuclei of the foetal human brain stem. *NeuroReport* **5**, 758-760.

GOYER, M., BUI, H., CHOU, L., EVANS, J., KEIL, L.C. & REID, I.A. (1994) Effect of inhibition of nitric oxide synthesis on vasopressin secretion in conscious rabbits. *American Journal of Physiology* **266**, H822-H828.

GRADY, E.F., SECHI, L.A., GRIFFIN, C.A., SCHAMBEIAN, M. & KALINYAK, J.E. (1991) Expression of AT_2 receptors in the developing rat fetus. *Journal of Clinical Investigation* **88**, 921-933.

GRANSTAM, E., WANG, L. & BILL, A. (1993) Vascular effects of endothelin-1 in the cat; modification by indomethacin and L-NAME. *Acta Physiologica Scandinavica* **148**, 165-176

GRAY, H. (1993) Anatomy. Descriptive and Surgical. *Parragon Press.*

GREEN, L.R., BENNET, L. & HANSON, M.A. (1994a) The role of endothelin-1 in peripheral vasoconstriction during acute hypoxaemia in the late gestation fetal sheep. *Proceedings of the Society for the Study of Fetal Physiology, Cairns, Australia* A32.

GREEN, L.R., BENNET, L. & HANSON, M.A. (1994b) Does inhibition of nitric oxide synthesis alter the haemodynamic responses to acute hypoxaemia in the late gestation fetal sheep? *Proceedings of The Thorburn Symposium, Hamilton Island, Australia* A36.

GREEN, L.R., BENNET, L. & HANSON, M.A. (1995a) Nitric oxide (NO) synthesis and haemodynamic responses to acute hypoxaemia in unanaesthetized fetal sheep. *Journal of Physiology* **483**, 92P

GREEN, L.R., BENNET, L. & HANSON, M.A. (1995b) The effect of chronic nitric oxide synthesis inhibition on cardiovascular control in late gestation fetuses. *Proceedings of the Society for the Study of Fetal Physiology, Malmo, Sweden* P10.

GREEN, L.R., BENNET, L., ROBSON, S. & HANSON, M.A. (1995c) Effect of carotid sinus denervation on renal blood flow and urine output during hypoxia in the late gestation ovine fetus. *Proceedings of the Society for the Study of Fetal Physiology, Malmo, Sweden* A17,

GREEN, L.R., MCGARRIGLE, H.H.G., BENNET, L. & HANSON, M.A. (1994c) The effect of acute hypoxaemia on plasma angiotensin II in intact and carotid sinus-denervated fetal sheep. *Journal of Physiology* **476**, 81P-82P

GREEN, L.R., MCGARRIGLE, H.H.G., BENNET, L. & HANSON, M.A. (1995d) Effect of carotid sinus denervation on plasma endothelin-1 during acute isocapnic hypoxemia in the late gestation ovine fetus. *Journal of The Society for Gynecologic Investigation* **2**, O46.

GREEN, L.R., MCGARRIGLE, H.H.G., BENNET, L. & HANSON, M.A. (1995e) The role of endothelin-1 in cardiovascular responses to acute hypoxia in late gestation fetal sheep. Japanese Journal of Physiology *in press.*

GREEN, L.R., ROBSON, S., BENNET, L. & HANSON, M.A. (1993) The effects of acute hypoxemia on the renal blood flow and urine output in late gestation fetal sheep. *Proceedings of the Congress of the International Union of Physiological Sciences, Glasgow.*

GRESORES, A., ROSENFELD, C.R., MAGNESS, R.R. & ROY, T.A. (1992) Metabolic clearance rate (MCR) of angiotensin II (AII) in fetal and pregnant sheep. *Pediatric Research* **31**, 60A.

GRIFFITHS, M.J.D., MESSENT, M., MACALLISTER, R.J. & EVANS, T.W. (1993) Aminoguanidine selectively inhibits inducible nitric oxide synthase. *British journal of Pharmacology* **110**, 963-968.

GRIMMINGER, F., SPRIESTERSBACH, R., WEISMANN, N., WALMRATH, D. & SEEGAR, W. (1995) Nitric oxide generation and hypoxic vasoconstriction in buffer-perfused rabbit lungs. *Journal of Applied Physiology* **78**, 1509-1515.

GRONE, H-J., LAUE, A. & FUCHS, E. (1990) Localization and quantification of [^{125}I]-endothelin binding sites in human fetal and adult kidneys-Relevance to renal ontogeny and pathophysiology. *Klinische Wochenschrift* **68**, 758-767.

GRONE, H-J., SIMON, M. & FUCHS, E. (1992) Autoradiographic characterization of angiotensin receptor subtypes in fetal and adult human kidney. *American Journal of Physiology* **262**, F326-F331.

GUILLERY, E.N. & ROBILLARD, J.E. (1993) The renin-angiotensin system and blood pressure regulation during infancy and childhood. *Pediatric Clinics of North America* **40**, 61-79.

GUILLERY, E.N., SEGAR, J.L., MERRILL, D.C., NAKAMURA, K.T., JOSE, P.A. & ROBILLARD, J.E. (1994) Ontogenic changes in renal response to α_1-adrenoceptor stimulation in sheep. *American Journal of Physiology* **267**, R990-R998.

HAEGERSTRAND, A., HEMSEN, A., GILLIS, C., LARSSON, O., & LUNDBURG, J.M. (1989) Endothelin: presence in human umbilical vessels, high levels in fetal blood and potent constrictor effect. *Acta Physiologica Scandinavica* **137**, 541-542.

HAHN, A.W.A., RESINK, T.J., SCOTT-BURDEN, T., POWELL, J., DOHI, Y. & BUHLER, F.R. (1990) Stimulation of endothelin mRNA and secretion in rat vascular smooth muscle cells: a novel autocrine function. *Cell Regulation* **1**, 649-659.

HAIGH, A.L. (1984) General Physiological Methods (A manual for advanced students). pp121-139.

HAMADA, Y., HAYAKAWA, T., HATTORI, H. & MIKAWA, H. (1993) Inhibitor of nitric oxide synthesis reduces hypoxic-ischemic brain damage in the neonatal rat. *Pediatric Research* **35**, 10-14.

HAMPL, V., ARCHER, S.L., NELSON, D.P. & WEIR, E.K. (1993) Chronic EDRF inhibition and hypoxia: effects on pulmonary circulation and systemic blood pressure. *Journal of Applied Physiology* **75**, 1748-1757.

HAN, X., SHIMONI, Y. & GILES, W.R. (1994) An obligatory role for nitric oxide in autonomic control of mammalian heart rate. *Jounal of Physiology* **476**, 309-314.

HANLEY, D.F., WILSON, D.A., FELDMAN, M.A. & TRAYSTMAN, R.J. (1988) Peripheral chemoreceptor control of neurohypophysial blood flow. *American Journal of Physiology* **254**, H742-H750.

HANSEN, J., JACOBSEN, T.N. & VICTOR, R.G. (1994) Is nitric oxide involved in the tonic inhibition of central sympathetic outflow in humans? *Hypertension* **24**, 439-444.

HANSON, M.A. (1988) The importance of baro- and chemoreflexes in the control of the fetal cardiovascular system. *Journal of Developmental Physiology* **10**, 491-511.

HANSON, M.A. (1993) The control of heart rate and blood pressure in the fetus: theoretical considerations. In: Fetus and Neonate, Physiology and Clinical Applications. The Circulation. M.A. Hanson, J.A.D. Spencer and C.H. Rodeck (eds.). *Cambridge University press* pp. 1-22.

HARPER, A.M. (1990) Physiological control of the cerebral circulation. In: Cerebral Blood Flow and Metabolism. A.M. Murray and S. Jennett (eds.) *Physiological Society Study Guides* **(number 5)** pp.1-26.

HARRIS, P.J., ZHUO, J., MENDELSOHN, F.A.O. & SKINNER, S.L. (1991) Haemodynamic and renal tubular effects of low doses of endothelin in anaesthetized rats. *Journal of Physiology* **433**, 25-39.

HARTTER & WOLOSZCUK (1989) Radioimmunoassay of endothelin. *The Lancet* **April 22,** 909.

HASHIGUCHI, K., TAKAGI, K., NAKABAYASHI, M., TAKEDA, Y., SAKAMOTO, S., NARUSE, M., NARUSE, K. & DEMURA, H. (1991) Relationship between fetal hypoxia and endothelin-1 in fetal circulation. *Journal of Cardiovascular Pharmacology* **17**, S509-S510.

HASUNUMA, K., RODMAN, D.M. & MCMURTRY, I.F. (1991) Effects of K^+ channel blockers on vascular tone in the perfused rat lung. *American Review of Respiratory Disease* **144**, 884-887.

HAY, W.W., MYERS, S.A., SPARKS, J.W., WILKENING, R.B., MESCHIA, G. AND BATTAGLIA, F.C. (1983) Glucose and lactate oxidation rates in the fetal lamb. *Proceedings of the Society for Experimental Biology and Medicine* **173**, 553-563.

HECKER, M., MITCHELL, J.A., HARRIS, H.J., KATSURA, M., THIEMERMANN, C. & VANE, J.R. (1990) Endothelial cells metabolize N^G-monomethyl-L-arginine to L-citrulline and subsequently to L-arginine. *Biochemical and Biophysical Research Communications* **167**, 1037-1043.

HECKER, M., MULSCH, A., BASSENGE, E. & BUSSE, R. (1993) Vasoconstriction and increased flow: two principle mechanisms of shear stress-dependent endothelial autacoid release. *American Journal of Physiology* **265**, H828-H833.

HECKER, M., MULSCH, A., BASSENGE, E., FORSTERMANN, U. & BUSSE, R. (1994) Subcellular localization and characterization of nitric oxide synthase(s) in endothelial cells: physiological implications. *Biochemical Journal* **299**, 247-252.

HERIN, P., KIM, J.K., SCHRIER, R.W., MESCHIA, G. & BATTAGLIA, F.C. (1988) Ovine fetal response to water deprivation: aspects on the role of vasopressin. *Quarterly Journal of Experimental Physiology* **73**, 931-940.

HEYMANN, M.A. (1984) Control of the pulmonary circulation in the perinatal period. *Journal of Developmental Physiology* **6**, 281-290.

HILL, D.J. & HAN, V.K.M. (1991) Paracrinology of growth regulation, *Journal of Developmental Physiology* **15**, 91-104.

HILL, K.J. & LUMBERS, E.R. (1988) Renal function in adult and fetal sheep. *Journal of Developmental Physiology* **10**, 149-159.

HISHIKAWA, K., NAKAKI, T., MARUMO, T., SUZUKI, H., KATO, R. & SARUTA, T. (1995) Pressure enhances endothelin-1 release from cultured human endothelial cells. *Hypertension* **25**, 449-452.

HOHIMER, A.R., BISSONNETTE, J.M., RICHARDSON, B.S. & MACHIDA, C.M. (1983) Central chemical regulation of breathing movements in fetal lambs. *Respiration Physiology* **52**, 99-111.

HOLDEN, K. (1994) Nitric oxide-from exhaust fumes to medical revolution. *The Pharmaceutical Journal* **252**,402-407.

HOLTZ, J. & GOETZ, R.M. (1994) The endothelium and the renin-angiotensin system. *Arzneimittelforschung* **44**, 397-401.

HONIG, A. (1989) Peripheral arterial chemoreceptors and reflex control of sodium and water homeostasis. *American Journal of Physiology* **257**, R1282-R1302.

HORIO, T., KOHNO, M., YOKOKAWA, K., MURAKAWA, K-I., YASUNARI, K., FUJIWARA, H., KURIHARA, N. & TAKEDA, T. (1991) Effect of hypoxia on plasma immunoreactive endothelin-1 concentration in anaesthetized rats. *Metabolism* **40**, 999-1001.

HOYER, D., WAEBER, C. & PALACOIS, J.M. (1989) [^{125}I]Endothelin-1 binding sites: Autoradiographic studies in the brain and periphery of various species including humans. *Journal of Cardiovascular Pharmacology* **13**, S162-S165.

HWA, J.J. & BEVAN, J.A. (1986) Stretch-dependent (myogenic) tone in rabbit ear resistance arteries. *American Journal of Physiology* **250**, H87-H95.

HYMAN, A., HEYMANN, M., LEVIN, D. & RUDOLPH, A. (1975) Angiotensin is not the mediator of hypoxia-induced pulmonary vasoconstriction in fetal lambs. *Circulation* **51** (suppl 2), A521.

HYNES, M.R., DANG, H. & PIPER DUCKLES, S. (1988) Contractile responses to adrenergic nerve stimulation are enhanced with removal of endothelium in rat caudal artery. *Life Sciences* **42**, 357-365.

IADECOLA, C., PELLIGRINO, D.A., MOSKOWITZ, M.A. AND LASSEN, N.A. (1994) Nitric oxide synthase inhibition and cerebrovascular regulation. *Journal of Cerebral Blood Flow and Metabolism* **14**, 175-192.

IBARRA-RUBIO, M.E., PENA, J.C. & PEDRAZA-CHAVERRI, J. (1989) Kinetic and inhibitory characteristics of serum angiotensin-converting enzyme from nine mammalian species. *Comparative Biochemistry and Physiology* **92B**, 399-403.

ICHIMORI, K., ISHIDA, H., FUKAHORI, M., NAKAZAWA, H. & MURAKAMI, E. (1994) Practical nitric oxide measurement employing a nitric oxide-selective electrode. *Reviews of Scientific Instrumentation* **65**, 1-5.

IGNARRO, L.J., BUGA, G.M., WOOD, K.S., BYRNS, R.E. & CHAUDHURI, G. (1987) Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. *Proceedings of the National Academy of Science USA* **84**, 9265-9269.

IGNARRO, L.J., RYRNS, R.E., BUGA, G.M. & WOOD, K.S. (1987) Mechanisms of endothelium-dependent vascular smooth muscle relaxation elicited by bradykinin and VIP. *American Journal of Physiology* **253**, H1047-H1082.

IHARA, M., NOGUCHI, K., SAEKI, T., FUKURODA, T., TSUCHIDA, S., KIMURA, S., FUKAMI, T., ISHIKAWA, K., NISHIKIBE, M. & YANO, M. (1992) Biological profiles of highly potent novel endothelin antagonists selective for the ET_A receptor. *Life Sciences* **50**, 247-255.

IKEDA, M., SASAGURI, M., MARUTA, H. & ARAKAWA, K. (1988) Formation of angiotensin II by tonin-inhibitor complex. *Hypertension* **11**, 63-70.

INOKUCHI, K. & MALIK, K.U. (1984) Attenuation by prostaglandins of adrenergically induced renal vasoconstriction in anaesthetized rats. *American Journal of Physiology* **246**, R228-R235.

ISAACSON, T.C., HAMPL, V., WEIR, NELSON, D.P. & ARCHER, S.L. (1994) Increased endothelium-derived NO in hypertensive pulmonary circulation of chronically hypoxic rats. *Journal of Applied Physiology* **76**, 933-940.

ISMAY, M.J.A., LUMBERS, E.R. & STEVENS, A.D. (1979) The action of angiotensin II on the baroreflex response of the conscious ewe and the conscious fetus. *Journal of Physiology* **288**, 467-479.

ISOZAKI-FUKUDA, Y., KOJIMA, T., HIRATA, Y., ONO, A., SAWARAGI, S., SAWARAGI, I & KOBAYASHI, Y. (1991) Plasma immunoreactive endothelin-1 concentration in human fetal blood: Its relation to asphyxia. *Pediatric Research* **30**, 244-247.

ITO, S., JUNCOS, L.A., NUSHIRO, N., JOHNSON, C.S. & CARRETERO, O.A. (1991) Endothelium-derived relaxing factor modulates endothelin action in afferent arterioles. *Hypertension* **17**, 1052-1056.

ITSKOVITZ, J., LAGAMMA, E.F., BRISTOW, J. & RUDOLPH, A.M. (1991) Cardiovascular responses to hypoxemia in sinoaortic-denervated fetal sheep. *Pediatric Research* **30**, 381-385.

ITSKOVITZ, J., LAGAMMA, E.F. & RUDOLPH, A.M. (1983) Baroreflex control of the circulation in chronically instrumented fetal lambs. *Circulation research* **52**, 589-596.

ITSKOVITZ, J. & RUDOLPH, A.M. (1982) Denervation of arterial chemoreceptors and baroreceptors in fetal lambs in utero. *American Journal of Physiology* **242**, H916-H920.

IVY, D.D., KINSELLA, J.P. & ABMAN, S.H. (1994) Physiological characterisation of endothelin A and B receptor activity in the ovine fetal pulmonary circulation. *Journal of Clinical Investigation* **93**, 2141-2148

IWAMOTO, H.S., KAUFMAN, T., KEIL, L.C. & RUDOLPH, A.M. (1989) Responses to acute hypoxemia in fetal sheep at 0.6-0.7 gestation. *American Journal of Physiology* **256**, H613-H620.

IWAMOTO, J. & MORIN, F.C. (1993) Nitric oxide inhibition varies with hemoglobin saturation. *Journal of Applied Physiology* **75**, 2332-2336.

IWAMOTO, H.S. & RUDOLPH, A.M. (1979) Effects of endogenous angiotensin II in the fetal circulation. *Journal of Developmental Physiology* **1**, 283-293.

IWAMOTO, H.S. & RUDOLPH, A.M. (1981a) Effects of angiotensin II on the blood flow and its distribution in fetal lambs. *Circulation Research* **48**, 183-189.

IWAMOTO, H.S. & RUDOLPH, A.M. (1981b) Role of renin-angiotensin system in response to hemorrhage in fetal sheep. *American Journal of Physiology* **240**, H848-H854.

IWAMOTO, H.S. & RUDOLPH, A.M. (1985) Metabolic responses of the kidney in fetal sheep: effect of acute and spontaneous hypoxemia. *American Journal of Physiology* **249**, F836-F841.

IWAMOTO, H.S., RUDOLPH, A.M., KEIL, L.C. & HEYMANN, M.A. (1979) Hemodynamic responses of the sheep fetus to vasopressin infusion. *Circulation Research* **44**, 430-436.

IWAMOTO, H.S., RUDOLPH, A.M., MIRKIN, B.L. & KEIL, L.C. (1983) Circulatory and humoral responses of sympathectomized fetal sheep to hypoxemia. *American Journal of Physiology* **245**, H767-H772.

IWAMOTO, J., YANG, S-P., YOSHINAGA, M., KRASNEY, E. & KRASNEY, J. (1992) N^{W}-nitro-L-arginine influences cerebral metabolism in awake sheep. *Journal of Applied Physiology* **73**, 2223-2240.

IWAMOTO, J., YOSHINAGA, M., YANG, S-P., KRASNEY, E. & KRASNEY, J. (1992) Methylene blue inhibits hypoxic cerebral vasodilatation in awake sheep. *Journal of Applied Physiology* **73**, 2226-2232.

JACKSON, B.J., MORRISON, S.H., COHN, H.E. & PIASECKI, G.J. (1989) Adrenal secretion of glucocorticoids during hypoxemia in fetal sheep. *Endocrinology* **125**, 2751-2757.

JANSEN, A.H., BELIK, J., IOFFE, S. AND CHERNICK, V. (1989) Control of organ blood flow in fetal sheep during normoxia and hypoxia. *American Journal of Physiology* **257**, H1132-H1139.

JENSEN, A., BAMFORD, O.S., DAWES, G.S., HOFMEYER, G. & PARKES, M.J. (1986) Changes in organ blood flow between high and low voltage electrocortical activity in fetal sheep. *Journal of Developmental Physiology* **8**, 187-194.

JENSEN, A. & BERGER, R. (1993) Regional distribution of cardiac output. In: Fetus and Neonate, Physiology and Clinical Applications. The Circulation. M.A. Hanson, J.A.D. Spencer and C.H. Rodeck (eds.). *Cambridge University press* pp. 23-74.

JENSEN, A., HOHMANN, M. & KUNZEL, W. (1987) Dynamic changes in organ blood flow and oxygen consumption during acute asphyxia in fetal sheep. *Journal of Developmental Physiology* **9**, 543-559.

JENSEN, A. & LANG, U. (1992) Foetal circulatory responses to arrest of uterine blood flow in sheep: effects of chemical sympathectomy. *Journal of Developmental Physiology* **17**, 75-86.

JIMBO, M., SUZUKI, H., ICHIKAWA, M., KUMAGAI, K., NISHIZAWA, M. & SARUTA, T. (1994) Role of nitric oxide in regulation of baroreceptor reflex. *Journal of the autonomic nervous system* **50**, 209-219.

JOHNSON, R.A. & FREEMAN, R.H. (1992) Pressure natriuresis in rats during blockade of the L-arginine/nitric oxide pathway. *Hypertension* **19**, 333-338.

JOHNSTON, B.M. (1991) Brain stem inhibitory mechanisms in the control of fetal breathing movements. In: The fetal and neonatal brain stem. Ed. M.A. Hanson. *Cambridge University Press* pp. 21-47.

JOHNSTON, C.I., CLAPPISON, B.H., ANDERSON, W.P. & YASUJIMA, M. (1982) Effect of angiotensin-converting enzyme inhibition on circulating and local kinin levels. *The American Journal of Cardiology* **49**, 1401-1404.

JOHNSTON, B.M. & GLUCKMAN, P.D. (1993) Peripheral chemoreceptors respond to hypoxia in pontine-lesioned fetal lambs in utero. *Journal of Applied Physiology* **75**, 1027-1034.

JONES, C.T., BODDY, K., ROBINSON, J.S. & RATCLIFFE, J.G. (1975) Pituitary and adrenal function in foetal sheep during the latter half of gestation. *Journal of Endocrinology* **67**, 26P.

JONES, C.T., RITCHIE, J.W.K. & WALKER, D. (1983) The effects of hypoxia on glucose turnover in the fetal sheep. *Journal of Developmental Physiology* **5**, 223-235.

JONES, C.T. & RITCHIE, J.W.K. (1983) The effects of adrenergic blockade on fetal response to hypoxia. *Journal of Developmental Physiology* **5**, 211-222.

JONES, C.T. & ROBINSON, R.O. (1975) Plasma catecholamines in foetal and adult sheep. *Journal of Physiology* **248**, 15-33.

JONES, C.T., ROEBUCK, M.M., WALKER, D.W., LAGERCRANTZ, H. & JOHNSTON, B.M. (1987) Cardiovascular, metabolic and endocrine effects of chemical sympathectomy and of adrenal demullation in fetal sheep. *Journal of Developmental Physiology* **9**, 347-367.

JONES, O.W. (1995) Endothelin-1 receptor blockade inhibits the cardiovascular response to exogenous big endothelin-1 and alters the response to hypoxia in the ovine fetus. *Journal of the Society for Gynecologic Investigation* **2**, 339.

JONES, O.W. & ABMAN, S.H. (1994) Systemic and pulmonary hemodynamic effects of big endothelin-1 and phosphoramidon in the ovine fetus. *American Journal of Physiology* **266**, R929-R935.

JONES, O.W., ABMAN, S.H. & WILKENING, R.B. (1994) Prolonged hypoxia increases plasma immunoreactive endothelin-1 levels in the ovine fetus. *Proceedings of The Society for Gynecologic Investigation* , 41st Annual Meeting, Chicago..

JOSE, P.A., SLOTKOFF, L.M., MONTGOMERY, S., CALCAGNO, P.L. AND EISNER, G. (1975) Autoregulation of renal blood flow in the puppy. *American Journal of Physiology* **229**, 983-988.

JOVER, B., HERIZI, A., VENTRE, F., DUPONT, M. & MIMRAN, A. (1993) Sodium and angiotensin in hypertension induced by long-term nitric oxide blockade. *Hypertension* **21**, 944-948.

KAKUCHI, J., ICHIKI, T., KIYAMA, S., HOGAN, B.L.M., FOGO, A., INAGAMI, T. & ICHIKAWA, I. (1995) Developmental expression of renal angiotensin II receptor genes in the mouse. *Kidney International* **47**, 140-147.

KANAI, A.J., STRAUSS, H.C., TRUSKEY, G.A., CREWS, A.L., GRUNFELD, S. & MALINSKI, T. (1995) Shear stress induces ATP-independent transient nitric oxide release from vascular endothelial cells, measured directly with polyphyrinic sensor. *Circulation Research* **77**, 284-293.

KANSE, S.M., TAKAHASHI, K., WARREN, J.B., GHATEI, M. & BLOOM, S.R. (1991) Glucocorticoids induce endothelin release form vascular smooth muscle cells but not endothelial cells. *European Journal of Pharmacology* **199**, 99-101.

KARIM, F. & AL-OBAIDI, M. (1993) Modification of carotid chemoreceptor-induced changes in renal haemodynamics and function by carotid baroreflex in dogs. *Journal of Phsyiology* **466**, 599-610.

KEETON, T.K., PETTINGER, W.A. & CAMPBELL, W.B. (1976) The effects of altered sodium balance and adrenergic blockade on renin release induced in rats by angiotensin antagonism. *Circulation Research* **38(6)**, 531-539.

KEITH, I.M., WILL, J.A. & WEIR, E.K. (1982) Captopril: Association with fetal death and pulmonary vascular changes in the rabbit. Proceedings of the Society for *Experimental Biology and Medicine* **170**, 378-383.

KENNEDY, R.H., HICKS, K.K., BRIAN, J.E. & SEIFEN, E. (1994) Nitric oxide has no chronotropic effect in right atria isolated from rat heart. *European Journal of Pharmacology* **255**, 149-156.

KIFOR, I. & DZAU, V.J. (1987) Endothelial renin-angiotensin pathway: Evidence for intracellular synthesis and secretion of angiotensins. *Circulation Research* **60**, 422-428.

KING, C.E., CURTIS, S.E., WINN, M.J., MEWBURN, J.D., CAIN, S.M. & CHAPLER, C.K. (1994) The role of endothelium-derived relaxing factor (EDRF) in the whole body and hindlimb vascular responses during hypoxic hypoxia. *Oxygen Transport to Tissue XVI M.C. Morgan et al. (Eds) Plenum Press New York.* 285-293.

KINGDOM, J.C.P., MCQUEEN, J., CONNELL, M.J. & WHITTLE, M.J. (1992) Maternal and fetal atrial natriuretic peptide levels at delivery from normal and growth retarded pregnancies. *British Journal of Obstetrics and Gynaecology* **99** 845-849.

KINGDOM, J.C.P., MCQUEEN, J., CONNELL, J.M.C. & WHITTLE, M.J. (1993) Fetal angiotensin II levels and vascular (type I) angiotensin receptors in pregnancies complicated by intrauterine growth retardation. *British Journal of Obstetrics and Gynecology* **100**, 476-482.

KINSELLA, J.P., IVY, D.D. & ABMAN, S.H. (1994) Ontogeny of NO activity and response to inhaled NO in the developing ovine pulmonary circulation. *American Journal of Physiology* **267**, H1955-H1961.

KINSELLA, J.P., MCQUESTON, J.A., ROSENBURG, A.A. & ABMAN, S.H. (1992) Hemodynamic effects of exogenous nitric oxide in ovine transitional pulmonary circulation. *American Journal of Physiology* **263**, H875-H880.

KITANAKA, T., ALONSO, J.G., GILBERT, R.D., SIU, B.L., CLEMONS, G.K. & LONGO, L.D. (1989) Fetal responses to long-term hypoxemia in sheep. *American Journal of Physiology* **256**, R1348-R1354.

KLIMASCHEWSKI, L., KUMMER, W., MAYER, B., COURAUD, J.Y., PREISSLER, U., PHILIPPIN, B. & HEYM, C. (1992) Nitric oxide synthase in cardiac nerve fibers and neurons of rat and guinea pig heart. *Circulation Research* **71**, 1533-1537.

KONDURI, G.G., GERVASIO, C.T. & THEODOROU, A.A. (1993) Role of adenosine triphosphate and adenosine in oxygen-induced pulmonary vasodilation in fetal lambs. *Pediatric Research* **33**, 533-539.

KOOS, B.J., MASON, B.A. & DUSCAY, C.A. (1993) Cardiovascular responses to adenosine in fetal sheep: autonomic blockade. *American Journal of Physiology* **264**, H526-H532.

KOOS, B.J., MASON, B.A., PUNLA, O. & ADINOLFI, A.M. (1994) Hypoxic inhibition of breathing in fetal sheep: relationship to brain adenosine concentrations. *Journal of Applied Physiology* **77**, 2734-2739.

KOOS, B., OGUNYEMI, D. & CHAU, A. (1995) Adenosine mediates metabolic and cardiovascular responses to hypoxia in fetal sheep. *Journal of the Society for the Gynecologic Investigation* **2**, 159 O45.

KOOS, B.J. & SAMESHIMA, H. (1988) Effects of hypoxaemia and hypercapnia on breathing movements and sleep state in sino-aortic denervated sheep. *Journal of Developmental Physiology* **10**, 131-144.

KOOS, B.J., SAMESHIMA, H. & POWER, G. (1987) Fetal breathing, sleep state, and cardiovascular responses to graded hypoxia in sheep. *Journal of Applied Physiology* **62**, 1033-1039.

KORENAGA, R., ANDO, J., TSUBOI, H., YANG, W., SAKUMA, I., TOYO-OKA, T. & KAMIYA, A. (1994) Laminar flow stimulates ATP- and shear stress-dependent nitric oxide production in cultures bovine endothelial cells. *Biochemical and Biophysical Research Communications* **198**, 213-219.

KOSEKI, C., IMAI, M., HIRATA, Y., YANAGISAWA, M. & MASAKI, T. (1989) Binding sites for endothelin-1 in rat tissues: An autoradiographic study. *Journal of Cardiovascular Pharmacology* **13**, S153-S154.

KOVACH, A.G., SZABO, C., BENYO, Z., CSAKI, C., GREENBERG, J.H. & REIVICH, M. (1992) Effects of N^G-nitro-L-arginine and L-arginine on regional cerebral blood flow in the cat. *Journal of Physiology* **449**, 183-196.

KOVITZ, K.L., ALESKOWITCH, T.D., SYLVESTER J.T. & FLAVAHAN, N.A. (1993) Endothelium-derived contracting and relaxing factors contribute to hypoxic responses of pulmonary arteries. *American Journal of physiology* **265**, H1139-H1148.

KOZNIEWSKA, E., OSEKA, M. & STYS, T. (1992) Effects of endothelium-derived nitric oxide on cerebral circulation during normoxia and hypoxia in the rat. *Journal of Cerebral Blood Flow and Metabolism* **12**, 311-317.

KUCHAN, M.J. & FRANGOS, J.A. (1994) Role of calcium and calmodulin in flow-induced nitric oxide production in endothelial cells. *American Journal of Physiology* **266**, C628-C636.

KUCHAN, M.J., JO, H. & FRANGOS, J.A. (1994) Role of G proteins in shear stress-mediated nitric oxide production by endothelial cells. *American Journal of Physiology* **267**, C753-C758.

KURIHARA, Y., KURIHARA, H., SUZUKI, H., KODAMA, T., MAEMURA, K., NAGAI, R., ODA, H., KUWAKI, T., CAO, W-H., KAMADA, N., JISHAGE, K., OUCHI, Y., AZUMA, S., TOYODA, Y., ISHIKAWA, T., KUMADA, M. & YAZAKI, Y. (1994) Elevated blood pressure and craniofacial abnormalities in mice deficient in endothelin-1. *Nature* **368**, 703-710.

KURTZ, A., KAISSLING, B., BUSSE, R. & BAIER, W. (1991) Endothelial cells modulate renin secretion from isolated mouse juxtaglomerular cells. *Journal of Clinical Investigation* **88**, 1147-1154.

LA GAMMA, E.F., ITSKOVITZ, J. AND RUDOLPH, A.M. (1982) Effects of naloxone on fetal circulatory responses to hypoxemia. *American Journal of Obstetrics and Gynecology* **143**, 933-940.

LAHERA, V. & KHRAIBI, A.A. (1994) Nitric oxide inhibition in hypertension. *NIPS* **9**, 268-271.

LAHERA, V., SALOM. M.G., MIRANDA-GUARDIOLA, F., MONCADA, S. & ROMERO, J.C. (1991) Effects of N^G-nitro-L-arginine methyl ester on renal function and blood pressure. *American Journal of Physiology* **261**, F1033-F1037.

LAUDIGNON, N., FARRI, E., BEHARRY, K., REX, J. & ARANDA, J.V. (1990) Influence of adenosine on cerebral blood flow during hypoxic hypoxia in the newborn piglet. *Journal of Applied Physiology* **68**, 1534-1541.

LEFFLER, C.W., BUSIJA, D.W., FLETCHER, A.M., BEASLEY, D.G., HESSLER, J.R. & GREEN, R.S. (1985) Effects of indomethacin upon cerebral hemodynamics of newborn pigs. *Pediatric Research* **19**, 1160-1164.

LERMAN, A., HILDEBRAND, F.L., AARHUS, L.L. & BURNETT, J.C. (1991) Endothelin has biological actions at pathophysiological concentrations. *Circulation* **83**, 1808-1814.

LEVENS, N.R., DE GASPARO, M., WOOD, J.M. & BOTTARI, S.P. (1992) Could the pharmacological differences observed between angiotensin II antagonists and inhibitors of

angiotensin converting enzyme be clinically beneficial? *Pharmacology and Toxicology* **71**, 241-249.

LEWIS, A.B., DONOVAN, M. & PLATZNER, A.C.G. (1980) Cardiovascular responses to autonomic blockade in hypoxemic fetal lambs. *Biology of the Neonate* **37**, 233-242.

LEWIS, A.B., HEYMANN, M.A. & RUDOLPH, A.M. (1976) Gestational changes in pulmonary vascular responses in fetal lambs in utero. *Circulation Research* **39**, 536-541.

LILLY, L.S., PRATT, R.E., ALEXANDER, R.W., LARSON, D.M., ELLISON, K.E., GIMBRONE, M.A. & DZAU, V.J. (1985) Renin expression by vascular endothelial cells in culture. *Circulation Research* **57**, 312-318.

LI, H., CHEN, S-J., CHEN, Y-F., MENG, Q.C., DURAND, J., OPARIL, S. & ELTON, T.S. (1994) Enhanced endothelin-1 and endothelin receptor gene expression in chronic hypoxia. *Journal of Applied Physiology* **77**, 1451-1459.

LIN, C-C., SHEIKH, Z. & LOPATA, R. (1990) The association between oligohydramnios and intrauterine growth retardation. *Obstetrics and Gynecology* **76**, 1100-1104.

LINGWOOD, B., HARDY, K.J., HORACEK, I., MCPHEE, M.L., SCOGGINS, B.A. & WINTOUR E.M. (1978) The effects of antidiuretic hormone on urine flow and composition in the chronically-cannulated ovine fetus. *Quarterly Journal of Experimental Physiology* **63**, 315-330.

LIPPTON, H.L., COHEN, G.A., MCMURTRY, I.F. & HYMAN, A.L. (1991) Pulmonary vasodilation to endothelin isopeptides in vivo is mediated by potassium channel activation. *Journal of Applied Physiology* **70**, 947-952.

LIPSKI, J., MCALLEN, R.M. & SPYER, K.M. (1975) The sinus nerve and baroreceptor input to the medulla of the cat. *Journal of Physiology* **251**, 61-78.

LONGO, L.D. & PEARCE, W.J. (1991) Fetal and newborn cerebral vascular responses and adaptations to hypoxia. *Seminars in Perinatology* **15**, 49-57.

LOWENSTEIN C.J. & SNYDER, S.H. (1992) Nitric oxide, a novel biologic messenger. *Cell* **70**, 705-707.

LUMBERS, E.R. (1983) A brief review of fetal renal function. *Journal of Developmental Physiology* **6**, 1-10.

LUMBERS, E.R., BURRELL, J.H., MENZIES, R.I. & STEVENS, A.D. (1993) The effects of a converting enzyme inhibitor (captopril) and angiotensin II on fetal renal function. *British Journal of Pharmacology* **110**, 821-827.

LUMBERS, E.R., KINGSFORD, N.M., MENZIES, R.I. & STEVENS, A.D. (1992) Acute effects of captopril, an angiotensin-converting enzyme inhibitor, on the pregnant ewe and fetus. *American Journal of Physiology* **262**, R754-R760.

LUMBERS, E.R. & STEVENS, A.D. (1983) Changes in fetal renal function in response to infusions of a hyperosmotic solution of mannitol to the ewe. *Journal of Physiology*, **343**, 439-446.

LUMBERS, E.R. & STEVENS, A.D. (1987) The effects of frusemide, saralasin and hypotension on fetal plasma renin activity and on fetal renal function. *Journal of Physiology*, **393**, 479-490.

MACPHERSON, R.D., MCLEOD, L.J. & RASIAH, R.L. (1991) Myogenic response of isolated pressurized rabbit ear artery is independent of endothelium. *American Journal of Physiology* **260**, H779-H784.

MACRAE, I.M., DAWSON, D.A., NORRIE, J.D. & MCCULLOCH, J. (1993) Inhibition of nitric oxide synthesis: effects on cerebral blood flow and glucose utilisation in the rat. *Journal of Cerebral Blood Flow and Metabolism* **13**, 985-992.

MACRAE, I., ROBINSON, M., MCAULEY, M., REI, J. & MCCULLOCH, J. (1991) Effects of intracisternal endothelin-1 injection on blood flow to the lower brain stem. *European Journal of Pharmacology* **203**, 85-01.

MALINSKI, T., BAILY, F., ZHANG, Z.G. & CHOPP, M. (1993) Nitric oxide measured by a porphyrinic microsensor in rat brain after transient middle cerebral artery occlusion. *Journal of Cerebral Blood Flow and Metabolism* **13**, 355-358.

MANNING, F.A. (1992) Biophysical profile scoring. *In: Fetal Behaviour. Developmental and Perinatal Aspects. J.G. Nijhuis (Ed.)* pp241-257

MARSHALL, J.J. & KONTOS, H.A. (1990) Endothelium-derived relaxing factors. A perspective from in vivo data. *Hypertension* **16**, 371-386.

MARSHALL, J.M., LLOYD, J. & MIAN, R. (1993b) The influence of vasopressin on the arterioles and venules of skeletal muscle of the rat during systemic hypoxia. *Journal of Phsyiology* **470**, 473-484.

MARSHALL, J.M., THOMAS, T. & TURNER, L. (1993a) A link between adenosine, ATP-sensitive K^+ channels, potassium and muscle vasodilatation in the rat in systemic hypoxia. *Journal of Physiology* **472**, 1-9.

MASAKI, T. (1991) Tissue specificity of the endothelin-induced responses. *Journal of Cardiovascular Pharmacology* **17**, S1-S4.

MASAKI, T. (1993) Overview: Reduced sensitivity of vascular response to endothelin. *Circulation* **87**, V33-V35.

MASAKI, T., KIMURA, S., YANAGISAWA, M. & GOTO, K. (1991) Molecular and cellular mechanism of endothelin regulation. Implications for vascular function. *Circulation* **84**, 1457-1468.

MATSON, J.R., STOKES, J.B. & ROBILLARD, J.E. (1981) Effects of inhibition of prostaglandin synthesis on fetal renal function. *Kidney International* **20**, 621-627.

MATSUDA, T., BATES, J.N., LEWIS, S.J., ABBOUND, F.M. & CHAPLEAU, M.W. (1995) Modulation of baroreceptor activity by nitric oxide and S-nitrocysteine. *Circulation Research* **76**, 426-433.

MATTHEWS, J.N.S., ALTMAN, D.G., CAMPBELL, M.J. & ROYSTON, P. (1990) Analysis of serial measurements in medical research. *British Medical Journal* **300**, 230-235.

MATTIOLI, L., CHIEN, S., VASSENON, T., CRIST, R. & LYNN, R. (1979) Effect of angiotensin II (A-2) blockade on the responses of the fetal lamb to acute hypoxia and hypovolemia. *Pediatric Research* **13**, 360 abstract 210.

MCCARRON, J.G. & HALPERN, W. (1990) Potassium dilates rat cerebral arteries by two independent mechanisms. *American Journal of Physiology* **259**, H902-H908.

MCCARTHY, A.L., WOOLFSON, R.G., RAJU, S.K. & POSTON, L. (1993) Abnormal endothelial cell function of resistance arteries from women with preeclampsia. *American Journal of Obstetrics and Gynaecology* **168**, 1323-1330.

MCCRABB, G.J. & HARDING, R. (1994) Cerebral blood flow is maintained during 12 hours of hypoxemia in the mid-gestation ovine fetus. *Proceedings of the Thorburn Symposium* A35.

MCINTYRE, T.M., ZIMMERMAN, G.A., SATOH, K. & PRESCOTT, S.M. (1985) Cultures endothelial cells synthesize both platelet-activating factor and prostacyclin in response to histamine, bradykinin and adenosine triphosphate. *Journal of Clinical Investigation* **76**, 271-280.

MCMURDO, L., LIDBURY, P.S., THIEMERMANN, C. & VANE, J.R. (1993) Mediation of endothelin-1-induced inhibition of platelet aggregation via the ET_B receptor. *British Journal of Pharmacology* **109**, 530-534.

MCPHERSON, R.W., KOEHLER, R.C. & TRAYSTMAN, R.J. (1994) Hypoxia, α_2-adrenergic, and nitric oxide-dependent interactions on canine blood flow. *American Journal of Physiology* **266**, H476-H482.

MCQUESTON, J.A., CORNFIELD, D.N., MCMURTRY, I.F. & ABMAN, S.H. (1993) Effects of oxygen and exogenous L-arginine on EDRF activity in fetal pulmonary circulation. *American Journal of Physiology* **264**, H865-H871.

MCQUEEN, D.S., DASHWOOD, M.R., COBB, V.J. & MARR, G. (1994) Effects of endothelins on respiration and arterial chemoreceptor activity in anaesthetised rats. *Advances in Experimental Medicine and Biology* **360**, 289-291.

MCQUEEN, D.S. & BELMONTE, C. (1974) The effects of prostaglandins E_2, A_2 and $F_{2\alpha}$ on carotid baroreceptors and chemoreceptors. *Quarterly Journal of Experimental Physiology* **59**, 63-71.

MEEI WEI, H., WEISS, H.R., SINHA, A.K. & CHI, Q.Z. (1993) Effects of nitric oxide synthase inhibition on regional cerebral blood flow and vascular resistance in conscious and isoflurane-anaesthetized rats. *Neurosurgical Anesthesia* **77**, 880-885.

MELKUMYANTS, A.M., BALASHOV, S.A. & KHAYUTIN, V.M. (1995) Control of arterial lumen by shear stress on endothelium. *NIPS* **10**, 204-210.

MELLOR, D.J. & SLATER, J.S. (1973) The use of chronic catheterization techniques in foetal sheep. *British Veterinary Journal* **129**, 260-266.

MESCHER, E.J., PLATZKER, A.C.G., BALLARD, P.L., KITTERMAN, J.A., CLEMENTS, J.A. & TOOLEY, W.H. (1975) Ontogeny of tracheal fluid, pulmonary surfactant, and plasma corticoids in the fetal lamb. *Journal of Applied Physiology* **39**, 1017-1021.

MEVORACH, R.A., BOGAERT, G.A. & KOGAN, B.A. (1994) Role of nitric oxide in fetal lower urinary tract function. *The Journal of Urology* **152**, 510-514.

MILLARD, R.W., BAIG, H. & VATNER, S.F. (1979) Prostaglandin control of the renal circulation in response to hypoxemia in the fetal lamb in utero. *Circulation Research* **45**, 172-179.

MILLER V.M. & BURNETT, J.C. (1992) Modulation of NO and endothelin by chronic increases in blood flow in canine femoral arteries. *American Journal of Physiology* **263**, H103-H108.

MINER, L.K., CHEUNG, C.Y. & BRACE, R.A. (1988) Autonomic modulation of fetal atrial natriuretic factor and urine flow responses to vascular volume expansion. *American Journal of Obstetrics and Gynecology* **159**, 1241-1246.

MINKES, R.K., BELLAN, J.A., SAROYAN, R.M., KERSTEIN, M.D., COY, D.H., MURPHY, W.A., NOSSMAN, B.D., MCNAMARA, D.B. & KADOWITZ, P.J. (1990) Ananlysis of cardiovascular and pulmonary responses to endothelin-1 and endothelin-3 in the anaesthetized cat. *The Journal of Pharmacology and Experimental Therapeutics* **253**, 1118-1125.

MITRA, S.C., GANESH, V. & APUZZIO, J.J. (1994) Effect of maternal cocaine abuse on renal arterial flow and urine output of the fetus. *American Journal of Obstetrics and Gynecology* **171**, 1556-1559.

MOLLACE,, V., SALVEMINI, D., ANGGARD, E. & VANE, J. (1991) Nitric oxide from vascular smooth muscle cells: regulation of platelet reactivity and smooth muscle cell guanylate cyclase. *British Journal of Pharmacology* **104**, 633-638.

MOLNAR, M., SUTO, T., TOTH, T. & HERTELENDY, F. (1994) Prolonged blockade of nitric oxide synthesis in gravid rats produces sustained hypertension, proteinuria, thrombocytopenia, and intrauterine growth retardation. *American Journal of Physiology* **170**, 1458-1466.

MONCADA, S., PALMER, R.M.J. & HIGGS, E.A. (1991) Nitric oxide: Physiology, pathophysiology, and pharmacology. *Pharmacological Reviews* **43**, 109-142.

MONCADA, S., REES, D.D., SCHULZ, R. & PALMER, R.M.J. (1991) Development and mechanism of a specific supersensitivity to nitrovasodilators after inhibition of vascular nitric oxide synthesis in vivo. *Proceedings of the National Academy of Science* **88**, 2166-2170.

MOORE, P.J. & HANSON, M.A. (1991) The role of peripheral chemoreceptors in the rapid response of the pulmonary vasculature of the late gestation sheep fetus to changes in PaO_2. *Journal of Developmental Physiology* **16**, 133-138.

MOORE, P.J. & HANSON, M.A. (1992) Animal Investigations. In: Fetal behaviour. Developmental and perinatal aspects. Ed. J.G. Nijhuis. *Oxford Medical Publications*, pp100-111.

MOORE, P.J., PARKES, M.J., NIJHUIS, J.G. & HANSON, M.A. (1989) The incidence of breathing movements of fetal sheep in normoxia and hypoxia after peripheral chemodenervation and brain-stem transection. *Journal of Developmental Physiology* **11**, 147-151.

MOORE, P.J., PARKES, M.J., NOBLE, R. & HANSON, M.A. (1991) Reversible blockade of the secondary fall of ventilation during hypoxia in anaesthetized newborn sheep by focal cooling of the brain stem. *Journal of Physiology* **438**, 242P

MOORE, P., VELVIS, H., FINEMAN, J.R., SOIFER, S.J. & HEYMANN, M.A. (1992) EDRF inhibition attenuates the increase in pulmonary blood flow due to oxygen ventilation in fetal lambs. *Journal of Applied Physiology* **73**, 2151-2157.

MOREIRA, E.D., IDA, F., PIRES, M.D. & KRIEGER, E.M. (1994) DuP 753 is more effective than captopril on baroreceptor function in high-renin hypertension. *Hypertension* **23**, I64-I67.

MORII, S., NGAI, A.C., KO, K.R. & WINN, R. (1987) Role of adenosine in regulation of cerebral blood flow: effects of theophylline during normoxia and hypoxia. *American Journal of Physiology* **253**, H165-H175.

MORRIS, N.H., SOORANNA, S.R., LEARMONT, J.G., POSTON, L., RAMSEY, B., PEARSON, J.D. & STEER, P.J. (1995) Nitric oxide synthase activities in placental tissue from normotensive, pre-eclamptic and growth retarded pregnancies. *British Journal of Obstetrics and Gynaecology*, **102**, 711-714.

MORTENSEN, L.H. & FINK, G.D. (1992) Captopril prevents chronic hypertension produced by infusion of endothelin-1 in rats. *Hypertension* **19**, 676-680.

MYERS, P.R., MINOR, R.L., GUERRA, R., BATES, J.N. & HARRISON, D.G. (1990) Vasorelaxant properties of the endothelium-derived relaxing factor more closely resemble S-nitrosocysteine than nitric oxide. *Nature* **345**, 161-163.

MYERS, D.A., ROBERTSHAW, D. & NATHANIELSZ, P.W. (1990) Effect of bilateral splanchnic nerve section on adrenal function in the ovine fetus. *Endocrinology* **127**, 2328-2335.

MACCUMBER, M.W., ROSS, C.A., GLASER, B.M. AND SNYDER, S.H. (1989) Endothelin: visualization of mRNAs by in situ hybridization provides evidence for local action. *Proceedings of the National Academy of Sciences* **86**, 7285-7289.

NADEN, R.P., COULTRUP, S., ARANT, B.S. & ROSENFELD, C.R. (1985) Metabolic clearance of angiotensin II in pregnant and nonpregnant sheep. *American Journal of Physiology* **249**, E49-E55.

NAESS, P.A., CHRISTENSEN, G. & KIIL, F. (1993) Inhibitory effect of endothelin on renin release in dog kidneys. *Acta Physiologica Scandinavica* **148**, 131-136.

NAESS, P.A., CHRISTENSEN, G., KIRKEBOEN, K.A. & KIIL, F. (1993) Effect on renin release of inhibiting renal nitric oxide synthesis in anaesthetized dogs. *Acta Physiologica Scandinavica* **148**, 137-142.

NAKAMURA, K.T., AYERS, N.A., GOMEZ, R.A. & ROBILLARD, J.E. (1985) Renal responses to hypoxemia during renin-angiotensin system inhibition in fetal lambs. *American Journal of Physiology* **249**, R116-R124.

NAKAMURA, T., KASAI, K., KONUMA, S., EMOTO, T., BANBA, N., ISHIKAWA, M. & SHIMODA, S-I. (1990) Immunoreactive endothelin concentrations in maternal and fetal blood. *Life Sciences* **46**, 1045-1050.

NAKAMURA, K.T., MATHERNE, G.P., MCWEENY, O.J., SMITH, B.A. & ROBILLARD, J.E. (1987) Renal hemodynamics and functional changes during the transition from fetal to newborn life in sheep. *Pediatric Research* **21**, 229-234.

NAVARRO, J., SANCHEZ, A., SAIZ, J., RUILOPE, L.M., GARCIA-ESTAN, J., ROMERO, J.C., MONCADA, S. AND LAHERA, V. (1994) Hormonal, renal, and metabolic alterations during hypertension induced by chronic inhibition of NO in rats. *American Journal of Physiology* **267**, R1516-R1521.

NELIN, L.D. & DAWSON, C.A. (1993) The effect of N^{ω}-nitro-L-arginine methylester on hypoxic vasoconstriction in the neonatal pig lung. *Pediatric Research* **34**, 349-353.

NELISSEN-VRANCKEN, M.H.J.M.G., LEENDERS, P.J.A., BOST, J.G.P.J., STRUIJKER-BOUDIER, H.A.J. & SMITS, J.F.M. (1992) Comparison of angiotensin-converting enzyme inhibitors in the rat in perfused hindlimbs and in vivo. *European Journal of Pharmacology* **217**, 49-55.

NEREM, R.M., HARRISON, D.G., TAYLOR, W.R. & ALEXANDER, R.W. (1993) Hemodynamics and vascular endothelial biology. *Journal of Cardiovascular Pharmacology* **21**, S6-S10.

NEYLON, M., MARSHALL, J.M. & JOHNS, E.J. (1995) The effects of systemic hypoxia on renal function in the anaesthetized rat. *Journal of Physiology* **487**, 497-511.

NICOLAIDES, K.H., PETERS, M.T., VYAS, S., RABINOWITZ, R., ROSEN, D.J.D. & CAMPBELL, S. (1990) Relation of urine production to oxygen tension in small-for-gestational-age fetuses. *American Journal Obstetrics and Gynecology* **162**, 387-391.

NIREI, H., HAMADA, K., SHOUBO, M., SOGABE, K., NOTSU, Y. & ONO, T. (1993) An endothelin ET_A receptor antagonist, FR139317, ameliorates cerebral vasospasm in dogs. *Life Sciences* **52**, 1869-1874.

NORIS, M., MORIGI, M., DONADELLI, R., AIELLO, SISTIANA, A., FOPPOLO, M., TODESCHINI, M., ORISIO, S., REMUZZI, G. & REMUZZI, A. (1995) Nitric oxide synthesis by cultures endothelial cells is modulated by flow conditions. *Circulation Research* **76**, 536-543.

NORTH, A.J., STAR, R.A., BRANNON, T.S., UJIIE, K., WELLS, L.B., LOWENSTEIN, C.J., SNYDER, S.H. & SHAUL, P.W. (1994) Nitric oxide synthase type I and type II gene expression are developmentally regulated in rat lung. *American Journal of Physiology* **266**, L635-L641.

NOVA, A., SIBAI, B.M., BARTON,, J.R., MERCER, B.M. & MITCHELL, M.D. (1991) Maternal plasma level of endothelin is increased in preeclampsia. *American Journal of Obstetrics and Gynecology* **165**, 724-727.

ORITA, Y., FUJIWARA, Y., OCHI, S., TAKAMA, T., FUKUNAGA, M. & YOKOYAMA, K. (1989) Endothelin-1 receptors in rat renal glomeruli. *Journal of Cardiovascular Pharmacology* **13**, S159-S161.

OZOLINS, I.Z., YOUNG, I.R. & MCMILLEN, I.C. (1992) Surgical disconnection of the hypothalamus from the fetal pituitary abolishes the corticotrophic response to intrauterine

hypoglycemia or hypoxemia in the sheep during late gestation. *Endocrinology* **130**, 2438-2445.

PALLER, M.S. & LINAS, S.L. (1984) Role of angiotensin II, α-adrenergic system, and arginine vasopressin on arterial pressure in rat. *American Journal of Physiology* **246**, H25-H30.

PALMER, R.M.J., FERRIGE, A.G. & MONCADA, S. (1987) Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature* **327**, 524-526.

PARISI, V.M. & WALSH, S.W. (1989) Fetal placental vascular responses to prostacyclin after angiotensin II-induced vasoconstriction. *American Journal of Physiology* **257**, E102-E107.

PATON, J.F.R., SILA-CARVALHO, L., GOLDSMITH, G.E. & SPYER, K.M. (1990) Inhibition of barosensitive neurones evoked by lobule IXb of the posterior cerebellar cortex in the decerebrate rabbit. *Journal of Physiology* **427**, 553-565.

PATON, J.F.R & SPYER, K.M. (1992) Cerebellar cortical regulation of circulation. *NIPS* **7**, 124-129.

PAULICK, R.P., MEYERS, R.L., RUDOLPH, C.D. & RUDOLPH, A.M. (1991a) Hemodynamic responses to alpha-adrenergic blockade during hypoxemia in the fetal lamb. *Journal of Developmental Physiology* **16**, 63-69.

PAULICK, R.P., MEYERS, R.L., RUDOLPH, C.D. & RUDOLPH, A.M. (1991b) Umbilical and hepatic venous responses to circulating vasoconstrictive hormones in fetal lamb. *Amercian Journal of Physiology* **260**, H1205-H1213.

PEARCE, W.J. (1995) Mechanisms of hypoxic cerebral vasodilatation. Pharmacology and Therapeutics **65**, 75-91.

PEARCE, W.J. & LONGO, L.D. (1991) Developmental aspects of endothelial function. *Seminars in Perinatology* **15**, 40-48.

PEARSON, P.J., LIN, P.J., EVORA, P.R.B. & SCHAFF, H.V. (1993) Endothelium-dependent responses of human internal mammary artery to hypoxia. *American Journal of Physiology* **264**, H376-H380.

PEETERS, L.L.H., SHELDON, R.E., JONES, M.D., MAKOWSKI, E.L. & MESCHIA, G. (1979) Blood flow to fetal organs as a function of arterial oxygen content. *American Journal of Obstetrics and Gynecology* **135**, 637-646.

PELLIGRINO, D.A., KOENIG, H.M. & ALBRECHT, R.F. (1993) Nitric oxide synthesis and regional cerebral blood flow responses to hypercapnia and hypoxia in the rat. *Journal of Cerebral Blood Flow and Metabolism* **13**, 80-87.

PERELLA, M.A., EDELL, E.S., KROWKA, M.J., CORTESE, D.A. & BURNETT, J.C. (1992) Endothelium-derived relaxing factor in pulmonary and renal circulations during hypoxia. *American Journal of Physiology* **263**, R45-R50.

PEREZ, R., ESPINOZA, M., RIQUELME, R., PARER, J.T. & LLANOS, A.J. (1989) Arginine vasopressin mediates cardiovascular responses to hypoxemia in fetal sheep. *American Journal of Physiology* **256**, R1011-R1018.

PERREAULT, T. & DE MARTE, J. (1991) Endothelin-1 has a dilator effect on neonatal pig pulmonary vasculature *Journal of Cardiovascular Pharmacology* **18**, 43-50.

PERSSON, M.G., GUSTAFSSON, L.E., WIKLUND, N.P., MONCADA, S. & HEDQVIST, P. (1990) Endogenous nitric oxide as a probable modulator of pulmonary circulation and hypoxic pressor response *in vivo*. *Acta Physiologica Scandinavica* **140**, 449-457.

PETERS, C.A., DOCIMO, S.G., LUETIC, T., REID, L.M., RETIK, A.B. & MANDELL, J. (1991) Effect of in utero vesicostomy on pulmonary hypoplasia in the fetal lamb with

bladder outlet obstruction and oligohydramnios: A morphometric analysis. *Journal of Urology* **146**, 1178-1183.

PIACQUADIO, K.M., BRACE, R.A. & CHEUNG, C.Y. (1990) Role of vasopressin in mediation of fetal cardiovascular responses to acute hypoxia. *American Journal of Obstetrics and Gynecology* **163**, 1294-1300.

POHL, U. & BUSSE, R. (1989) Hypoxia stimulates release of endothelium-derived relaxant factor. *American Journal of Physiology* **256**, H1595-H1600.

POHL, U., HOLTZ, J., BUSSE, R. & BASSENGE, E. (1986) Crucial role of endothelium in the vasodilator response to increased flow in vivo. *Hypertension* **8**, 37-44.

POHL, U. & KAAS, J. (1994) Interactions of hormones with the vascular endothelium. *Arzneimittelforschung* **44**, 459-461.

POLLOCK, D.M. & OPGENORTH, T.J. (1993) Evidence for endothelin-induced renal vasoconstriction independent of ET_A receptor activation. *American Journal of Physiology* **264**, R222-R226.

PRADO, R., WATSON, B.D., KULUZ, J. & DIETRICH, D. (1992) Endothelium-derived nitric oxide synthase inhibition. Effects on cerebral blood flow, pial artery diameter, and vascular morphology in rats. *Stroke* **23**, 1118-1124.

PRADO, R., WATSON, B.D., WESTER, P. (1993) Effects of nitric oxide synthase inhibition on cerebral blood flow following bilateral carotid artery occlusion and recirculation in the rat. *Journal of Cerebral Blood Flow and Metabolism* **13**, 720-723.

PRYDE, P.G., SEDMAN, A.B., NUGENT, C.E. & BARR, M. (1993) Angiotensin-converting enzyme inhibitor fetopathy. *Journal of the American Society of Nephrology* **3**, 1575-1582.

QIU, C., ENGELS, K. & BAYLIS, C. (1994) Angiotensin II and a1-adrenergic tone in chronic nitric oxide blockade-induced hypertension. *American Journal of Physiology* **266**, R1470-R1476.

RADOMSKI, M.W., PALMER, R.M.J. & MONCADA, S. (1987) The anti-aggregating properties of vascular endothelium: interactions between prostacyclin and nitric oxide. *British Journal of Pharmacology* **92**, 639-646.

RADOMSKI, M.W., PALMER, R.M.J. & MONCADA, S. (1990) Glucocorticoids inhibit the expression of an inducible, but not the constitutive, nitric oxide synthase in vascular endothelial cells. *Proceedings of the National Academy of Sciences* **87**, 10043-10047.

RAFF, H., KANE, C.W. & WOOD, C.E. (1991) Arginine vasopressin responses to hypoxia and hypercapnia in late-gestation fetal sheep. *American Journal of Physiology* **260**, R1077-R1081.

RAFF, H., SHINSAKO, J. & DALLMAN, M.F. (1984) Renin and ACTH responses to hypercapnia and hypoxia after chronic carotid chemodenervation. *Amercian Journal of Physiology* **247**, R412-R417.

RAFF, H., TZANKOFF, S.P. & FITZGERALD, R.S. (1982) Chemoreceptor involvement in cortisol responses to hypoxia in ventilated dogs. *Journal of Applied Physiology* **52**, 1092-1096.

RAKUGI, H., TABUCHI, Y., NAKAMARU, M., NAGANO, M., HIGASHIMORI, K., MIKAMI, H., OGIHARA, T. & SUZUKI, N. (1990) Evidence for endothelin-1 release from resistance vessels of rats in response to hypoxia. *Biochemical and Biophysical Research Communications* **169**, 973-977.

RAMSAY, M., BROUGHTON PIPKIN, F. & RUBIN, P. (1992) Comparative study of pressor and heart rate responses to angiotensin II and noradrenaline in pregnant and non-pregnant women. *Clinical Science* **82**, 157-162.

RAIMBACH, S.J. & THOMAS, A.L. (1990) Renin and angiotensin converting enzyme concentrations in the fetal and neonatal guinea-pig. *Journal of Physiology* **423**, 441-451.

RANG, H.P. & DALE, M.M. (1987) Pharmacology. *Churchill and Livingstone, UK.* pp. 265-266.

RANKIN, J.H.G., LANDAUER, M., TIAN, Q. & PHERNETTON, T.M. (1987) Ovine fetal electrocortical activity and regional cerebral blood flow. *Journal of Developmental Physiology* **9**, 537-542.

RAWASHDEH, N.M., RAY, N.D., SUNDBERG, D.K. & ROSE, J.C. (1988) Comparison of hormonal responses to hypotension in mature and immature fetal lambs. *Amercian Journal of Physiology* **255**, R67-R72.

RAWASHDEH, N.M., ROSE, J.C. & RAY, N.D. (1988) Differential maturation of β-adrenoceptor-mediated responses in the lamb fetus. *American Journal of Physiology* **255**, R794-R798.

RAWASHDEH, N.M., ROSE, J.C. & RAY, N.D. (1990) Renin secretion by fetal lamb kidneys in vitro. *Amercian Journal of Physiology* **258**, R388-R392.

RE, R., FALLON, J.T., DZAU, V., QUAY, S.C. AND HABER, E. (1982) Renin synthesis by canine aortic smooth muscle cells in culture. *Life Sciences* **30**, 99-106.

REES, D.D., CELLEK, S., PALMER, R.M.J. & MONCADA, S. (1990) Dexamethasone prevents the induction by endotoxin of a nitric oxide synthase and the associated effects on vascular tone: An insight into endotoxin shock. *Biochemical and Biophysical Research Communications* **173**, 541-547.

REES, D.D., PALMER, R.M.J., HODSON, H.F. & MONCADA, S. (1989) A specific inhibitor of nitric oxide formation from L-arginine attenuates endothelium-dependent relaxation. *British Journal of Pharmacology* **96**, 418-424.

REES, D.D., PALMER, R.M.J. & MONCADA, S. (1989) Role of endothelium-derived nitric oxide in the regulation of blood pressure. *Proceedings of the National Academy of Sciences* **86**, 3375-3378.

REES, D.D., PALMER, R.M.J., SCHULZ, R., HODSON, H.F. & MONCADA, S. (1990) Characterization of three inhibitors of endothelial nitric oxide synthase in vitro and in vivo. *British Journal of Pharmacology* **101**, 746-752.

REID, I.A. & CHOU, L. (1990) Analysis of the action of angiotensin II on the baroreflex control of heart rate in conscious rabbits. *Endocrinology* **126**, 2749-2756.

REID, I.A., BUI, H. & CHOU, L. (1994) Role of nitric oxide in the renin and heart rate responses to β-adrenergic stimulation. *Hypertension* **23**, I49-I53.

REILLY, C.F., TEWKSBURY, D.A., SCHECHTER, N.M. & TRAVIS, J. (1982) Rapid conversion of angiotensin I to angiotensin II by neutrophil and mast cell proteinases. *The Journal of Biological Chemistry* **257**, 8619-8622.

REUSS, M.L., PARER, J.T., HARRIS, J.L. & KRUEGER, T.R. (1982) Hemodynamic effects of alpha-adrenergic blockade during hypoxia in fetal sheep. *American Journal of Obstetrics and Gynecology* **142**, 410-415.

RIBEIRO, M.O., ANTUNES, E., DE NUCCI, G., LOVISOLO, S.M. & ZATZ, R. (1992) Chronic inhibition of nitric oxide synthesis. A new model of arterial hypertension. *Hypertension* **20**, 298-303.

RICHARD, V., GOSGNACH, M., DRIEU LA ROCHELLE, C., GIUDICELLI, J.F. & BERDEAUX, A. (1991) The L-arginine-nitric oxide pathway in the canine femoral vascular bed: *in vitro* and *in vivo* experiments. *Fundamental and Clinical Pharmacology* **5**, 777-788.

RICHARDSON, B.S., PATRICK, J.E. & ABDULJABBAR, H. (1985) Cerebral oxidative metabolism in the fetal lamb: Relationship to electrocortical state. *American Journal of Obstetrics and Gynecology* **153**, 426-431.

RIDDIHOUGH, G. (1994) A twist in the tail of human endothelin. *Nature* **369**, 84.

ROBERTS, J.D., CHEN, T-Y., KAWAI, N., WAIN, J., DUPUY, P., SHIMOUCHI, A., BLOCH, K., POLANER, D. & ZAPOL, W.M. (1993) Inhaled nitric oxide reverses pulmonary vasoconstriction in the hypoxic and acidotic newborn lamb. *Circulation Research* **72**, 246-254.

ROBILLARD, J.E., GOMEZ, R.A., MEERNIK, J.G., KUEHL, W.D. & VAN ORDEN, D. (1982) Role of angiotensin II on the adrenal and vascular responses to hemorrhage during development in fetal lambs. *Circulation Research* **50**, 645-650.

ROBILLARD, J.E., GOMEZ, A., VANORDEN, D. & SMITH, F.G. (1982) Comparison of the adrenal and renal responses to angiotensin II in fetal lambs and adult sheep. *Circulation Research* **50**, 140-147.

ROBILLARD, J.E. & NAKAMURA, K.T. (1988) Neurohormonal regulation of renal function during development. *American Journal of Physiology* **254**, F771-F779.

ROBILLARD, J.E., NAKAMURA, K.T. & DIBONA, G.F. (1986) Effects of renal denervation on renal responses to hypoxemia in fetal lambs. *American Journal of Physiology* **250**, F294-F301.

ROBILLARD, J.E., NAKAMURA, K.T. & LAWTON, W.J. (1985) Effects of aldosterone on urinary kallikrein and sodium excretion during fetal life. *Pediatric Research* **19**, 1048-1052.

ROBILLARD, J.E., NAKAMURA, K.T., VARILLE, V.A., ANDRESSEN, A.A., MATHERNE, G.P. & VANORDEN, D.E. (1988b) Ontogeny of the renal response to natriuretic peptide in sheep. *American Journal of Physiology* **254**, F634-F641.

ROBILLARD, J.E., NAKAMURA, K.T., VARILLE, V.A., MATHERNE, G.P. & MCWEENY, O.J. (1988a) Plasma and urinary clearance rates of atrial natriuretic factor during ontogeny in sheep. *Journal of Developmental Physiology* **10**, 335-346.

ROBILLARD, J.E., PORTER, C.C. & JOSE, P.A. (1994) Structure and function of the developing kidney. In: Holliday MA, Barratt TM, Avner ED, Kogan BA (eds) *Pediatric Nephrology*. Williams & Wilkins, pp 21-39

ROBILLARD, J.E., SCHUTTE, B.C., PAGE, W.V., FEDDERSON, J.A., PORTER C.C. & SEGAR, J.L. (1994) Ontogenic changes and regulation of renal angiotensin II type I receptor gene expression during fetal and newborn life. *Pediatric Research* **36**, 755-762.

ROBILLARD, J.E., SMITH, F.G., SEGAR, J.L., GUILLERY, E.W. & JOSE, P.A. (1994) Renal function in the fetus. Eds. G.D. Thorburn and R. Harding. *Oxford Medical Publications* pp 196-204.

ROBILLARD, J.E., SMITH, F.G., SEGAR, J.L., MERRILL, D.C. & JOSE, P.A. (1992) Functional role of renal sympathetic innervation during fetal and postnatal development. *NIPS* **7**, 130-133.

ROBILLARD, J.E., WEISMANN, D.N., GOMEZ, R.A., AYERS, N.A., LAWTON, W.J. & VAN ORDEN, D.E. (1983) Renal and adrenal responses to converting-enzyme inhibition in fetal and newborn life. *American Journal of Physiology* **244**, R249-R256.

ROBILLARD, J.E., WEISMANN, D.N. & HERIN, P. (1981) Ontogeny of single glomerular perfusion rate in fetal and newborn lambs. *Pediatric Research* **15**, 1248-1255.

ROBILLARD, J.E. & WEITZMAN, R.E. (1980) Developmental aspects of the fetal renal response to exogenous arginine vasopressin. *American Journal of Physiology* **238**, F407-F414.

ROBILLARD, J.E., WEITZMAN, R.E., BURMEISTER, L. & SMITH, F.G. (1981) Developmental aspects of the renal response to hypoxemia in the lamb fetus. *Circulation Research* **48**, 128-138.

RODRIGO, J., SPRINGALL, D.R., UTTENTHAL, O., BENTURA, M.L., ABADIA-MOLINA, F., RIVEROS-MORENO, V., MARTINEZ-MURILLO, R., POLAK, J.M. & MONCADA, S. (1994) Localization of nitric oxide synthase in the adult rat brain. *Philosophical Transactions of the Royal Society of London* **345**, 175-221.

RONGEN, G.A., SMITS, P. & THIEN, TH. (1994) Endothelium and the regulation of vascular tone with emphasis on the role of nitric oxide. *Netherlands Journal of Medicine* **44**, 26-35.

ROSE, J.C., MACDONALD, A.A., HEYMANN, M.A. & RUDOLPH, A.M. (1978) Developmental aspects of the pituitary-adrenal axis response to hemorrhage stress in lamb fetuses in utero. *The Journal of Clinical Investigation* **61**, 424-432.

ROSE, J.C., MEIS, P.J. & MORRIS, M. (1981) Ontogeny of endocrine (ACTH, vasopressin, cortisol) responses to hypotension in lamb fetuses. *American Journal of Physiology* **240**, E656-E661.

ROSE, C.E., VANCE, J.E., DACUS, W.S., BRASHERS, V.L., PEACH, M.J. & CAREY, R.M. (1991) Role of intrarenal angiotensin II and α-adrenoceptors in renal vasoconstriction with acute hypoxemia and hypercapnic acidosis in conscious dogs. *Circulation Research* **69**, 142-156.

ROSENFELD, C.R., COX, B.E., MAGNESS, R.R. & SHAUL, P.W. (1993) Ontogeny of angiotensin II vascular smooth muscle receptors in ovine fetal aorta and placental uterine arteries. *American Journal of Obstetrics and Gynecology* **168**, 1562-1569.

ROSENFELD, C.R., GRESORES, A., ROY, T.A. & MAGNESS, R.R. (1995) Comparison of angiotensin II in fetal and pregnant sheep: metabolic clearance and vascular sensitivity. *American Journal of Physiology* **268**, E237-E247.

ROSENFELD, C.R., SAMSON, W.K., ROY, T.A., FAUCHER, D.J. & MAGNESS, R.R. (1992) Vasoconstrictor-induced secretion of ANP in fetal sheep. *American Journal of Physiology* **263**, E526-E533.

ROSS, M.G., ERVIN, M.G., RAPPAPORT, V.J., YOUSSEF, A., LEAKE, R.D. & FISHER, D.A. (1988) Ovine fetal urine contribution to amniotic and allantoic compartments. *Biology of the Neonate* **53**, 98-104.

RUBANYI, G.M. (1993) The role of endothelium in cardiovascular homeostasis and diseases. *Journal of cardiovascular Pharmacology* **22**, S1-S14.

RUDOLPH, A.M. (1984) The fetal circulation and its response to stress. *Journal of Developmental Physiology* **6**, 11-19.

RUDOLPH, A.M. & HEYMANN, M.A. (1970) Circulatory changes during growth in the fetal lamb. *Circulation Research* **XXVI**, 289-299.

RUDOLPH, A.M. & HEYMANN, M.A. (1976) Cardiac output in the fetal lamb: The effects of spontaneous and induced changes of heart rate on right and left ventricular output. *American Journal of Obstetrics and Gynecology* **124**, 183-192.

RURAK, D.W. (1978) Plasma vasopressin levels during hypoxaemia and the cardiovascular effects of exogenous vasopressin in foetal and adult sheep. *Journal of Physiology* **277**, 341-357.

RURAK, D.W. & GRUBER, N.C. (1983) Increased oxygen consumption associated with breathing activity in fetal lambs. *Journal of Applied Physiology* **54**, 701-707.

RURAK, D.W., STOBBS, K.S., KWAN, E. & HALL, C. (1995) The effect of arginine vasopressin (AVP) on hindlimb blood flow and O_2 consumption in the fetal lamb. *Journal of the Society of Gynecologic Investigation* **2**, 331 P229.

RURAK, D.W., RICHARDSON, B.S., PATRICK, J.E., CARMICHAEL, L. AND HOMAN, J. (1990) Blood flow and oxygen delivery to fetal organs and tissues during sustained hypoxemia. *American Journal of Physiology* **258**, R1116-R1122.

SAID, S.I. (1992) Nitric oxide and vasoactive intestinal peptide: cotransmitters of smooth muscle relaxation. *NIPS* **7**, 181-183.

SAKUMA, I., TOGASHI, H., YOSHIOKA, M., SAITO, H., YANAGIDA, M., TAMURA, M., KOBAYASHI, T., YASUDA, H., GROSS, S.S. & LEVI, R. (1992) N^G-methyl-L-arginine, an inhibitor of L-arginine-derived nitric oxide synthesis, stimulates renal sympathetic nerve activity in vivo. *Circulation Research* **70**, 607-611.

SAKURAI, T., YANAGISAWA, M. & MASAKI, T. (1992) Molecular characterization of endothelin receptors. *TIPS* **13**, 103-108.

SAMESHIMA, H. & KOOS, B.J. (1986) Effects of moderate hypoxia on fetal electrocortical activity, eye movements, and breathing activity in sheep. *Journal of Developmental Physiology* **8**, 411-419.

SCHIFF, E., WEINER, E., ZALEL, Y., MASHIACH, S., SIBAI, B.M. & SHALEV, E. (1994) Endothelin-1,2 levels in umbilical vein serum of intra-uterine growth retarded fetuses as detected by cordocentesis. *Acta Obstetrica Gynecologica Scandinavica* **73**, 21-24.

SCHINI, V.B., BUSSE, R. & VANHOUTTE, P.M. (1994) Inducible nitric oxide synthase in vascular smooth muscle. *Arzneimittelforschung* **44**, 432-435.

SCHMIDT, R.F. & THEWS, G. (eds.) (1987) Human Physiology (2nd Ed.).

SCHOEFFTER, P., RANDRIANTSOA, A., JOST, B. & BRUTTEL, K. (1993) Comparative effects of the two endothelin ET_A receptor antagonists, BQ-123 and FR139317, on endothelin-1-induced contraction in guinea-pig iliac artery. *European Journal of Pharmacology* **241**, 165-169.

SCHRODER, H., GILBERT, R.D. & POWER, G.G. (1984) Urinary and hemodynamic responses to blood volume changes in fetal sheep. *Journal of Developmental Physiology* **6**, 131-141.

SCHUIJERS, J.A., WALKER, D.W., BROWNE, C.A. & THORBURN, G.D. (1986) Effect of hypoxemia on plasma catecholamines in intact and immunosympathectomized fetal lambs. *American Journal of Physiology* **251**, R893-R900.

SCHWIELER, J.H., KAHAN, T., NUSSBERGER, J. & HJEMDAHL, P. (1993) Converting enzyme inhibition modulates sympathetic neurotransmission in vivo via multiple mechanisms. *American Journal of Physiology* **264**, E631-E637.

SCOGGINS, B.A., SPENCE, C.D., PARKES, D.G., MCDONALD, M., WADE, J.D. & COGHLAN, J.P. (1989) Cardiovascular actions of human endothelin in conscious sheep. *Clinical and Experimental Pharmacology and Physiology* **16**, 235-238.

SCROGIN, K.E., VEELKEN, R. & LUFT, F.C. (1994) Sympathetic baroreceptor responses after chronic N^G-nitro-L-arginine methyl ester treatment in conscious rats. *Hypertension* **23**, 982-986.

SCROOP, G.C., STANKEWYTSCH-JANUSCH, B. & MARKER, J.D. (1992) Renin-angiotensin and autonomic mechanisms in cardiovascular homeostasis during haemorrhage in fetal and neonatal sheep. *Journal of Developmental Physiology* **18**, 25-33.

SEGAR, J.L., BEDELL, K., PAGE, W.V., MAZURSKY, J.E., NUYT, A-M. & ROBILLARD, J.E. (1995) Effect of cortisol on gene expression of the renin-angiotensin system in fetal sheep. *Pediatric Research* **37**, 741-746.

SEGAR, J.L., MAZURSKY, J.E. & ROBILLARD, J.E. (1994) Changes in ovine renal sympathetic nerve activity and baroreflex function at birth. *American Journal of Physiology* **267**, H1824-H1832.

SEGAR, J.L., MERRILL, D.C. & ROBILLARD, J.E. (1994) Role of endogenous ANG II on resetting arterial baroreflex during development. *American Journal of Phsyiology* **266**, H52-H59.

SENN, S. (1993) Cross-over trials in clinical research. *John Wiley & Sons.*

SHANMUGAN, S., CORVOL, P. & GASC, J-M. (1994) Ontogeny of the two angiotensin II type I receptor subtypes in rats. *American Journal of Physiology* **267**, E828-E836.

SHANMUGAM, S., MONNOT, C., CORVOL, P. & GASC, J-M. (1994) Distribution of type I angiotensin II receptor subtype messenger RNAs in the rat fetus. *Hypertension* **23**, 137-141.

SHAUL, P.W., CAMPBELL, W.B., FARRAR, M.A. & MAGNESS, R.R. (1992) Oxygen modulates prostacyclin synthesis in ovine fetal pulmonary arteries by an effect on cyclooxygenase. *Journal of Clinical Investigation* **90**, 2147-2155.

SHAUL, P.W., FARRAR, M.A. & MAGNESS, R.R. (1993) Pulmonary endothelial nitric oxide production is developmentally regulated in the fetus and newborn. *American Journal of Physiology* **265**, H1056-H1063.

SHAUL, P.W., FARRAR, M.A. & ZELLERS, T.M. (1992) Oxygen modulates endothelium-derived relaxing factor production in fetal pulmonary arteries. *American Journal of Physiology* **262**, H355-H364.

SHAUL, P.W., MAGNESS, R.R., MUNTZ, K.H., DEBELTZ, D. & BUJA, L.M. (1990) α_1-adrenergic receptors in pulmonary and systemic vascular smooth muscle. *Circulation Research* **67**, 1193-1200.

SHAUL, P.W. & WELLS, L.B. (1994) Oxygen modulated nitric oxide production selectively in fetal pulmonary endothelial cells. American Journal of Respiratory Cell and Molecular Biology **11**, 432-438.

SHETTY, S.S., OKADA, T., WEBB, R.L., DELGRANDE, D. & LAPPE, R.W. (1993) Functionally distinct endothelin B receptors in vascular endothelium and smooth muscle. *Biochemical and Biophysical Research Communications* **191**, 459-464.

SHIBA, R., YANAGISAWA, M., MIYAUCHI, T., ISHII, Y., KIMURA, S., UCHIYAMA, Y., MASAKI, T & GOTO, K. (1989) Elimination of intravenously injected endothelin-1 from the circulation of the rat. *Journal of Cardiovascular Pharmacology* **13**, S98-S101.

SHINE, P., MCDOUGALL, J.G., TOWSTOLESS, M.K. & WINTOUR, E.M. (1987) Action of atrial natriuretic peptide in the immature ovine kidney. *Pediatric Research* **22**, 11-15.

SHIRAKAMI, G., NAKAO, K., SAITO, Y., MAGARIBUCHI, T., JOUGASAKI, M., MUKOYAMA, M., ARAI, H., HOSODA, K., SUGA, S-I., OGAWA, Y., YAMADA, T., MORI, K. & IMURA, H. (1991) Acute pulmonary alveolar hypoxia increases lung and plasma endothelin-1 levels in conscious rats. *Life Sciences* **48**, 969-976.

SIDDIQI, T.A., AUSTIN, J.E., HOLROYD, J.C. & CLARK, K.E. (1983) Modulation of angiotensin II pressor responsiveness by circulating levels of angiotensin II in pregnant sheep. *American Journal of Obstetrics and Gynecology* **145**, 458-464.

SIEGEL, S.R. & FISHER, D.A. (1980) Ontogeny of the renin-angiotensin-aldosterone system in the fetal and newborn lamb. *Pediatric Research* **14**, 99-102.

SIGMON, D.H., CARRETERO, O.A. & BEIERWALTES, W.H. (1992) Endothelium-derived relaxing factor regulates renin release in vivo. *American Journal of Physiology* **263**, F256-F261.

SIGMON, D.H., CARRETERO, O.A. & BEIERWALTES, W.H. (1993) Renal versus femoral hemodynamic response to endothelium-derived relaxing factor synthesis inhibition. *Journal of Vascular Research* **30**, 218-223.

SIMONSON, M.S. & DUNN, M.J. (1991) Endothelins: A family of regulatory peptides. *Hypertension* **17**, 856-863.

SISSON, S. & GROSSMAN, J.D. (1938) The Anatomy of the Domestic Animals. *W.B. Saunders Company.*

SKIMMING, J.W., DEMARCO, V.G. & CASSIN, S. (1994) The effects of nitric oxide inhalation on the pulmonary circulation of preterm lambs. *Pediatric Research* **37**, 35-40.

SLOTTEN, P., PHERNETTON, T.M. & RANKIN, J.H.G. (1989) Relationship between fetal electrocorticographic changes and umbilical blood flow in the near-term sheep fetus. *Journal of Developmental Physiology* **11**, 19-23.

SMIESKO, V. & JOHNSON, P.C. (1993) The arterial lumen is controlled by flow-related shear stress. *NIPS* **8**, 34-38.

SMITH, C.C.T., CURTIS. L.D., DELAMOTHE, A.P., PRICHARD, B.N.C. & BETTERIDGE, D.J. (1985) The distribution of catecholamines between platelets and plasma in normal human subjects, *Clinical Science* **69**, 1-6.

SMITH, F.G., LUPU, A.N., BARAJAS, L., BAUER, R. & BASHORE, R.A. (1974) The renin-angiotensin system in the fetal lamb. *Pediatric Research* **8**, 611-620.

SMITH, F.G., SATO, T., MCWEENY, O.J., KLINKEFUS, J.M. & ROBILLARD, J.E. (1990) Role of renal sympathetic nerves in response of the ovine fetus to volume expansion. *American Journal of Physiology* **259**, R1050-R1055.

SMITH, F.G., SMITH, B.A., GUILLERY, E.N. & ROBILLARD, J.E. (1991) Role of sympathetic nerves in lambs during the transition from fetal to newborn life. *Journal of Clinical Investigation* **88**, 1988-1994.

SOGABE, K., NIREI, H., SHOUBO, M., HAMADA, K., NOMOTO, A., HNMI, K., NOTSU, Y. & ONO, T. (1992) A novel endothelial receptor antagonist. *Journal of Vascular Research* **29**, 201-202.

SOGABE, K., NIREI, H., SHOUBO, M., NOMOTO, A., AO, S., NOTSU, Y. & ONO, T. (1993) Pharmacological profile of FR139317, a novel, potent endothelin ET_A receptor antagonist. *The Journal of Pharmacology and Experimental Therapeutics*, **264**, 1040-1046.

SOLHAUG, M.J., WALLACE, M.R. & GRANGER, J.P. (1993) Endothelium-derived nitric oxide modulates renal hemodynamics in the developing piglet. *Pediatric Research* **34**, 750-754.

SPENCER, J.A.D. (1993) Fetal heart rate monitoring: application in clinical practice. In: Fetus and Neonate, Physiology and Clinical Applications. The Circulation. M.A. Hanson, J.A.D. Spencer and C.H. Rodeck (eds.). *Cambridge University press* pp. 281-295.

SPYER, K.M. (1981) Neural organisation and control of the baroreceptor reflex. *Reviews of Physiology, Biochemistry and Pharmacology* **88**, 23-124.

SPYER, K.M. (1994) Central nervous mechanisms contributing to cardiovascular control. *Journal of Physiology* **474**, 1-19.

SPYER, K.M., MCQUEEN, D.S., DASHWOOD, M.R., SYKES, R.M., DALY, M. DE B. & MUDDLE, J.R. (1991) Localization of [^{125}I] endothelin binding sites in the region of the carotid bifurcation and brainstem of the cat: possible baro- and chemoreceptor involvement. *Journal of Cardiovascular Pharmacology* **17**, S385-S389.

STALCUP, S.A., LIPSET, J.S., LEGANT, P.M., LEUENBERGER, P.J. & MELLINS, R.B. (1979) Inhibition of converting enzyme activity by acute hypoxia in dogs. *Journal of Applied Physiology* **46**, 227-234.

STARK, R.I., WARDLAW, S.L. AND DANIEL, S.S. (1986) Characterization of plasma beta-endorphin immunoreactivity in the fetal lamb: effects of gestational age and hypoxia. *Endocrinology* **119**, 755-761.

STEVENS, A.D. & LUMBERS, E.R. (1981) The relationship between plasma renin activity and renal electrolyte excretion in the fetal sheep. *Journal of Developmental Physiology* **3**, 101-110.

STEVENSON, K.M. & LUMBERS, E.R. (1992) Effects of indomethacin on fetal renal function, renal and umbilicoplacental blood flow and lung liquid production. *Journal of Developmental Physiology* **17**, 257-264.

STEVENSON, K.M. & LUMBERS, E.R. (1995) Effects of angiotensin II in fetal sheep and modification of its actions by indomethacin. *Journal of Physiology* **487**, 147-158.

STIGTER, R.H., VISSER, G.H.A. & MULDER, E.J.H. (1995) Hourly fetal urine production rate: Is it really higher during fetal behavioural state 1F? *Proceedings of the 22nd Annual Meeting of The Society for the Study of Fetal Physiology, Sweden* A11.

SUNG, C-P., ARLETH, A.J., SHIKANO, K. & BERKOWITZ, B.A. (1988) Characterization and function of bradykinin receptors in vascular endothelial cells. *The Journal of Pharmacology and Experimental Therapeutics* **247**, 8-13.

TAGAWA, T., IMAIZUMI, T., HARADA, S., ENDO, T., SHIRAMOTO, M., HIROOKA, Y. & TAKESHITA, A. (1994) Nitric oxide influences neuronal activity in the nucleus tractus solitarius of rat brainstem slices. *Circulation Research* **75**, 70-76.

TANAKA, K., GOTOH, F., GOMI, S., TAKSHIMA, S., MIHARA, B., SHIRAI, T., NOGAWA, S. & NAGATA, E. (1991) Inhibition of nitric oxide synthesis induces a significant reduction in local cerebral blood flow. *Neuroscience letters* **127**, 129-132.

TANGALAKIS, K., LUMBERS, E.R., MORITZ, K.M., TOWSTOLESS. M.K. & WINTOUR, E.M. (1992) Effect of cortisol on blood pressure and vascular reactivity in the ovine fetus. *Experimental Physiology* **77**, 709-717.

TENNEY, S.M. (1990) Hypoxic vasorelaxation. *NIPS* **5**, 40.

THORESEN, M., DAHLIN, I., LUNDBURG, J.M. AND LAGERCRANTZ, H. (1992) Neuropeptide Y and catecholamine release in the piglet during hypoxia; enhancement by theophylline. *Journal of Developmental Physiology* **18**, 187-191.

THORNBURG, K.L. & MORTON, M.J. (1993) Growth and development of the heart. In: Fetus and Neonate, Physiology and Clinical Applications. The Circulation. M.A. Hanson, J.A.D. Spencer and C.H. Rodeck (eds.). *Cambridge University press* pp. 137-159.

THORBURN, G.D. & HARDING, R. (1994) Textbook of Fetal Physiology. G.D. Thorburn and R.Harding (eds.). *Oxford Medical Publications* pp. 126-128.

THORNBURY, K.D., HOLLYWOOD, M.A. & MCHALE, N.G. (1992) Mediation by nitric oxide of neurogenic relaxation of the urinary bladder neck muscle in sheep. *Journal of Physiology* **451**, 133-144.

TIKTINSKY, M.H., CUMMINGS, J.J. & MORIN, F.C. (1992) Acetylcholine increases pulmonary blood flow in intact fetuses via endothelium-dependent vasodilation. *American Journal of Physiology* **262**, H406-H410.

TIKTINSKY, M.H. & MORIN, F.C. (1993) Increasing oxygen tension dilates fetal pulmonary circulation via endothelium-derived relaxing factor. *American Journal of Physiology* **265**, H376-H380.

TIMMERMANS, P.B.M.W.M, WONG, P.C., CHIU, A.T. & HERBIN, W.F. (1991) Nonpeptide angiotensin II receptor antagonists. *TIPS* **12**, 55-62.

TOD, M.L. & CASSIN, S. (1992) Endothelin-1-induced pulmonary arterial dilation is reduced by N^{ω}-nitro-L-arginine in fetal lambs. *Journal of Applied Physiology* **72**, 1730-1734.

TOGASHI, H., SAKUMA, I., YOSHIOKA, M., KOBAYASHI, T., YASUDA, H., KITABATAKE, A., SAITO, H., GROSS, S.S. & LEVI, R. (1992) A central nervous system action of nitric oxide in blood pressure regulation. *The Journal of Pharmacology and Experimental Therapeutics* **262**, 343-347.

TOLINS, J.P., PALMER, R.M.J., MONCADA, S. & RAIJ, L. (1990) Role of endothelium-derived relaxing factor in regulation of renal hemodynamic responses. *American Journal of Physiology* **258**, H655-H662.

TOLINS, J.P. & RAIJ, L. (1991) Effects of amino acid infusion on renal hemodynamics. Role of endothelium-derived relaxing factor. *Hypertension* **17**, 1045-1051.

TOLINS, J.P., SCHULTZ, P.J. & RAIJ, L. (1991) Role of endothelium-derived relaxing factor in regulation of vascular tone and remodelling. Update on humoral regulation of vascular tone. *Hypertension* **17**, 909-916.

TOWELL, M.E., FIGUEROA, J., MARKOWITZ, S., ELIAS, B. & NATHANIELSZ, P.W. (1987) The effect of mild hypoxemia maintained for twenty-four hours on maternal and fetal glucose, lactate, cortisol, and arginine vasopressin in pregnant sheep at 122 to 139 days' gestation. *American Journal of Obstetrics and Gynecology* **157**, 1550-7.

TRESHAM, J.J., DUSTING, G.J., COGHLAN, J.P. & WHITWORTH, J.A. (1991) Haemodynamic and hormonal effects of N-nitro-L-arginine, an inhibitor of nitric oxide biosynthesis, in sheep. *Clinical and Experimental Pharmacology and Physiology* **18**, 327-330.

TRZEBSKI, A., SATO, Y., SUZUKI, A. & SATO, A. (1995) Inhibition of nitric oxide synthesis potentiates the responsiveness of carotid chemoreceptors to systemic hypoxia in the rat. *Neuroscience Letters* **190**, 29-32.

TUFRO-MCREDDIE, A. & GOMEZ, R.A. (1993) Ontogeny of the renin-angiotensin system. *Seminars in Nephrology* **13**, 519-530.

TUFRO-MCREDDIE, A., HARRISON, J.K., EVERETT, A.D. & GOMEZ, R.A. (1993) Ontogeny of type I angiotensin II receptor gene expression in the rat. *Journal of Clinical Investigation* **91**, 530-537.

UJIIE, K., YUEN, J., HOGARTH, L., DANZIGER, R. & STAR, R.A. (1994) Localization and regulation of endothelial NO synthase mRNA expression in rat kidney. *American Journal of Physiology* **267**, F296-F302.

UZUNER, K. & BANKS, R.O. (1993) Endothelin-induced natriuresis and diuresis are pressure-dependent events in the rat. *American Journal of Physiology* **265**, R90-R96.

VALLET, B., CUTIS, S.E., WINN, M.J., KING, C.E., CHAPLER, C.K. & CAIN, S.M. (1994) Hypoxic vasodilation does not require nitric oxide (EDRF/NO) synthesis. *Journal of Applied Physiology* **76**, 1256-1261.

VAN BEL, F., ROMAN, C., KLAUTZ, R.J.M., TEITEL, D.F. & RUDOLPH, A.M. (1994) Relationship between brain blood flow and carotid arterial flow in the sheep fetus. *Pediatric Research* **35**, 329-333.

VANHOUTTE, P.M. (1994) A matter of life and breath. *Nature* **368**, 693-694.

VAN RENTERGHAM, C., VIGNE, P., BARHANIN, J., SCHMID-ALLIANA, A., FRELIN, C. & LAZDUNSKI, M. (1989) Molecular mechanism of endothelin-1 action on aortic cells. *Journal of Cardiovascular Pharmacology* **13**, S186-S187.

VARGAS DA SILVA, S., DIAS DA SILVA, V.J., BALLEJO, G., SALGADO, M.C.O. & SALGADO, H.C. (1994) Blockers of the L-arginine-nitric oxide-cyclic GMP pathway facilitate baroreceptor resetting. *Hypertension* **23**, I60-I63.

VASQUEZ, E.C., CUNHA, R.S. & CABRAL, A.M. (1994) Baroreceptor reflex function in rats submitted to chronic inhibition of nitric oxide synthesis. *Brazilian Journal of Biological Research* **27**, 767-774.

VIJAYRAGHAVAN, J., SCICLI, A.G., CARRETERO, O.A., SLAUGHTER, C., MOOMAW, C., & HERSH, L.B. (1990) The hydrolysis of endothelins by neutral endopeptidase 24.11 (enkephalinase). *The Journal of Biological Chemistry* **265**, 14150-14155.

VO, P.A., REID, J.J. & RAND, M.J. (1991) Endothelial nitric oxide attenuates vasoconstrictor responses to nerve stimulation and noradrenaline in the rat tail artery. *European Journal of Physiology* **199**, 123-125.

VOELKER, C.A., MILLER, M.J.S., ZHANG, X-J., ELOBY-CHILDRESS, S., CLARK, D.A. & PIERCE, M.R. (1995) Perinatal nitric oxide synthase inhibition retards neonatal growth by inducing hypertrophic pyloric stenosis in rats. *Pediatric Research* **38**, 768-774.

VYAS, S., NICOLAIDES, K.H. & CAMPBELL, S. (1989) Renal artery flow-velocity waveforms in normal and hypoxemic fetuses. *American Journal of Obstetrics and Gynecology* **161**, 168-172.

WAGERLE, L.C., KUMAR, S.P., BELIK, J. & DELIVORIA-PAPADOPOULOS, M. (1988) Blood-brain barrier to hydrogen ion during acute metabolic acidosis in piglets. *Journal of Applied Physiology* **65**, 776-781.

WAGERLE, L.C., KURTH, C.D. & ROTH, R.A. (1990) Sympathetic reactivity of cerebral arteries in developing fetal lamb and adult sheep. *American Journal of Physiology* **258**, H1432-H1438.

WAGERLE, L.C. & MISHRA, O.P. (1988) Mechanism of CO_2 response in cerebral arteries of the newborn pig: Role of phospholipase, cyclooxygenase, and lipoxygenase pathways. *Circulation Research* **62**, 1019-1026.

WAGERLE, L.C., MOLIKEN, W., & RUSSO, P. (1995) Nitric oxide and β-adrenergic mechanisms modify contractile responses to norepinephrine in ovine fetal and newborn cerebral arteries. *Pediatric Research* **38**, 237-242.

WAGNER, O.F., CHRIST, G., WOJTA, J., VIERHAPPER, H., PARZER, S., NOWOTNY, P.J., SCHNEIDER, B.S., WAKATSUKI, A., MURATA, Y., NINOMIYA, Y., MASAOKA, N., TYNER, J.G. & KUTTY, K.K. (1992) Physiologic baroreceptor activity in the fetal lamb. *American Journal of Obstetrics and Gynecology* **167**, 820-827.

WALDHAUSL, W. & BINDER, B.R. (1992) Polar secretion of endothelin-1 by cultured endothelial cells. *The Journal of Biological Chemistry* **267**, 16066-16068.

WALDER, C.E., THIEMERMANN, C. & VANE, J.R. (1991) The involvement of endothelium-derived relaxing factor in the regulation of renal cortical blood flow in the rat. *British Journal of Pharmacology* **102**, 967-973.

WALKER, A.M. (1993) Circulatory transitions at birth and the control of the neonatal circulation. In: Fetus and Neonate, Physiology and Clinical Applications. The Circulation. M.A. Hanson, J.A.D. Spencer and C.H. Rodeck (eds.). *Cambridge University press* pp. 160-196.

WALKER, D.W. (1977) Renal function and micturition in fetal lambs, and the effects of hypoxia. *Annales Recherches Veterinaires* **8**, 497-498.

WALKER, V., BENNET, L., MILLS, G.A., GREEN, L.R., GNANAKUMARAN, K. AND HANSON, M.A. (1996) Effects of hypoxia on urinary organic acid and hypoxanthine excretion in fetal sheep. *Pediatric Research* In press.

WALKER, A.M., FLEMING, J., SMOLICH, J., STUNDEN, R., HORNE, R. & MALONEY, J. (1984) Fetal oxygen consumption, umbilical circulation and electrocortical activity transitions in fetal lambs. *Journal of Developmental Physiology* **6** 267-274.

WALKER, M.P.R., MOORE, T.R. & BRACE, R.A. (1992) Urinary and cardiovascular responses to indomethacin infusion in the ovine fetus. *American Journal of Obstetrics and Gynecology* **167**, 834-843.

WALKER, M.P.R., MOORE, T.R. & BRACE, R.A. (1994) Indomethacin and arginine vasopressin interaction in the fetal kidney: A mechanism of oliguria. *American Journal of Obstetrics and Gynecology* **171**, 1234-1241.

WALKER, M.P.R., MOORE, T.R., CHEUNG, C.Y. & BRACE, R.A. (1992) Indomethacin-induced urinary flow rate reduction in the ovine fetus is associated with reduced free water clearance and elevated plasma arginine vasopressin levels. *American Journal of Obstetrics and Gynecology* **167**, 1723-1731.

WALLENSTEIN, S. (1982) Regression models for repeated measurements. *Biometrics* **38**, 849-853.

WALLENSTEIN, S., ZUCKER, C.L. & FLEISS, J.L. (1980) Some statistical methods useful in circulation research. *Circulation Research* **47**, 1-9.

WANG, Y. & COCEANI, F. (1992) Isolated pulmonary resistance vessels from fetal lambs. Contractile behaviour and responses to indomethacin and endothelin-1. *Circulation Research* **71**, 320-330.

WANG, Y., COE, Y., TOYODA, O. & COCEANI, F. (1995) Involvement of endothelin-1 in hypoxic pulmonary vasoconstriction in the lamb. *Journal of Physiology* **482**, 421-434.

WANG, Z.Z., STENSAAS, L.J., DINGER, B.G. & FIDONE, S.J. (1995) Nitric oxide mediates chemoreceptor inhibition in the cat carotid body. *Neuroscience* **65**, 217-229.

WEI, H.M., WEISS, H.R., SINHA, A.K. & CHI, O.Z. (1993) Effects of nitric oxide synthase inhibition on regional cerebral blood flow and vascular resistances in conscious and isoflurane-anaesthetized rats. *Neurosurgical Anesthesia* **77**, 880-885.

WEISMANN, D.N., HERRIG, J.E., MCWEENY, O.J. & ROBILLARD, J.E. (1983) Organ tissue blood flow responses to hypoxemia in lambs: effect of angiotensin converting enzyme inhibitor. *Pediatric Research* **17**, 195-199.

WENNMALM, A., BENTHIN, G. & PETERSSON, A.-S. (1992) Dependence of the metabolism of nitric oxide (NO) in healthy human whole blood on the oxygenation of its red cell haemoglobin. *British Journal of Pharmacology* **106**, 507-508.

WHITE, D.G., DREW, G.M., GURDEN, J.M., PENNY, D.M., ROACH, A.G. & WATTS, I.S. (1993) The effect of N^G-nitro-L-arginine methyl ester upon basal blood flow and endothelium-dependent vasodilatation in the dog hindlimb. *British Journal of Pharmacology* **108**, 763-768.

WHITTLE, B.J.R., LOPEZ-BELMONTE, J. & REES, D.D. (1989) Modulation of the vasodepressor actions of acetylcholine, bradykinin, substance P and endothelin in the rat by a specific inhibitor of nitric oxide formation. *British Journal of Pharmacology* **98**, 646-652.

WILKES, B.M., KRIM, E. & MENTO, P.F. (1985) Evidence for a functional renin-angiotensin system in full-term fetoplacental unit. *American Journal of Physiology* **249**, E366-E373.

WILLETTE, R.N. & SAUERMELCH, C.F. (1990) Abluminal effects of endothelin in cerebral microvasculature assessed by laser-Doppler flowmetry. *American Journal of Physiology* **259**, H1688-H1693.

WINTOUR, E.M., BELL, R.J., CONGUI, M., MACISAAC, R.J. & WANG, X. (1985) The value of urine osmolality as an index of stress in the ovine fetus. *Journal of Developmental Physiology* **7**, 347-354.

WINTOUR, E.M. & SHANDLEY, L. (1993) Effects of fetal fluid balance on amniotic fluid volume. *Seminars in perinatology* **17**, 158-172.

WIRIYATHIAN, S., PORTER, J.C., NADEN, R.P. & ROSENFELD, C.R. (1983) Cardiovascular effects and clearance of arginine vasopressin in the fetal lamb. *American Journal of Physiology* **245**, E24-E31.

WLODEK, M.E., CHALLIS, J.R.G. & PATRICK, J. (1988) Urethral and urachal urine output to the amniotic and allantoic sacs in fetal sheep. *Journal of Developmental Physiology* **10**, 309-319.

WLODEK, M.E., CHALLIS, J.R.G., RICHARDSON, B. & PATRICK, J. (1989) The effects of hypoxemia with progressive acidemia on fetal renal function in sheep. *Journal of Developmental Physiology* **12**, 323-328.

WLODEK, M.E., HARDING, R. & THORBURN, G.D. (1992) The influence of gestational age and onset of labour on determinants of fetal-maternal fluid and electrolyte balance in sheep. *Journal of Developmental Physiology* **18**, 111-119.

WLODEK, M.E., HARDING, R. & THORBURN, G.D. (1994) Effects of inhibition of prostaglandin synthesis on flow and composition of fetal urine, lung liquid, and swallowed fluid in sheep. *American Journal of Obstetrics and Gynecology* **170**, 186-195.

WLODEK, M.E., THORBURN, G.D. & HARDING, R. (1989) Bladder contractions and micturition in fetal sheep: their relation to behavioural states. *American Journal of Physiology* **257**, R1526-R1532.

WOLFF, D.J. & LUBESKIE, A. (1995) Aminoguanidine is an isoform-selective, mechanism-based inactivator of nitric oxide synthase. *Archives of Biochemistry and Biophysics* **316**, 290-301.

WONG, J., FINEMAN, J.R. & HEYMANN, M.A. (1994) The role of endothelin receptor subtypes in regulation of fetal pulmonary vascular tone. *Pediatric Research* **35**, 664-670.

WONG, J., VANDERFORD, P.A., FINEMAN, J.R., CHANG, R. & SOIFER, S.J. (1993) Endothelin-1 produces pulmonary vasodilation in the intact newborn lamb. *American Journal of Physiology* **265**, H1318-H1325.

WONG, J., VANDERFORD, P.A., FINEMAN, J.R. & SOIFER, S.J. (1994) Developmental effects of endothelin-1 on the pulmonary circulation in sheep. *Pediatric Research* **36**, 394-401.

WOOD, C.E. (1989) Sinoaortic denervation attenuates the reflex responses to hypotension in fetal sheep. *American Journal of Physiology* **256**, R1103-R1110.

WOOD, C.E. (1993) Local and endocrine factors in the control of the circulation. In: Fetus and Neonate. The Circulation. M.A. Hanson, J.A.D. Spencer, C.H. Rodeck (eds.). *Cambridge University Press* pp. 100-115.

WOOD, C.E. & CHEN, H-G. (1989) Acidemia stimulates ACTH, vasopressin, and heart rate responses in fetal sheep. *American Journal of Physiology* **257**, R344-R349.

WOOD, C.E., CHEN, H-G & BELL, M.E. (1989) Role of vagosympathetic fibres in the control of adrenocorticotropic hormone, vasopressin, and in renin responses to hemorrhage in fetal sheep. *Circulation Research* **64**, 515-523.

WOOD, C.E., CHEUNG, C.Y. & BRACE, R.A. (1987) Fetal heart rate, arterial pressure, and blood volume responses to cortisol infusion. *American Journal of Physiology* **253**, R904-R909.

WOOD, C.E., KANE, C. & RAFF, H. (1990) Peripheral chemoreceptor control of fetal renin responses to hypoxia and hypercapnia. *Circulation Research* **67**, 722-732.

WOOD, C.E., KEIL, L.C. & RUDOLPH, A.M. (1984) Physiological inhibition of ovine fetal plasma renin activity by cortisol. *Endocrinology* **115**, 1792-1796.

WOOD, C.E., SHINSAKO, J. & DALLMAN, M.F. (1982) Comparison of canine corticosteroid responses to mean and phasic increases in ACTH. *American Journal of Physiology* **242**, E102-E108.

WOOD, C.E., SHINSAKO, J., KEIL, L.C., RAMSAY, D.J. & DALLMAN, M.F. (1982) Apparent dissociation of adrenocorticotropin and corticosteroid responses to 15ml/kg hemorrhage in conscious dogs. *Endocrinology* **110**, 1416-1421.

WOOLFSON, R.G. & POSTON, L. (1990) Effect of N^G-monomethyl-L-arginine on endothelium-dependent relaxation of human subcutaneous resistance arteries. *Clinical Science* **79**, 273-278.

WYATT, J.S., EDWARDS, A.D. & REYNOLDS, E.O.R. (1993) Assesment of cerebral haemodynamics and oxygenation in the newborn human infant. In: Fetus and Neonate, Physiology and Clinical Applications. The Circulation. M.A. Hanson, J.A.D. Spencer and C.H. Rodeck (eds.). *Cambridge University press* pp. 339-364.

YALLAMPALLI, C. & GARFIELD, R.E. (1993) Inhibition of nitric oxide synthesis in rats during pregnancy produces signs similar to those of preeclampsia. *American Journal of Obstetrics and Gynecology* **169**, 1316-1320.

YANAGISAWA, M., KURIHARA, H., KIMURA, S., TOMOBE, Y., KOBAYASHI, M., MITSUI, Y., YAZAKI, Y., GOTO, K. & MASAKI, T. (1988) A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature* **332**, 411-415.

YONEYAMA, Y. & POWER, G.G. (1992) Plasma adenosine and cardiovascular responses to dipyridamole in fetal sheep. *Journal of Developmental Physiology* **18**, 203-209.

YANG, B.C. & METHA, J.L. (1995) Critical role of endothelium in sustained arterial contraction during prolonged hypoxia. *American Journal of Physiology* **268**, H1015-H1020.

YARDLEY, R.W., BOWES, G., WILKINSON, M., MALONEY, J.E., RITCHIE, B.C. & WALKER, A.M. (1983) Increased arterial pressure variability after arterial baroreceptor denervation in fetal lambs. *Circulation Research* **52**, 580-588.

YATES, F. (1982) Regression models for repeated measurements. *Biometrics* **38**, 850-853.

YESBURG, N.E., HENDERSON, M., WILSON, K., LAW, S. & CROSS, R.B. (1982) The effects of an angiotensin blocker (saralasin) on kidney function in dehydrated sheep. Quarterly Journal of Experimental Physiology **67**, 97-103.

YOSHIDA, H., TAMAKI, T., AKI, Y., KIMURA, S., TAKENAKA, I. & ABE, Y. (1994) Effects of angiotensin II in isolated rabbit afferent arterioles. *Japanese Journal of Pharmacology* **66**, 457-464.

YOSHIDA, K., YASUJIMA,, M., KOHZUKI, M., KANAZAWA, M., YOSHINAGA, K. & ABE, K. (1992) Endothelin-1 augments pressor response to angiotensin II infusion in rats. *Hypertension* **20**, 292-297.

YOSHIMOTO, ISHIZAKI, Y., MORI, SASAKI, T., TAKAKURA, K. & MUROTA, S-I. (1991) The role of cerebral microvessel endothelium in regulation of cerebral blood flow through production of endothelin-1. *Journal of Cardiovascular Pharmacology* **17**, S260-S263.

YOSHIMURA, T., MAGNESS, R.R. & ROSENFELD, C.R. (1990a) Angiotensin II and α-agonist I. Responses of ovine fetoplacental vasculature. *American Journal of Physiology* **259**, H464-H472.

YOSHIMURA, T., MAGNESS, R.R. & ROSENFELD, C.R. (1990b) Angiotensin II and α-agonist II. Effects on ovine fetoplacental prostaglandins. *American Journal of Physiology* **259**, H473-H479.

YOSHIZUMI, M., KURIHARA, H., SUGIYAMA, T., TAKAKU, F., YANAGISAWA, M., MASAKI, T. & YAZAKI, Y. (1989) Hemodynamic shear stress stimulates endothelin production by cultured endothelial cells. *Biochemical and Biophysical Research Communications* **161**, 859-864.

APPENDIX 1
ORGAN WEIGHTS

Animal	Age	single /twin (S/T)	Male /Female (M/F)	CRL (cm)	WEIGHTS					
					Fetus (Kg)	Heart (g)	Lungs (g)	Liver (g)	Kidney 1 (g)	Kidney 2 (g)
					CHAPTER 3 Renal					
INTACT										
92-53	120	T	-	-	2.8	-	-	-	-	-
92-21	134	S	F	48	3.38	34	100	116	12(op)	15
92-26	122	S	M	47	3.26	29	106	106	15(op)	13
92-30	136	S	F	47	3.16	29	85	116	22(op)	17
92-31	133	S	F	45.3	3.02	35	94	132	13(op)	13
92-36	134	S	F	45	3.98	32	120	112	12(op)	12
CSD										
94-39	133		F		4.66	37	143	149	18	18
93-75	128	S	M	48	3.54	26	100	133	21	22
93-79	128	S	M	49	4.54	30	126	142	18(op)	15
93-100	126	T	F	45	4.22	43	156	154	35(op)	44
94-01	126	S		48	3.48	42	97	150	16	28
94-40	134	T	M	48	3.9	38	106	124	20	18
94-44	131	S	F	48.5	4.18	30	129	176	17(op)	19
94-45	132	T	M	56	4.52	44	124	116	26	17
94-53	133	S	-	-	2.52	24.2	73	72	9.7	10.5
93-94	125	S	F	43.5	2.74	26	84	161	13	14
93-95	131	T	M	-	4.26	42	86	150	11	20
93-101	127	T	F	38.5	2.42	26	77	105	14	14
					CHAPTER 4 Angiotensin II					
Intact										
93-03	132	S	M	41	4.54	37	94	113	15	15
93-05	132	S	M	44	4.82	31	152	155	9	9
93-08	131	T	- (op)	-	3.8	27	126	113	14	15
			-	-	3.48	-	-	-	-	-
93-16	131	T	M (op)	-	3.58	34	103	105	18	15
			F	-	4.28	34	144	99	18	16
93-23	120	T	-(op)	48	3.86	28	143	97	14	15
			F	-	3.4	23	101	65	14	14
93-44	128	T	-(op)	-	3.76	29	117	134	21	21
			-	-	2.88	23	70	80	20	X
CSD										
93-12	131	S	M	43.5	3.7	28	138	104	10	11
93-30	130	T	aborted	-	-	-	-	-	-	
93-33	131	T	M(op)	50.5	3.28	22	104	97	12	12
			F	50	3.78	24	142	100	13	14
93-42	133	T	-(op)	45.5	3.48	34	103	124	11	15
			-	44	3.16	30	118	98	15	17
93-49	125	S	M	46	3.29	28	80	139	19	18
93-53	121	S	M	40	2.48	24.2	79.6	107.2	14.7	12.9
93-58	131	S	-	-	4.74	33	126	132	17	19
93-62	131	T	M (op)	41	2.84	26	86	91	12	12
			F	44	3.22	22	124	79	16	14
93-66	128	S	F	47	3.64	30	115	112	18	17

Animal	Age	single /twin (S/T)	Male /Female (M/F)	CRL (cm)	WEIGHTS					
					Fetus (Kg)	Heart (g)	Lungs (g)	Liver (g)	Kidney 1 (g)	Kidney 2 (g)
			CHAPTER 5 AND 6 Endothelin-1 and Nitric oxide studies							
94-18	131	T	F (op)	-	3.88	47	106	112	25	16
			-	-	3.9	36	144	114	20	21
94-20	131	T	M (op)	47	3.16	32	114	108	18	17
			M	44	3.54	36	117	108	22	20
94-21	126	T	M (op)	50	3.54	39	104	78	18	18
			F	48	3.1	29	100	52	12	14
94-29	127	T	M (op)	43	2.74	29	87	96	14	14
			M	42.5	2.52	23	56	77	10	15
94-30	127	T	M(op)	43	2.74	29	81	96	14	14
			M	42.5	2.52	23	56	77	10	15
94-33	132	S	F	48	4.48	31	139	162	21	20
94-35	125	S	-	-	3.22	26	110	123	18	19
			CHAPTER 7 Long-term nitric oxide study							
94-64	127	S		-	3.16	24.92	81.481	120.4	9.07	9.64
95-07		S	M	48	4.52	31.93	127	141	19.4	21
95-17	128	T	M (op)	49	4.16	26.6	110.9	118.8	16.4	18
			M	45.8	3	19.27	108.61	88.3	13.1	13.4
95-14	133	T	M (op)	46.5	3.64	21.67	120.9	83.3	11.7	11.6
			M	50.5	3.74	26.63	152	115.1	12	12.65
95-32	132	S	F	47	4.18	39	115.6	165.8	18.4	16.3
95-33	131	S	F	47	3.46	25.4	89.8	110.2	14.1	14
95-37	133	T	F (op)	55	3.92	24.7	123	109.5	12.9	14
			F	48.5	3.4	24.1	102.6	102.4	14.7	13.6
95-43	134	S	F	-	4.54	39	138.7	141.2	14.5	14.7
94-44	134	T	F (op)	49.7	3.38	24.1	98.7	88.5	11.2	10
			F	49	4.1	30.1	107.6	124.4	13.7	14.9

APPENDIX 2
POWER CALCULATION

In Chapter 3, a nomogram (Figure A2.1) was used to calculate the chance of detecting, as a statistically significant, a change in UO.

From the data presented in Chapter 3 the mean and standard deviation of UO measurements from 6 intact fetuses was 7.33 and 4.29, respectively. Standardised difference is calculated from:

the difference of interest ÷ standard deviation

A 4ml change in UO from the given data would yield a standardised difference of 4÷4.29 (=0.93). With a sample size (N) of 6 at the 5% level of significance there is a 21% probability of detecting a such a change in UO (Figure A2.1).

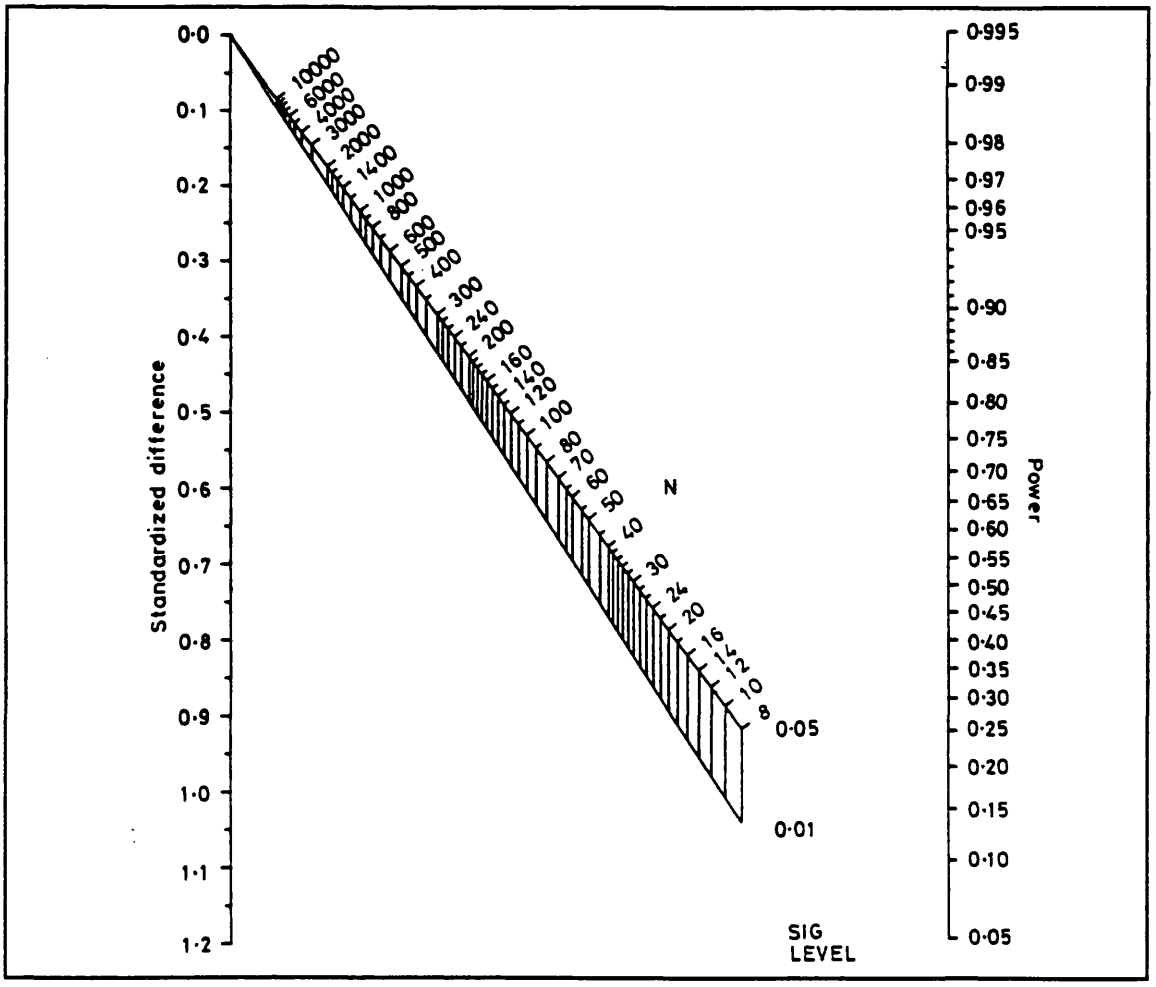


Figure A2.1 Nomogram for calculating sample size or power (Taken from Altman, 1991).

APPENDIX 3

BLOOD GAS and CO-OXIMETER PARAMETERS

Derivation of calculated parameters

HCO_3^- :

$\log_{10} [HCO_3^-] = pH + \log_{10} PCO_2 - 7.604$

BE :

$BE = (1-0.014[Hb]) ([HCO_3^-]-24 + (1.43[Hb] +7.7) (pH-7.4)$

Hb (or total Hb):

$Hb = [O_2Hb] + [RHb] + [COHb] + [metHb]$

sO_2 :

$sO_2m = [\% O_2Hb / (100-(\%CO_2Hb + \%metHb))] \times 100$

O_2 content

$O_2ct = 1.39 [Hb] \times \% O_2Hb/100$

Temperature correction equations

pH:

$\Delta pH / \Delta T = -0.0147 + 0.0065 (7.4-pH)$

PCO_2:

$(\Delta \log_{10} PCO_2) / \Delta T = 0.019$

PO_2:

$(\Delta \log_{10} PO_2) / \Delta T = (5.49 \times 10^{-11} PO_2^{3.88} + 0.071) / (9.72 \times 10^{-9} PO_2^{3.88} + 2.3)$

APPENDIX 4
ANTIBIOTIC SPECIFICATIONS

Streptopen (Pitman-Moore Ltd., UK)

Per ml:	Procaine penicillin G	250mg
	Digydrostreptomycin	250mg

Active against Gram-positive (penicillin and streptomycin) and Gram-negative (streptomycin) bacteria.

Gentamicin (DBL, UK)

Per ml:	gentamicin sulphate	40mg
	sodium metabisulphate	3.2mg
	disodium edetate	0.1mg

Active against a wide range of Gram-positive and Gram-negative bacteria. Particularly active against *Pseudomonas* species.

Crystapen (Britannia Pharmaceuticals Ltd., UK)

600mg sodium benzylpenicillin. Dissolved in 4ml sterile saline.

Bactericidal activity in infections due to penicillin-sensitive organisms. Particularly active against Gram-positive organisms.

APPENDIX 5
HORMONE ASSAY TECHNIQUES

Blood was collected onto chilled EDTA tubes, chil-spun at 4°C, the plasma was aspirated off and stored in plastic tubes at -20°C prior to hormonal analysis [2.4.4].

Catecholamine assay

The catecholamine assay contained in Chapter 3 was performed by Dr C. Smith, Department of Pharmacology and Medicine, University College London.

2ml portions of plasma were treated with 20mg alumina and 400µl of 2M Tris buffer and 5pmol (25µl) DHBA internal standard. HPLC with electrochemical detection was used to determine [catecholamine] (adrenaline and noradrenaline) (Smith, Curtis, Delamothe, Prichard and Betteridge, 1985). The chromatographic system consisted of a Waters "resolve" C18 reversed-phase column (3.9mm x 150mm, particle 5µM), a Waters Model 460 electrochemical detector with glossy carbon electrode set at a potential of +0.58V vs. KCl and a sensitivity of 0.5nA and a Waters Model 510 HPLC pump. Data are recorded on a waters 740 Data Module (Waters, Division of Millipore Ltd., Middlesex, UK). Separation was carried out using an isocratic solvent system consisting of an acetate-citrate buffer, containing sodium octane sulphonate (5.8mmol.L^{-1}), EDTA (3mmol.L^{-1}) and 14% methanol with flow rate maintained at 1ml.min^{-1}.

Intact and CSD samples were assayed in separate batches. For adrenaline and noradrenaline recoveries were >80% and the sensitivity of the assay was 0.1 pmol per 70 µl injection onto the HPLC column. Interassay coefficients of variation (COV) were 13 and 8%, respectively, and intraassay COV were 5 and 4%, respectively.

Angiotensin II assay

The AII assay contained in Chapters 4, 5 and 6 were performed by Dr. H.H.G. McGarrigle, Department of Obstetrics and Gynaecology, University College London.

Angiotensin II was measured by a sensitive and specific competitive protein-binding RIA, following its separation from plasma proteins by methanol extraction and chromatography, using a kit supplied by Nichols Institute (Diagnostics B.V., Saffron Walden, Essex, UK).

Extraction procedure: C18 chromatography columns (Sep Pak, Waters Associates, Millford, MA, USA) were mounted on a Super Separator-24 manifold, containing a vacuum facility for eluting the columns. The columns were washed with 10ml methanol and 10ml phosphate buffer. 0.5ml aliquots of plasma were mixed with equal volumes of 10mmol phosphate buffer pH 7.4 and added to the column. Columns were then washed with 10ml phosphate buffer. 2.5ml methanol was added to the column to elute the AII. The methanol was evaporated with a jet of air (at 37°C) and the residue reconstituted with 0.84ml of Tris buffer.

RIA procedure: Duplicate 0.4ml aliquots of extract in Tris buffer were transferred to polystyrene tubes and 0.1ml of anti-AII (rabbit) antiserum added. Tubes were mixed, covered and incubated for 6 h at 2-8°C. 0.1ml iodinated-AII was added to tubes, mixed and incubated for 18 h at 4°C. 0.1ml anti-rabbit-antiserum (donkey) precipitant was added and tubes were mixed and incubated at room temperature for 30 min. Deionized water (1ml) was added to each sample, and the tubes centrifuged at room temperature for 15 min (2000g). The supernatant was decanted and the tubes containing the residue were transferred to a gamma counter to determine radioactive content.

For a given study samples were assayed in a single batch. Recoveries averaged 83% (range 75-91%). The assay sensitivity was 3.8 $pg.ml^{-1}$. The intra and interassay COV's reported of the assay were 4% for a value of 42$pg.ml^{-1}$, and 5.1% and 9.3% for values of 31$pg.ml^{-1}$ and 96$pg.ml^{-1}$, respectively.

Endothelin-1 assay

The ET-1 assay reported in Chapter 5 was performed by Dr. H.H.G. McGarrigle, Department of Obstetrics and Gynaecology, University College London using a kit purchased from Nichols Institute (Diagnostics B.V., Saffron Walden, Essex, UK).

Endothelin-1 was measured by a sensitive and specific competitive protein-binding RIA, following its extraction from plasma and purification on C18 columns.

Extraction procedure:

0.6ml aliquots of plasma were acidified with 1.0ml of 4% acetic acid. The acidified samples were then passed through C18 columns and the columns rinsed with 3ml of 25% ethanol. The columns were then washed with 1ml of 4% acetic acid in 86% ethanol (to extract the endothelin) and the eluate collected in glass tubes. The eluate was evaporated at 37°C in a stream of air. The dried extracts were reconstituted in 0.22ml of assay buffer.

RIA procedure: 0.2ml aliquots of each extract were transferred to polystyrene tubes and 0.1ml of anti-endothelin (rabbit) antiserum, and 0.1ml iodinated-endothelin tracer were added to each tube. The tubes were mixed and incubated for 18 h at 4°C. 0.1ml anti-rabbit antibody was added, 1ml of deionised water was added to each tube and the tubes were mixed and incubated at room temperature for 30 min. The tubes were centrifuged at 4°C for 10 min (2000g). The supernatant was decanted and the residue counted in a gamma counter for 3 min to determine radioactive content.

For a given study samples were assayed in a single batch. Recoveries averaged 88%. The assay sensitivity was 8pg.ml^{-1}. The intra and inter assay COV's reported for the assay are 4.5% (14.7pg.ml^{-1}) and 6.8% (10.9pg.ml^{-1}), respectively. The assay was specific for ET1-21 and cross-reacted with ET-1 (100%), -2 (144%) and -3 (52%).

ACTH assay

The ACTH assay contained in Chapters 5 and 6 was carried out by Dr H.H.G. McGarrigle, Department of Obstetrics and Gynaecology, University College London.

Plasma [ACTH] was measured by a double-antibody ^{125}I RIA using an assay kit (Diagnostics Products Ltd., Abington UK)

RIA procedure: 100µl plasma samples were incubated with 100µl of rabbit anti-human ACTH antiserum and 100µl of [^{125}I]ACTH for 24 h. The bound and free hormone fractions were separated by mixing with 1ml antibody-precipitating mixture of goat anti-rabbit γ-globulin antiserum and dilute polyethylene glycol in saline. The precipitate was removed and the radioactive content assessed (Giussani, McGarrigle, Moore, Bennet, Spencer and Hanson, 1994a).

The sensitivity of the assay was 8pg.ml^{-1}. The anti-ACTH antiserum showed 0.2% cross-reactivity against α-melanocyte-stimulating hormone and no detectable cross-reactivity against β-endorphin (human), [met^5]enkephalin, [leu^5]enkephalin, neurotensin, substance-P and somatostatin.

Cortisol assay

Measurement of plasma [cortisol] contained in Chapters 5 and 6 was carried out by Dr H.H.G. McGarrigle, Department of Obstetrics and Gynaecology, University College London by RIA using ^{125}I cortisol.

Extraction procedure: 50µl plasma samples were mixed with equal volumes of sodium carbonate solution (1.7M, pH 10.5) and extracted with 2.0ml of diethyl ether. After

freezing the ether was decanted and evaporated. The residue was reconstituted in 500µl of phosphate-buffered saline (PBS; pH 7.4).

RIA procedure: Aliquots were removed and made up to 400µl with PBS and incubated with 16000 dpm [1,2,6,7-3H]cortisol (Amersham International, Aylesbury, UK) and 100ml of anti-cortisol antiserum (Steranti Ltd, St Albans, UK). Bound and free steroid were separated using dextran-coated charcoal and, after centrifugation, a 500µl aliquot was removed for measuring the radioactive content.

The recovery of the assay was 90%. The sensitivity of the assay was 30fmol.ml^{-1}. The anti-cortisol antiserum showed cross-reactivity with 21-deoxycortisol (58%), 11-deoxycortisol (15.3%), corticosterone (2.8%), cortisone (2%), deoxycortisone (<0.6%), aldosterone (<0.6%), progesterone (2.4%), 17-β-oestradiol (<0.6%) and oestrone (<0.6%).

The specificity of the assay for sheep cortisol has already been established (Giussani, Spencer, Moore, Bennet and Hanson, 1994a).

APPENDIX 6

ANALYSIS OF FETAL URINE USING PROTON NMR SPECTROSCOPY

All the spectroscopic analysis carried out in this appendix was performed by Dr. P. Foxall, Department of Chemistry, Birkbeck College, University of London using urine samples obtained from intact and CSD fetuses included in Chapter 3.

A6.1 Introduction

During acute hypoxia there is a redistribution of blood flow favouring the heart, brain and adrenal glands at the expense of peripheral vascular beds. This has been characterised by a rapid initial bradycardia, a fall in FBF and a slower rise in MAP. It was the aim of Chapter 3 to put the haemodynamic changes (i.e. RBF) occurring in hypoxia in the context of organ function (i.e. UO). However, while the results presented in Chapter 3 confirmed a fall in RBF during hypoxia, there were no consistent changes in UO. An alternative approach was to measure the metabolic changes during hypoxia.

A traditional approach has been to define biochemical reactions in the blood by measurement of pH and other parameters of acid-base status. A large number of metabolic processes, including the Kreb's cycle, are reliant on an adequate supply of oxygen. It is possible to exploit this dependency when assessing the dynamics of metabolism, since under pathophysiological conditions of inadequate oxygenation metabolic processes will be disrupted leading to the accumulation of a number of metabolic intermediates. A variety of low MW metabolites (e.g. organic acids, amino acids, amines and sugars) can be excreted by the kidney and therefore can potentially be detected in urine. Analysis of metabolites accumulated in urine may therefore provide an index not only of metabolic status of the fetus but of renal function. In the fetus such measurements may be preferable to similar measurements in the blood, where the detection of metabolic changes may be minimised by placental clearance. Gas chromatography and mass spectrometry of fetal sheep urine has already shown an organic acid disturbance in response to acute hypoxia (Walker, Bennet, Mills, Green, Gnanakumaran and Hanson, 1996, Appendix 18). The aim of the present study was to investigate the low MW composition of late gestation fetal urine during normoxia and one hour of hypoxia using the technique of ^{1}H NMR spectroscopy.

Carotid chemoreceptors have been implicated in the rapid bradycardia and fall in peripheral (e.g. femoral) blood flow at the onset of hypoxia (Giussani, Spencer, Moore, Bennet and Hanson, 1993). Furthermore removal of the carotid chemoreflex reveals an initial rise in RBF and subsequent fall during hypoxia (see Chapter 3), similar to that observed with renal denervation (Robillard, Nakamura and DiBona, 1986). Previous work has shown that the rise in fetal urinary lactate after 45 min of acute hypoxia is reduced in CSD fetuses (Walker *et al.*, 1996). The smaller reduction in femoral blood flow in hypoxia after CSD (Giussani *et al.*, 1993) indicates that the reduction in oxygenation of this vascular bed was probably less than in intact fetuses which might account for the lower lactate levels in the CSD fetuses. In the present study the ^{1}H NMR spectroscopy technique was used to monitor lactate, and other metabolites, during normoxia and hypoxia in intact fetuses and in those which had undergone CSD .

A6.2 Methods

Urine samples of intact (n=4) and CSD (n=6) fetuses were analysed in separate batches. In both instances there were four urine samples for each fetus (refer to Figure 3.1): 1) Normoxia (A. 15 to 45 min), 2) Hypoxia 1 (B. 60 to 90 min), 3) Hypoxia 2 (C. 90 to 120 min), and 4) Recovery (D. 135 to 165 min).

CSD samples

A 675µl aliquot of each urine sample was placed in a high grade 5mm NMR tube and 75µl of deuterium oxide (D_2O) was added to each to provide a field frequency lock. The D_2O contained 1mM/l fumarate to act as a chemical shift reference (δ6.54) and internal standard for quantification. Single pulse ^{1}H NMR measurements were made on a Bruker AMX600 spectrometer operating at 600.13 MHz ^{1}H resonance frequency. For each sample, 128 free induction decays (FIDs) were collected into 32768 computer points with a spectral width of 8000Hz. The data acquisition time per FID was 2.26 s and the total pulse recycle time was 4.26 s. The water signal was suppressed by application of the 1-dimensional NOESY (nuclear Overhauser effect spectroscopy) presaturation pulse sequence applied at the water resonance frequency for 1.5 s in the delay between 90° pulses. Total analysis time was ~10 min. Prior to Fourier transformation, an exponential apodization function was applied to the FID corresponding to a line broadening of 0.5Hz (Foxall, Bewley, Neild, Rodeck and Nicholson, 1995).

Intact samples

A 675µl aliquot of each urine sample was lyophilised and reconstituted in 675µl of D_2O and placed in a high grade 5mm NMR tube. Single pulse ^{1}H NMR measurements were made on a JEOL GSX500 spectrometer operating at 500.13 MHz ^{1}H resonance frequency. For each sample, 128 FIDs were collected into 32768 computer points with a

spectral width of 6000Hz. The data acquisition time per FID was 2.73s and a further delay of 2.27s was added between the 40° pulses to permit full T_1 relaxation, The residual water signal was suppressed by application of a secondary irradiation field (gated off during acquisition) at the water resonance frequency. Prior to Fourier transformation, an exponential apodization function was applied to the FID corresponding to a line broadening of 0.4Hz.

A6.3 Data analysis

A6.3.1 Assignment of resonances

In intact and CSD groups, assignment of resonances was made by consideration of chemical shifts relative to fumarate, spin-spin coupling patterns and pH dependence of the chemical shifts. All spectra were scaled to the same signal-to-noise ratio so that resonance intensities were directly comparable between urine samples. Quantification of urinary lactate was by peak height measurement relative to that of creatinine and consideration of the number of protons contributing to the signals. The quantification of other urinary metabolites are currently under investigation (Foxall, Bewley, Neild, Rodeck and Nicholson, 1995).

A6.3.2 Statistical analysis

Urinary lactate during hypoxia 2 and recovery were compared to normoxia by Student's Paired t-test. The Bonferroni method of correction was used for multiple comparisons.

A6.4 Results

Blood gas and haemodynamic data relating to these experiments are contained in Chapter 3.

In CSD fetuses plasma lactate rose during hypoxia and recovery, and plasma glucose rose by hypoxia 2 (Table A6.1). No glucose/lactate measurements were available for intact fetuses.

In both groups of fetuses urinary lactate levels rose by hypoxia 2 and remained elevated during recovery (Figure A6.1 to A6.4) although this only reached significance during recovery in the CSD group.

	93-75	*93-79*	*93-100*	*94-45*	*94-53*	*94-40*	*mean± S.E.M.*
			Plasma Lactate (mmol.L^{-1})				
normoxia	0.74	0.92	0.67	0.67	0.92	-	0.79± 0.06
hypoxia1	1.58	1.92	1.02	1.95	2.75	1.65	1.81± 0.23*
hypoxia2	3.48	3.67	4.02	5.02	5.28	3.93	4.23± 0.30*
recovery	3.70	3.44	4.63	5.69	5.33	4.24	4.50± 0.36*
			Plasma Glucose (mmol.L^{-1})				
normoxia	0.72	0.70	0.66	0.85	0.78	-	0.74± 0.03
hypoxia1	0.91	0.93	0.59	1.27	0.99	1.00	0.95± 0.09
hypoxia2	1.02	0.88	1.08	1.75	1.03	1.15	1.15± 0.13*
recovery	0.92	0.72	1.05	1.04	0.68	1.05	0.91± 0.07

Table A6.1 Plasma lactate and glucose in CSD fetuses during normoxia, hypoxia (1 and 2) and recovery. *P<0.017, significantly different from normoxia by unpaired t-test.

In CSD, but not intact, fetuses there was a significant relationship between the magnitude of change in UO and the magnitude of change in urinary lactate observed from normoxia to hypoxia 2 (Figure A6.5). However there was no relationship between the magnitude of the rise in urinary lactate and the magnitude of the change in plasma lactate (CSD fetuses only), PaO_2 or MAP observed from normoxia to hypoxia 2.

No comparisons were made between metabolites and RBF since the number of animals in which both measurements were obtained were too few.

From qualitative assessment, hypoxia caused an increase in urinary citrate, histidine and succinate and changes in N-acetylglycoproteins in both groups. In some, but not all fetal urine there was a rise in acetate, pyruvate, taurine and trimethylamine-N-oxide. In addition fumarate rose during hypoxia in samples where it was not used as an internal standard.

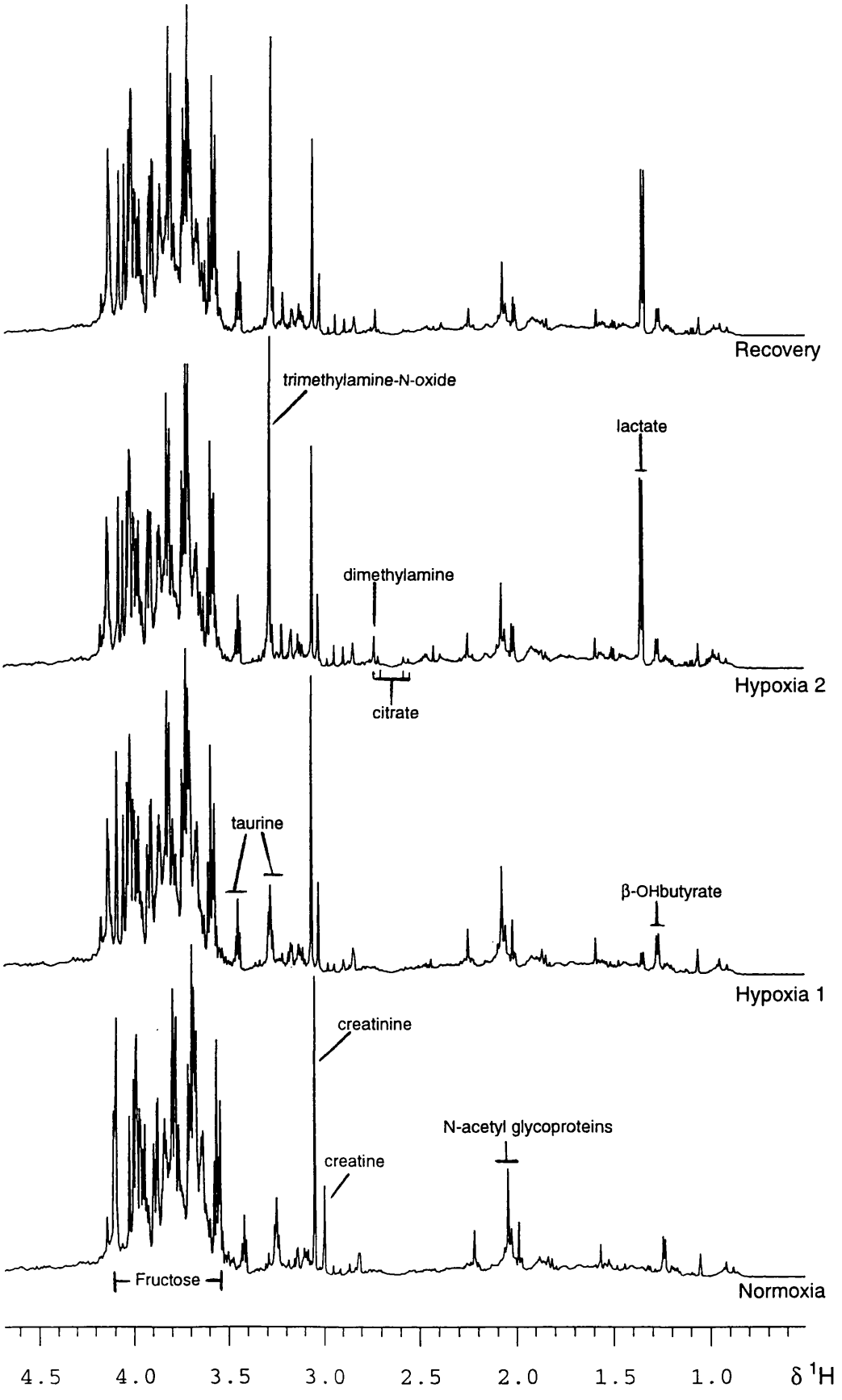


Figure A6.1 Partial 600 MHz ^{1}H NMR spectra of urine collected from one CSD fetus (94-40) during normoxia, hypoxia 1, hypoxia 2 and recovery.

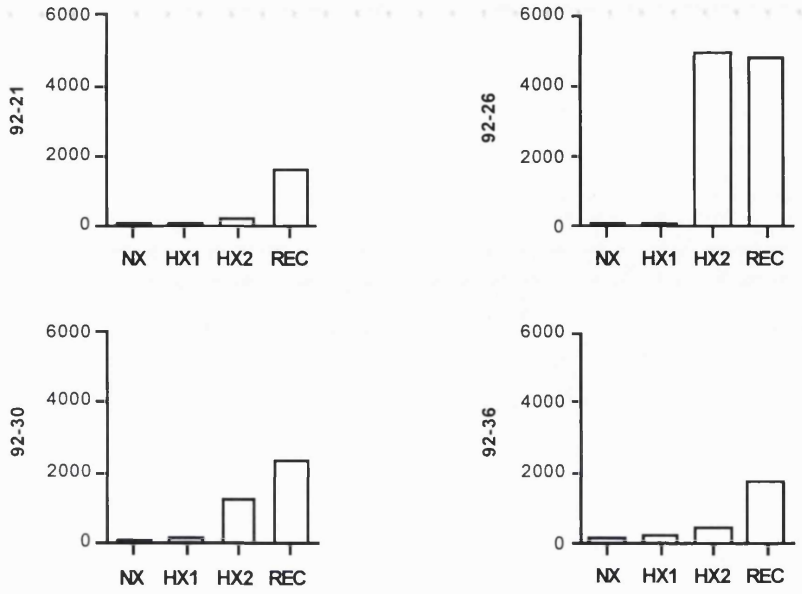


Figure A6.2 Urinary Lactate/creatinine (μM/mM, ordinate) in individual intact (n=4) fetuses during normoxia (NX), hypoxia (HX1 and HX2) and recovery (REC).

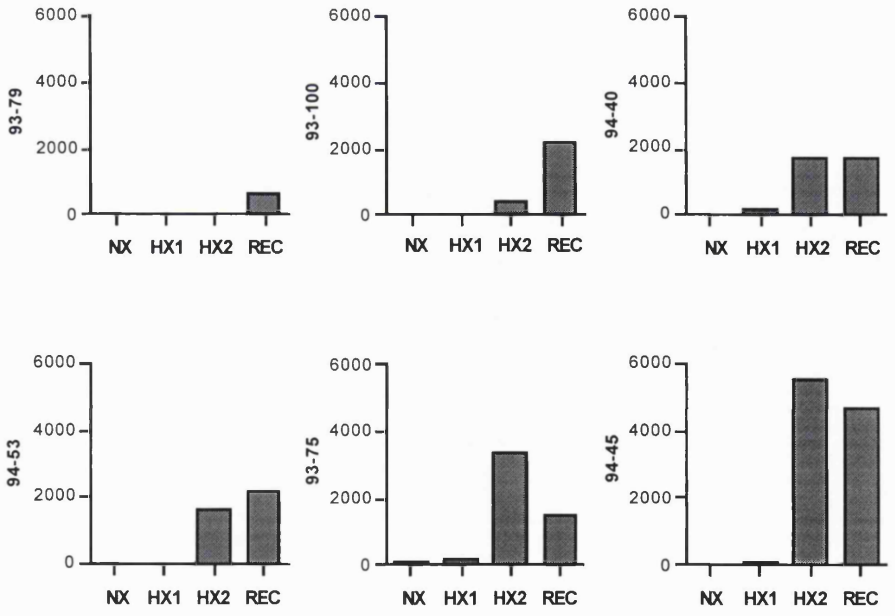


Figure A6.3 Urinary Lactate/creatinine (μM/mM, ordinate) in individual CSD (n=6) fetuses during normoxia (NX), hypoxia (HX1 and HX2) and recovery (REC).

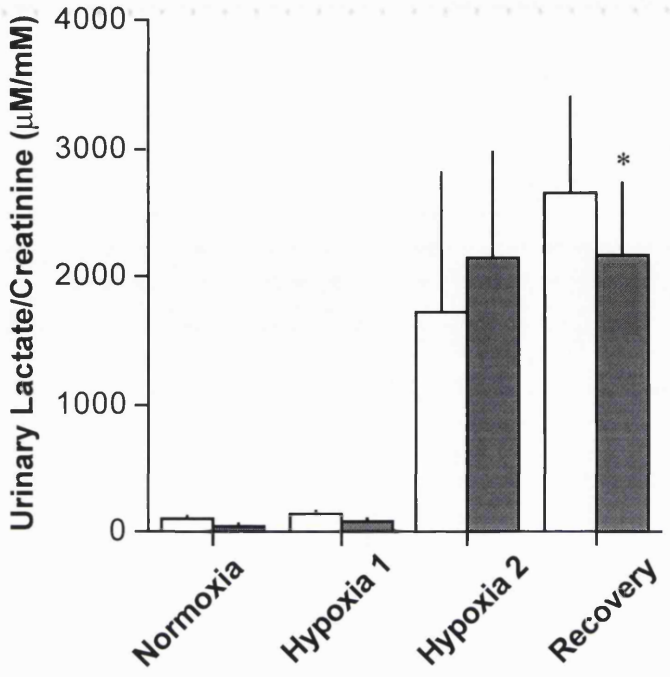


Figure A6.4 Urinary Lactate/ Creatinine (μM/mM) in intact (n=4, open bars) and CSD (n=6, filled bars) during normoxia, hypoxia (1 and 2) and recovery. Values are shown as Mean±S.E.M. *P<0.025, significantly different from normoxia by paired t-test.

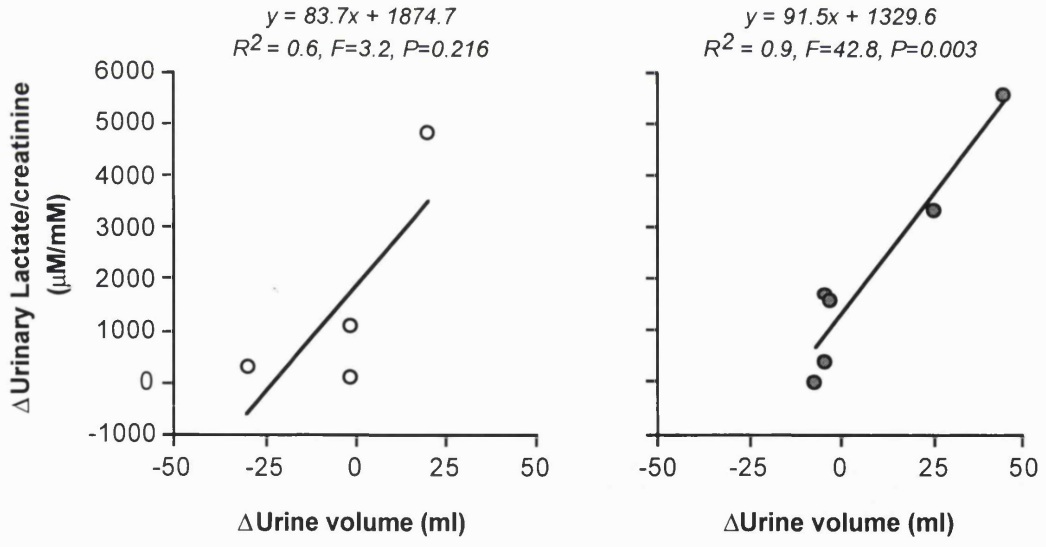


Figure A6.5 Scatter plots of magnitude of change in urinary lactate/creatinine (ordinate) against magnitude of change in UO (abscissa) between normoxia and hypoxia 2 in intact (○,n=4) and CSD (●, n=6) fetuses. There was a significant relationship between urinary lactate and UO in CSD (slope significantly different from zero, p<0.01 by regression analysis) but not intact fetuses.

A6.5 Discussion

The results of the present study show an increase in urinary lactate in late hypoxia in both intact and CSD fetuses which persist throughout the recovery period. There was no difference in urinary lactate levels between intact and denervated groups throughout the protocol. Qualitative assessment of the results showed that hypoxia caused a rise in urinary citrate, histidine and succinate and changes in N-acetylglycoproteins. In some, but not all, fetuses (from both groups) urinary acetate, pyruvate, fumarate, taurine and trimethylamine-N-oxide rose during hypoxia.

Methodological considerations

High resolution ^{1}H NMR spectroscopy is able to provide information on both the structure and composition of metabolites in biological fluids. One advantage of the technique over more traditional methods such as gas chromatography-mass spectroscopy is that it does not require metabolite pre-selection and therefore eliminates the need for *a priori* judgements to be made by the investigator. In addition the technique is non-destructive, so that the samples can be used for subsequent conventional analysis. Sample sizes required for the technique are small, which is an important consideration in fetal studies.

In this preliminary study no measurements were made of plasma glucose or lactate in intact fetuses, however previous work has shown that the rise in plasma lactate in intact fetuses is greater than in CSD fetuses (see Walker, Bennet, Mills, Green, Gnanakumaran and Hanson, 1996, Appendix 18).

Hypoxia causes a large rise in urinary lactate

The major feature of the results contained in this appendix were the changes in lactate excretion. Lactate is the major end-product of anaerobic metabolism. Fetal plasma lactate is known to rise during acute (see Chapters 4, 5 and 6) and mild-chronic (Towell, Figueroa, Markowitz, Elias and Nathanielsz, 1987) hypoxia. The results presented in this appendix have confirmed, using the ^{1}H NMR spectroscopic technique, a prominent rise in *urinary* lactate by late hypoxia and recovery. The fact that this rise only reached significance during recovery in the CSD group may simply reflect the large variation observed between fetuses, or it may reflect altered peripheral blood flow changes. It is possible that some of the rise in urinary lactate during hypoxia was due to altered renal function or tubular damage. However this seems unlikely since there was no evidence of markers of tubular damage, such as proteinuria, amino acidurea and glucosurea. Finally, creatinine levels were found to be constant throughout the protocol which indicates that the handling of metabolites by the kidney was unlikely to have been altered. Active

tubular secretion of organic acids and bases has previously been shown to be absent in the fetal sheep (see Lumbers, 1983). It seems more likely therefore that the urinary lactate was an overspill from the high plasma levels that are seen during hypoxia. It has previously been suggested that a large component of this rise is due to a peripheral (e.g. femoral) vasoconstriction, mainly in skeletal muscle, since lower urinary lactate levels were observed in CSD fetuses where femoral vasoconstriction was reduced (Walker, Bennet, Mills, Green, Gnanakumaran and Hanson, 1996). However there was no apparent difference between urinary lactate of intact and CSD fetuses in the present study. The reason for the discrepancy between these two studies is not obvious but since the CSD urine used in the two studies was from the same fetuses it is likely to be accounted for by the smaller size of the intact group in the present study and/or the different analytical techniques. In the intact, but not CSD, group urinary lactate tended to continue to rise during the recovery period. This is in accordance with Walker *et al.* (1996) and may be caused by a 'wash-out' of metabolites from the skeletal muscle bed by the rebound rise in blood flow which is sometimes seen following the cessation of hypoxia.

In the present study a relationship was observed between UO changes and urinary lactate changes by late hypoxia in CSD but not intact fetuses. Thus with low UO small amounts of lactate were detected. This result is difficult to interpret but it seems unlikely that urinary lactate is UO-dependent since all the urinary lactate measurements quoted in this Appendix were expressed in relation to urinary creatinine to take volume changes into account. On the other hand it may be that this correlation reflects the dependency of urinary lactate on filtered, rather than tubular secreted, lactate.

Pyruvate (salt of pyruvic acid, derived from the breakdown of glucose), along with acetylcoenzyme A, is metabolised via the Kreb's cycle. However a number of amino acids can also be converted to intermediates of the Kreb's cycle by deamination, for example histidine. Since the Kreb's cycle requires oxygen it is reasonable to predict that this metabolic process may have declined during acute hypoxia in the present study. Thus the rise in excretion of histidine during hypoxia in the present study might be by virtue of its decreased incorporation into the Kreb's cycle. The rise in urinary succinate (salt of succinic acid, an intermediate in the Kreb's cycle) also observed by Walker *et al.* (1996) and in urinary citrate (intermediate in the Kreb's cycle) by late hypoxia may also be indicative of altered metabolic processes. They observed a delay in the rise in organic acid excretion during hypoxia in CSD fetuses. This might be explained by the attenuated fall in FBF in CSD fetuses during hypoxia. Full quantitative analysis of organic acid changes will be required before similar comparisons between intact and CSD fetuses can be made in the present study.

In summary ^{1}H NMR analysis showed a rise in fetal urinary lactate levels during acute hypoxia along with increases in other low MW metabolites. In future studies it will be important to characterise the ^{1}H NMR metabolic "fingerprints" of normal fetal sheep urine as a standard against which the metabolic profile arising from experimental manipulations can be compared. ^{1}H NMR of urine may be of clinical importance in detecting metabolic changes in response to fetal asphyxia or indeed in accurately diagnosing birth asphyxia particularly since urine samples, unlike blood samples, contain an accumulation of metabolites. Furthermore studies on the urine of human fetuses have indicated the need of such a technique for monitoring renal development and abnormalities *in utero* (Foxall, Bewley, Neild, Rodeck and Nicholson, 1995).

APPENDIX 7

XXXII CONGRESS OF THE INTERNATIONAL UNION OF PHYSIOLOGICAL SCIENCES. GLASGOW 1993

214.7/P

THE EFFECTS OF ACUTE HYPOXEMIA ON THE RENAL BLOOD FLOW AND URINE OUTPUT IN THE LATE GESTATION FETAL SHEEP.

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Renal blood flow (RBF) and urinary output decrease during acute hypoxemia. In order to establish any correlation between these two parameters we have continuously monitored RBF and urinary output during acute hypoxia in fetal sheep (n=6, 119-127d, term 147d). Under general maternal anaesthesia we implanted catheters and a blood flow probe (Transonic Systems Ltd.) around the left renal artery. Five days post-operatively fetuses were subjected to an hour of hypoxia (PaO_2 ca. 13 mmHg) induced by changing maternal inspired gases. RBF was monitored continuously and urine collected at 15 min intervals. RBF was variable during hypoxia, but fell to below control ($p<0.05$) after 45 min. Urine flow also fell during hypoxia but for the group as a whole this was not significant. In individual animals the change in RBF did not correlate with that in urinary flow. This suggests that the fall in urine output is not explained simply by a fall in RBF. We are currently investigating the endocrine correlates of the changes. Supported by The Wellcome Trust and The Medical Research Council.

APPENDIX 8

Journal of Physiology (1994) 476, 81-82P.

The effect of acute hypoxaemia on plasma angiotensin II in intact and carotid sinus-denervated fetal sheep

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In late gestation fetal sheep hypoxaemia produces peripheral vasconstriction by neuronal and hormonal mechanisms. Arterial chemoreceptors have been implicated in the former (Giussani *et al.* 1993) but do not play a significant role in the rise in [AVP], [ACTH] or [cortisol] after 45 min of isocapnic hypoxaemia. We have now measured fetal plasma angiotensin II concentration ([AII]) and femoral blood flow (FBF) during acute isocapnic hypoxaemia in intact and carotid sinus-denervated (CSD) fetuses, with and without the angiotensin converting enzyme inhibitor, captopril.

Ten fetal sheep at 113–126 days (term = 147 days) were instrumented under general anaesthesia (1 g thiopentone I.V. then 2% halothane in O_2 to ewe) with catheters in a jugular vein and a carotid artery, ECG electrodes and blood flow probes (Transonic) around a femoral and a carotid artery. In five fetuses the carotid sinus nerves were cut bilaterally. After 5 days post-operative recovery fetuses underwent 1 h of isocapnic hypoxaemia (P_{a,O_2} *ca* $12 \pm 0{\cdot}3$ mmHg, mean ± S.E.M.), produced by reducing maternal F_{I,O_2}, on two consecutive days. On the first day saline vehicle was infused I.V. and on the second day a 1 mg bolus of captopril followed by an I.V. infusion of 3 mg h^{-1} was given. This dose blocked the pressor response to 5 μg of angiotensin I. FBF was monitored continuously and arterial blood was collected at 15 min intervals for blood gas and [AII] analysis.

As previously reported (Giussani *et al.* 1993), the fall in FBF was delayed in CSD fetuses during hypoxaemia. [AII] rose in both intact (control: 37 ± 11; after 15 min hypoxaemia: 92 ± 16 pg ml^{-1}, $P < 0{\cdot}05$ by paired t test) and CSD fetuses (control: 30 ± 8; after 15 min hypoxaemia: 128 ± 35 pg ml^{-1}, $P < 0{\cdot}05$), but there was no significant difference between these two groups. Captopril blocked the rise in [AII] during hypoxaemia in intact and CSD fetuses. It had no significant effect on FBF in normoxaemia or the fall during hypoxaemia in intact fetuses. In contrast, captopril attenuated any fall in FBF in CSD fetuses (control: $48{\cdot}4 \pm 10{\cdot}2$; after 30 min hypoxaemia: $43{\cdot}6 \pm 8{\cdot}5$, $P > 0{\cdot}05$).

The rise in [AII] during hypoxia is consistent with previous findings (Broughton Pipkin *et al.* 1974) in the late gestation fetal sheep. However, our results indicate that this rise is not due to a carotid chemoreflex. Furthermore, AII contributes to hypoxic vasconstriction in the CSD but not the intact fetus, presumably because in the latter its effect is masked by chemoreflex vasoconstrictor mechanisms.

Work supported by the Wellcome Trust. L.R.G. is a MRC scholar.

REFERENCES

Broughton Pipkin, B., Lumbers, E.R. & Mott, J.C. (1974). *J. Physiol.* 243, 619–636.

Giussani, D.A., McGarrigle, H.H.G., Moore, P.J., Bennet, L., Spencer, J.A.D. & Hanson, M.A. (1993). *J. Physiol.* 461, 431–449.

APPENDIX 9

*Journal of Physiology (1995) **483**, 92P.*

Nitric oxide (NO) synthesis and haemodynamic responses to acute hypoxaemia in unanaesthetized fetal sheep

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NO is implicated in the regulation of vascular resistance in many tissues (Moncada *et al.* 1991). However, a role for NO in the systemic vascular responses to hypoxaemia in the fetus has not been investigated.

Seven fetal sheep at 114–121 days (term = 147 days) were instrumented under general anaesthesia (1 g thiopentone I.V. then 2% halothane in O_2 to ewe) with jugular vein, carotid artery and brachial vein catheters, ECG electrodes and probes (Transonic) to measure femoral (FBF) and carotid (CBF) artery blood flows. After > 5 days post-operative recovery, fetal hypoxaemia (P_{a,O_2} to 12·4 mmHg) was induced by reducing maternal F_{I,O_2} for 1 h on two separate days during I.V. infusion of either the NO-synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME, 20 mg bolus then 100 mg h^{-1} infusion) or vehicle.

In normoxaemia, L-NAME caused a fall in fetal heart rate (FHR) (control: 162 ± 3·8 *vs.* 135 ± 7·7 beats min^{-1} after 15 min L-NAME, $P < 0·05$, paired t test), a slower rise in mean arterial pressure (MAP) (control: 48 ± 1·9 *vs.* 60 ± 3·3 mmHg after 45 min L-NAME, $P < 0·05$), a fall in CBF (control: 72·3 ± 10·6 *vs.* 46·9 ± 4·8 after 45 min L-NAME, $P < 0·05$), and a lower FBF (vehicle: 48·5 ± 0·6 *vs.* NAME: 39·0 ± 1·0, $P < 0·01$, ANOVA) (Fig. 1). In hypoxaemia, FBF fell to a lower level with L-NAME than with vehicle ($P < 0·01$, ANOVA). The rise in CBF during hypoxaemia with vehicle ($P < 0·01$) was absent with L-NAME infusion (Fig. 1). FHR fell to similar levels during L-NAME and vehicle infusion at the onset of hypoxaemia; in both cases FHR returned to pre-hypoxic values. MAP rose to a greater level during hypoxaemia with L-NAME than with vehicle infusion ($P < 0·01$).

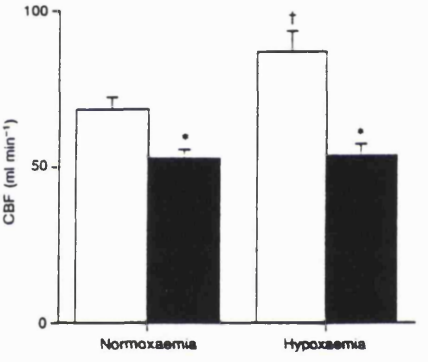

Fig. 1. CBF (mean ± S.E.M.) with vehicle (□) or L-NAME (■) infusion during normoxaemia and hypoxaemia. * $P < 0·01$, L-NAME *vs.* vehicle; † $P < 0·05$, normoxaemia *vs.* hypoxaemia ($n = 7$, ANOVA).

Our data suggest a role for endogenous NO in the tonic regulation of FHR. NO also plays a role in the regulation of peripheral vascular resistance in both normoxaemia and hypoxaemia. Even more strikingly, NO synthesis appears to be responsible for the rise in CBF in hypoxaemia in the late gestation fetal sheep.

Supported by the Wellcome Trust. L.R.G. is a MRC scholar.

REFERENCE

Moncada, S., Palmer, R.M.J. & Higgs, E.A. (1991). *J. Am. Soc. Pharm. Exp. Ther.* **43** (2), 109–142.

APPENDIX 10

The 21st Annual Meeting of The Society for the Study of Fetal Physiology. Cairns, Australia. 30 July- 3 August, 1994.

A32

THE ROLE OF ENDOTHELIN-1 IN PERIPHERAL VASOCONSTRICTION DURING ACUTE HYPOXAEMIA IN THE LATE GESTATION FETAL SHEEP.

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Endothelin (ET-1) has been implicated in the regulation of fetal systemic vascular tone[1]. Administration of exogenous ET-1 produces a rise in systemic blood pressure[2]. We found that i.v. infusion of ET-1 (1.5 or 2 μg) produced a fall in fetal heart rate (FHR), and a fall in femoral blood flow (FBF) and a rise in mean arterial (MAP) and venous pressures which were blocked by administration of the specific ET_A receptor antagonist, FR139317 (Fujisawa Pharmaceutical Ltd., 300μg bolus followed by 50μg/min infusion). Using this antagonist we have now investigated the role of endogenous ET-1 in fetal cardiovascular responses to acute hypoxaemia.

Five fetal sheep (118-121d, term=147d) were instrumented under general anaesthesia with jugular vein, carotid artery and brachial vein catheters, ECG electrodes and Transonic flow probes around a femoral and a carotid artery. After 5 days post-operative recovery, fetal hypoxaemia (PaO2 12±0.4 mmHg mean ± S.E.M.) was induced by reducing maternal FiO2 for 1h on 2 consecutive days during infusion of either FR139317 (dissolved in 10ml saline + 5 drops 150mM NaOH) or vehicle alone at 4.2 ml/h. Blood pressure, blood flows and heart rate were monitored continuously and blood was collected at 15 min intervals for blood gas and hormone analysis.

In normoxaemia, FR139317 caused a significant rise in FHR (control: 163±4.13 vs. 193±10.96 bpm after 45 mins FR139317, $p<0.05$ by paired t-test). In addition there was a tendency for carotid flow to rise, however FBF and MAP were not altered. At the onset of hypoxaemia FHR fell to similar levels in both FR139317 and vehicle infused fetuses. CBF during hypoxaemia was greater with FR139317 than with vehicle infused fetuses ($p<0.05$ by ANOVA). FBF fell significantly ($p<0.05$ by ANOVA) during hypoxaemia in both FR139317 and vehicle infused fetuses but the fall in FR139317 infused fetuses was less ($p<0.05$ by ANOVA). The rise in MAP during hypoxaemia was similar in both groups.

The effect of FR139317 on FHR during normoxaemia suggests a role for endogenous ET-1 in the tonic regulation of FHR. This may be via direct action at the SA node, possibly by nitric oxide-mediated mechanisms (see Han *et al.*, 1994). In addition ET-1 plays a role in the peripheral vasoconstriction during hypoxaemia, since FR139317 reduced the femoral vasoconstriction and increased the carotid vasodilatation.

Supported by The Wellcome Trust. LRG is a Medical Research Council Scholar.
We are grateful to Fujisawa Pharmaceuticals Ltd. for the gift of FR139317.

1. Haegerstrand, A., Hemsen, A., Larsson, O. and Lundberg, J.M. Endothelin: presence in human umbilical vessels, high levels in fetal blood and potent constrictor effect. Acta Physiol Scand (1989)**137**:541-542.
2. Chatfield, B.A., McMurtry, I.F., Hall, S.L. and Abman, S.H. Hemodynamic effects of endothelin-1 on ovine fetal pulmonary circulation. Am J Physiol (1991) **261**: R182-R187.
3. Han, X., Shimoni,Y. and Giles, W.R. An obligatory role for nitric oxide in autonomic control of mammalian heart rate. J Physiol (1994) **476.2**:309-314.

APPENDIX 11

The Thorburn Symposium. Hamilton Island, Queensland, Australia. 5-9 August, 1994.

A36

DOES INHIBITION OF NITRIC OXIDE SYNTHESIS ALTER THE HAEMODYNAMIC RESPONSES TO ACUTE HYPOXAEMIA IN THE LATE GESTATION FETAL SHEEP?

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Nitric oxide (NO) is implicated in the regulation of the fetal systemic circulation. NO-synthase inhibitors increase basal systemic mean arterial pressure (MAP)[1] and basal renal vascular resistance[2] in the late gestation fetal sheep and attenuate the effect of ET-1 and TX in the fetal-placental circulation[3]. However a role for NO in the systemic vascular responses to hypoxaemia in the fetal sheep has not been established.

Five fetal sheep (118-121d, term=147d) were instrumented under general anaesthesia with jugular vein, carotid artery and brachial vein catheters, ECG electrodes and Transonic flow probes around a femoral and a carotid artery. After 5 days post-operative recovery, fetal hypoxaemia (PaO_2 12±0.4 mmHg, mean ± S.E.M.) was induced by reducing maternal FiO_2 for 1h on 2 separate days during i.v. infusion of either the specific NO-synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME, 20 mg in 2 ml bolus followed by 3 ml/h infusion of 33mg/ml in saline) or vehicle alone.

In normoxaemia, L-NAME caused a significant fall in fetal heart rate (FHR) (control: 153±3.9 vs. 139±4.8 b.p.m. after 15 mins L-NAME, $p<0.05$, paired t-test), a slower rise in MAP (control: 46±1.5 vs. 58.2 ±2.8 mmHg after 45 mins L-NAME) and a large fall in carotid blood flow (CBF) (control: 70.3±7.1 vs. 43±3.3 ml/min after 45 mins L-NAME, $p<0.05$). During hypoxaemia, femoral blood flow (FBF) fell significantly with both L-NAME and vehicle infusion ($p<0.01$, by ANOVA); FBF during hypoxaemia was less with L-NAME than with vehicle ($p<0.01$). The rise in CBF during hypoxaemia with vehicle ($p<0.01$) was absent with L-NAME infusion. FHR fell to similar levels during L-NAME and vehicle infusion at the onset of hypoxaemia; in both cases FHR returned to its pre-hypoxic value. There was a significant rise in MAP during hypoxaemia with L-NAME and vehicle infusion ($p<0.01$) but MAP was greater during hypoxaemia with L-NAME infusion ($p<0.01$).

The effect of L-NAME on FHR in normoxaemia suggests a role for endogenous NO in the tonic regulation of FHR, as in the adult[4]. We do not know the mechanism of this effect but an interaction with ET-1 may be involved (see Green *et al.*, 1994, SSFP). NO also plays a role in the regulation of peripheral vascular resistance in both normoxaemia and hypoxaemia, as seen from the significantly greater MAP and smaller FBF and CBF under both conditions. Even more strikingly, NO synthesis appears to be responsible for the rise in CBF in hypoxaemia in the late gestation fetal sheep.

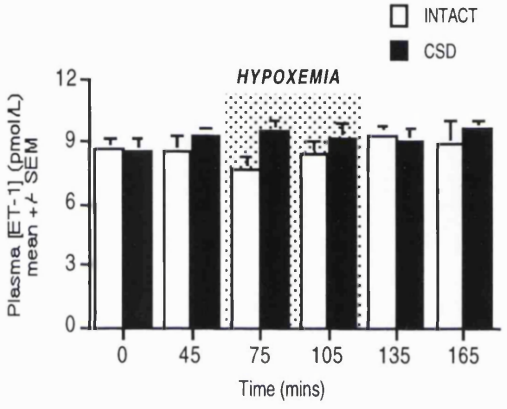
Supported by The Wellcome Trust. LRG is a Medical Research Council scholar.

1. Moore, P., Velvis, H., Fineman, J.R., Soifer, S.J. and Heymann, M.A. EDRF inhibition attenuates the increase in pulmonary blood flow due to oxygen ventilation in fetal lambs. J Appl Physiol (1992) **73(5)**:2151-2157.
2. Bogaert, G.A., Kogan, B.A. and Mevorach, R.A. Effects of endothelium-derived nitric oxide on renal hemodynamics and function in the sheep fetus. Pediatr Res (1993) **34**:755-761.
3. Myatt, L., Brewer, A.S., Langdon, G. and Brockman, D.E. Attenuation of the vasoconstrictor effects of thromboxane and endothelin by nitric oxide in the human fetal-placental circulation. Am J Obstet Gynecol (1992) **166**:224-230.
4. Fernandez, N., Garcia, J.L., Garcia-Villalon, A.L., Monge, L., Gomez, B. and Dieguez, G. Cerebral blood flow and cerebrovascular reactivity after inhibition of nitric oxide synthesis in conscious goats. Br J Pharmacol (1993) **110**:428-434.

APPENDIX 12

Journal of The Society for Gynecologic Investigation
2 (2), 046 1995

EFFECT OF CAROTID SINUS DENERVATION ON PLASMA ENDOTHELIN-1 DURING ACUTE ISOCAPNIC HYPOXEMIA IN THE LATE GESTATION OVINE FETUS. L.R. Green*, H.H.G. McGarrigle*, L. Bennet* and M.A. Hanson*. Departments of Obstetrics & Gynaecology and Physiology, University College, London WC1E 6HX, U.K. (SPON: G.C.L. Lachelin)



The fetus responds to isocapnic hypoxemia with a rise in peripheral vascular resistance which is attenuated by carotid sinus denervation (CSD) and modified by endothelin receptor-A blockade. We investigated a role for changes in plasma ET-1 ([ET-1]) in this response, in intact and CSD fetuses. **Methods:** 12 fetuses (113-121d, term=147d) were instrumented under general anaesthesia to measure continuously mean arterial pressure, carotid and femoral arterial blood flows (Transonic) and heart rate. 6 fetuses underwent CSD. After >5d recovery, hypoxemia (PaO_2 to ca.12 mmHg) was induced for 1h by reducing maternal FiO_2. Arterial blood was sampled for PO_2, PCO_2 and pH measurement and plasma ET-1 analysis by radioimmunoassay (Nichols Institute). **Results:** [ET-1] in intact fetuses was not elevated from baseline by 1h isocapnic hypoxemia. CSD did not alter [ET-1] during either normoxemia or hypoxemia. **Conclusions:** A rise in [ET-1] cannot account for the rise in vascular resistance in acute hypoxemia and ET-1 is not chemoreflexly released. ET-1 modulation of vascular resistance at local tissue level is likely to be more important. *Supported by The Wellcome Trust and MRC.*

APPENDIX 13

Japanese Journal of Physiology (1995). In press

The Role Of Endothelin-1 In Cardiovascular Responses to Acute Hypoxaemia In Late Gestation Fetal Sheep.

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Endothelin (ET-1) is implicated in the regulation of fetal systemic vascular tone (Chatfield *et al.*, 1989). We found that 1.5 or 2 μg ET-1 i.v. reduced fetal heart rate (FHR) and femoral (FBF) & carotid (CBF) blood flow, and elevated mean arterial (MAP) and venous pressures. These responses were blocked by the specific ET_A receptor antagonist, FR139317 (Fujisawa Pharmaceutical Ltd., 300μg bolus then 50μg/min infusion). We have now investigated the role of endogenous ET-1 in fetal cardiovascular responses to acute isocapnic hypoxaemia (HX).

Seven fetal sheep (114-121d, term=147d) were instrumented under general anaesthesia (1g thiopentone i.v. then 2% halothane in O_2 to ewe) with jugular vein, carotid artery and brachial vein catheters, ECG electrodes and blood flow probes (Transonic) around a femoral and a carotid artery. After >5 days post-operative recovery, fetal HX (PaO_2 12±0.4 mmHg, mean±S.E.M.) was induced by reducing maternal FiO_2 for 1h on 2 consecutive days, during infusion of either FR139317 or vehicle (10ml saline+5 drops 150mM NaOH). MAP, FHR, FBF and CBF were monitored continuously and blood was collected for blood gas and plasma [ET-1] analysis.

In normoxaemia (NX), FR139317 caused a rise in FHR (control: 165.7±3.9 vs. 196.7±9.9 b.p.m. after 45 min; $P<0.05$, paired t-test) and CBF (control: 70.3±11.2 vs. 85.0±13.1 $ml.min^{-1}$ after 45 min; $P<0.05$). However FBF and MAP were not altered. At the onset of HX FHR fell to similar levels with both FR139317 and vehicle infusion. During HX CBF was greater with FR139317 than with vehicle infusion ($P<0.05$, ANOVA), and FBF fell to lower levels with vehicle than with FR139317 ($P<0.05$, ANOVA). MAP rose to similar levels during HX in both groups. Plasma [ET-1] was not altered by FR139317 in NX and during HX [ET-1] did not change from NX levels with either vehicle or FR139317.

The effect of FR139317 during NX suggests a role for endogenous ET-1 in the regulation of basal FHR and carotid vascular tone. In addition, ET-1 modulates the peripheral vasoconstriction during HX, since FR139317 reduced the femoral vasoconstriction and increased the carotid vasodilatation. The lack of a rise in [ET-1] after 1h of HX agrees with Jones *et al.* (1994) who found a rise only after 3h of HX. Thus ET-1 may modulate, but does not mediate, the fetal cardiovascular responses to acute HX for 1h.

Supported by The Wellcome Trust. LRG is an MRC Scholar. FR139317 was a gift from Fujisawa Pharmaceuticals Ltd.

Chatfield, B.A *et al.* (1991). *Am. J. Physiol.* **261,** R182-R187.

Jones, O.W *et al.* (1994). *Proc. Soc. Gynec. Invest., USA.* p.116, 059.

APPENDIX 14

22nd Annual Meeting of The Society for the Study of Fetal Physiology. Malmo, Sweden, June 11-14, 1995.

A17

EFFECT OF CAROTID SINUS DENERVATION ON RENAL BLOOD FLOW AND URINE OUTPUT DURING HYPOXIA IN THE LATE GESTATION OVINE FETUS.

Green, L.R., Bennet, L., Robson, S. and Hanson, M.A. *Depts of Obstetrics & Gynaecology and Physiology, University College London, UK.*

In hypoxia (HX) there is a decrease in fetal renal blood flow (RBF) (Robillard *et al.*, 1986) and urine output (UO) (Nicolaides *et al.*, 1990). We examined whether the change in RBF & UO during acute HX correlated and investigated a role for the carotid sinus nerves (CSN) in mediating these responses.

17 fetuses were instrumented to monitor mean arterial pressure (MAP), heart rate (FHR), RBF (Transonic) and UO. 11 fetuses underwent bilateral CSN denervation (CSD). After >5 days post-operative recovery fetuses under went 1h isocapnic HX (PaO_2 to ca. 13mmHg) by reducing maternal FiO_2. Urine was collected over consecutive 15 min intervals.

During HX, RBF fell in intact fetuses (18.7±1.7 to 13.5±1.4 $ml.min^{-1}$. $p<0.05$, ANOVA). In CSD fetuses RBF showed an initial significant rise after 5 min HX (22±2 to 27±3 $ml.min^{-1}$. $p<0.01$ by paired t-test) and then tended to fall, but this was not significant. UO fell in HX in intact (0.49±0.07$ml.min^{-1}$ to 0.29±0.05$ml.min^{-1}$. $p<0.05$, ANOVA), but not in CSD fetuses. RBF & UO were correlated in intact fetuses ($r=0.98$) during HX but not in CSD fetuses .

The rise in RBF in early HX in CSD fetuses implies a vasodilatation, possibly due to prostaglandins (Robillard *et al.*, 1986). In intact fetuses this is masked by a vasoconstriction with a CSN afferent limb & renal nerve efferent limb (Robillard *et al.*, 1986). The absence of a correlation between RBF & UO in HX in CSD fetuses suggests that neural influences on renal function are more important during HX.

Supported by The Wellcome Trust. LRG is a MRC scholar.

Robillard *et al.* (1986). Am. J. Physiol. **250**:F294-F301.
Nicolaides *et al.* (1990). Am. J. Obstet. Gynecol. **162**:387-391.

APPENDIX 15

22nd Annual Meeting of The Society for the Study of Fetal Physiology. Malmo, Sweden. June 11-14, 1995.

P10

THE EFFECT OF CHRONIC NITRIC OXIDE SYNTHESIS INHIBITION ON CARDIOVASCULAR CONTROL IN LATE GESTATION OVINE FETUSES

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We have implicated nitric oxide (NO) synthesis in the regulation of tonic fetal heart rate (FHR) and vascular tone in the femoral and carotid beds using a 1h infusion of N^G-nitro-L-arginine methyl ester (L-NAME; Green *et al.*, 1995). We are now investigating the effect of chronic infusion of L-NAME on fetal cardiovascular control.

5 fetuses were instrumented under general anaesthesia to measure blood pressure (MAP), carotid (CBF) and femoral (FBF) arterial blood flows (Transonic) and FHR. After 5 days recovery L-NAME (99mg/h in saline at 1.6ml/h) or saline was infused for 4-5 days and fetuses were monitored daily for 1h. They were challenged with 1h isocapnic hypoxia (HX; PaO_2 to ca. 13) pre-infusion and after 1h (acute) & ca. 90h (chronic) of infusion.

After 1h L-NAME MAP rose and FHR fell but, while MAP had returned to pre-infusion levels after 26±3h NAME, FHR remained low throughout the infusion period. In one fetus, CBF fell markedly by 26h but returned towards pre-infusion levels as the infusion of L-NAME continued. During HX, the initial reflex fall in FHR was similar before and after L-NAME. However FHR in late HX tended to be lower after acute but not after chronic NAME. MAP tended to rise to a higher and FBF to fall to a lower level in HX after acute but not after chronic L-NAME.

The initial effects of L-NAME and its effects on the response to HX confirm our earlier findings (Green *et al.*, 1995). While FHR remained lower, MAP and CBF showed a degree of adaptation after chronic NAME which may be due to an up regulation of NO synthesis. Despite this, there are differences in the responses to HX after chronic L-NAME infusion which merit further study.

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Green, L.R., Bennet, L. & Hanson, M.A. (1995). J. Physiol. **483**, 92P.

APPENDIX 16

22nd Annual Meeting of The Society for the Study of Fetal Physiology. Malmo, Sweden. June 11-14, 1995.

A36

EFFECT OF ACIDAEMIA ON HEART RATE VARIATION IN HYPOXIA IN FETAL SHEEP

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Fetal heart rate variation (FHRV) increases in acute hypoxia (0-15 min) in fetal sheep, related to the chemoreflex bradycardia (Kozuma et al, 1994). The aim of this study was to measure FHRV during sustained hypoxia with or without a decrease in pH in intact and carotid sinus-denervated fetuses.

Sixteen fetal sheep were chronically instrumented at 113-127 days gestational age and the carotid sinus nerve was sectioned bilaterally in seven of them. At least 5 days after post-operative recovery, fetal hypoxia (PaO2 11-14 mmHg) was induced by reducing maternal inspired oxygen fraction for 60 minutes. FHRV was calculated by the method of Dawes et al (1981). In both intact and denervated fetuses, FHRV increased but then returned to control at 30-60 minutes of hypoxia if the decrease in pH was less than 0.03. However if the decrease in pH was more than 0.03 , FHRV remained high. The preliminary experiment in which fetal hypoxia was induced by maternal uterine artery occlusion showed that hypoxia for 9 hours reduced FHRV despite development of acidaemia. However, during this period acute reduction in PaO2 (<14 mm Hg) still increased FHRV.

In conclusion, low pH plays an important role in determining FHRV by a mechanism which is independent of the peripheral chemoreflex. FHRV appears to decline in longer term hypoxia despite continuous acidaemia.

Supported by The Wellcome Trust

1.Kozuma S, Watanabe T, Bennet L, Green L & Hanson M. Abstracts of the 21st Annual Meeting of the SSFP. Cairns, Australia. 1994.
2.Dawes GS, Visser GHA, Goodman JDS & Redman CWG. Am J Obstet Gynaecol 1981;141: 43-52

APPENDIX 17

22nd Annual Meeting of The Society for the Study of Fetal Physiology. Malmo, Sweden. June 11-14, 1995.

P14

CHANGE IN THE COMPONENT OF POWER SPECTRUM IN HEART RATE DURING HYPOXAEMIA IN FETAL LAMB

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Aims: It is reported that fetal heart rate variability is increased by acute hypoxaemia in the fetus. However there has been no report regarding the change in the frequency component in heart rate variability during acute hypoxaemia in the fetus. The aim of this study is to see the change in the component of power spectrum in heart rate during hypoxaemia in the fetus.
Methods: Fetal sheep were chronically instrumented at 115-120 days gestational age and at least 5 days after post-operative recovery, fetal hypoxaemia was induced by reducing maternal inspired oxygen fraction for 60 minutes. Power spectral analysis was performed by means of an autoregressive method before, during and after the hypoxaemic period. The area under the peak between 0.04 and 0.12 Hz, 0.12-0.5 Hz and 0.8-2.0 Hz were calculated as the measure of very low frequency (VL), low frequency (L) and high frequency (H) bands, respectively. Experiments were performed on intact and carotid sinus nerve denervated animals.
Results: There was fluctuations in the power for each frequency band before, during and after hypoxaemia. During hypoxaemic period increase in the low frequency band was seen. VL and H/L changed as mirror image during hypoxaemic period however, these changes were not seen in carotid sinus denervated animals.
Conclusion: Fetal hypoxaemia may be estimated by analyzing the power spectrum in heart rate.

APPENDIX 18

EFFECTS OF HYPOXIA ON URINARY ORGANIC ACID AND HYPOXANTHINE EXCRETION IN FETAL SHEEP

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(Pediatric Research; In press)

ABSTRACT

Severe birth asphyxia leads to a transient organic aciduria and increased hypoxanthine excretion. To investigate its origin and timing, we analysed urine from 12 late gestation fetal sheep *in utero* subjected to moderately severe isocapnic hypoxia for 1 h. In 6 fetuses the carotid sinus nerves were cut to determine whether reflex peripheral vasoconstriction contributed to the changes in excretion. Following a control period of 1 h, maternal inspired oxygen was reduced for 1 h so that fetal arterial oxygen tension fell significantly from 2.86 ± 0.12 kPa (mean ± SEM) to 1.55 ± 0.04 kPa. The ewes were returned to normoxia and monitoring continued for 1 h. Fetal heart rate, arterial blood pressure and femoral arterial blood flow (intact fetuses only) were recorded and arterial pH, blood gases and lactate measured. Urine collected via a bladder catheter was analysed for organic acids and hypoxanthine with gas chromatography-mass spectrometry. In intact fetuses, hypoxia increased excretion of hypoxanthine and several organic acids, notably lactic acid and intermediates of valine catabolism. Changes were apparent by 15 min, significant by 45 min and maximal after re-oxygenation. In denervated fetuses, there were small, significant, increases in organic acids and hypoxanthine by 45 min of hypoxia, but there was no surge in excretion post-hypoxia. Hypoxia caused a large, significant, fall in femoral arterial blood flow in intact fetuses. We conclude that the extent of the reflex peripheral vasoconstriction, particularly in skeletal muscle, determines the amount of organic acid and hypoxanthine excretion and may explain similar biochemical disturbances after birth asphyxia. Urinary lactic acid measurement has potential value for grading birth asphyxia.

INTRODUCTION

It is still difficult to decide whether a newborn baby has suffered perinatal hypoxia or asphyxia of a severity likely to cause permanent brain damage. Currently available clinical and biochemical indicators, including cord blood pH and other measures of acid-base status, do not predict neurological outcome well (1-3). Because many metabolic processes are oxygen-dependent, severe tissue hypoxia causes wide-ranging biochemical disturbances

with accumulation of a variety of intermediary metabolites. These include organic acids, which are excreted by the kidneys and accumulate in urine. We previously observed pronounced, transient, abnormalities in the urinary organic acid profiles of infants during metabolic stress (4), and speculated that organic acid analysis might have potential value for detecting severe perinatal hypoxia. We went on to study 50 term newborns with fetal distress or birth asphyxia and 27 controls, and observed statistically significant abnormalities for the group of 12 severely asphyxiated babies. These predicted long-term outcome successfully in 9 of them (5). They were not seen in less severely distressed babies. Organic acid analysis of the first samples of urine passed after birth might be a useful adjunct to other measures of birth asphyxia. However, we needed to know more about the origin of the organic acid disturbance in order to define its clinical significance.

Adenosine triphosphate (ATP) is degraded rapidly during hypoxia producing adenosine, a vasodilator and putative neuroprotective agent (6) and, in muscle, inosine monophosphate. Hypoxanthine is a metabolite of both compounds. Adenosine has been measured in cord blood (7) but its short half life (< 10 s) precludes its use diagnostically. Hypoxanthine has a longer half life (40 min in pigs (8)) and has been measured in blood (9-13), cerebrospinal fluid (11), amniotic fluid (11,14) and urine (11,15-17) to detect perinatal asphyxia. Because the placenta clears hypoxanthine efficiently (18), levels in cord blood may underestimate severe self-limiting intra-uterine hypoxic events. Although often harmless, these sometimes cause problems neonatally and may even result in brain damage. Once hypoxanthine has been excreted by the kidneys it accumulates in urine. Thus urine provides a cumulative record of events (11) and its analysis is more likely to detect a previous serious episode. It is known that arterial plasma hypoxanthine levels increase in fetal hypoxia (18,19), but urinary excretion has not been studied *in utero*.

In this observational study, we monitored late gestation fetal sheep *in utero* stressed by moderately severe isocapnic hypoxia for 60 min. We aimed to investigate firstly whether fetal hypoxia induced under controlled conditions produces an organic aciduria similar to that of severely birth asphyxiated human newborns and secondly, the origin and timing of changes in urinary organic acid and hypoxanthine excretion with hypoxia. Our hypothesis was that biochemical disturbances are not due to hypoxia *per se*, but to chemoreceptor-drive reflexes that decrease blood flow to peripheral tissues, particularly skeletal muscle, causing ischaemia. To this end, we analysed urine from acutely hypoxic fetal sheep after section of both carotid sinus nerves, which reduces hypoxia-induced peripheral vasoconstriction dramatically (20,21). For the study, we developed a new specific assay for urinary hypoxanthine using gas chromatography-mass spectrometry.

METHODS

Surgical Preparation. All procedures were conducted under licence and in accordance with the 1986 Home Office regulations on animal experimentation. Twelve cross-bred pregnant ewes were studied at 119-133 d (median 126 d) of gestation (term is 147 d) using

general anaesthesia (1 g sodium thiopentone for induction; 2-3% halothane in oxygen for maintenance) and sterile techniques. The fetus was partially exteriorised through a uterine incision. Multistranded stainless steel wire electrodes (Cooner Wire Co, USA) were sewn into the fetal chest to record the electrocardiogram. Polyvinyl catheters (i.d. 1.0, o.d. 2.0 mm, Portex Ltd, Hythe, Kent, UK) were placed in a carotid artery, a jugular vein and the amniotic sac and a catheter was placed in the bladder via a puncture incision and secured by a purse string suture. A maternal femoral vein was catheterised. All leads were exteriorised through a maternal flank and secured to the ewe's back in a plastic bag. All catheters had a blunt needle in the distal end which was attached to a 3-way stopcock. The animals studied were primarily involved in other research which involved slightly different additional instrumentation. In the six *intact* fetuses, ultrasonic flow transducers (Transonics Inc, Ithaca, NY, USA) were implanted around one femoral artery. In the six *denervated* fetuses both carotid sinus nerves were sectioned (21). Treatment of the animals prior to our study did not differ. Post-operatively, the vascular catheters were maintained patent by a slow infusion of heparinised saline (50 IU mL^{-1} at 0.125 mL h^{-1}). Sodium benzylpenicillin (Crystapen, Glaxo Ltd, Middlesex, UK) was administered intravenously, 600 mg to the ewe, 200 mg to the fetus and into the amniotic sac (300 mg). The antibiotics were given daily for 4-5 d post-operatively, but not on the day of experimentation. Results are also presented for femoral blood flow of twelve denervated fetuses that we studied previously under identical experimental conditions. Details of their surgical preparation have been reported (21).

Experimental Procedure. At least 4 d elapsed after surgery before the experiments. These were based on a 3 h protocol (21). Following a control period of 1 h, fetal isocapnic hypoxia was induced for 1 h by reducing maternal inspired oxygen fraction to 0.09 (18 L min^{-1} of air, 22 L min^{-1} nitrogen and 1.2 L min^{-1} of carbon dioxide). The ewes were then returned to normoxia and monitoring continued for 1 h. During hypoxia, fetal arterial oxygen pressure (PaO_2) was reduced from 2.86 ±0.12 kPa to 1.55 ± 0.04 kPa (mean ± SEM). At 15 min intervals throughout the protocol, arterial blood samples (0.5 mL) were taken anaerobically from the fetus for determination of pH, blood gases and haematocrit, (model 1302; Instrumentation Laboratory, Warrington, Cheshire, UK, measurements corrected to 39.5°C) and whole blood lactate (11 fetuses) (YS1 model 2300 glucose and L lactate analyser; Yellow Springs Instrument Co. Inc, Yellow Springs, Ohio, USA). In addition, up to five 1 mL arterial blood samples were collected for the other studies (results not reported here). To collect urine samples the stopcock was opened and urine drained into a sterile measuring container. Up to 3 ml was removed for analysis and this volume was replaced with isotonic saline. Urine and saline were returned to the amniotic sac. Urine was collected before hypoxia, after 15 and 45 min of hypoxia and 15 and 45 min (intact fetuses) post-hypoxia. Arterial blood pressure (ABP), fetal heart rate (FHR) and femoral blood flow (FBF) were recorded continuously on a chart recorder (Linearcorder FWR 3701, Graphtec, UK).

Post-mortem examination. On completion of the experiments, ewes and fetuses were sacrificed with an overdose of sodium pentobarbitone to determine fetal and organ weights and to examine the organs macroscopically for evidence of damage.

Analytical Methods. Creatinine was measured by a kinetic alkaline picrate method using a discretionary autoanalyser (CX7 analyser; Beckman Instruments, High Wycombe, Bucks, UK). Organic acids were measured by a published gas chromatographic method (22), but using a heated split injector, split ratio 20:1, instead of cool on-column injection. An aliquot of urine equivalent to 1 μmol of creatinine was oximated with hydroxylamine hydrochloride, acidified to pH 1.0, saturated with sodium chloride, then extracted successively with ethyl acetate and diethyl ether. After evaporation, the urinary organic acids were derivatised with bis(trimethylsilyl)-trifluoroacetamide (BSTFA) and analysed by capillary gas chromatography with flame ionisation detection. Unknown compounds were identified by combined gas chromatography-electron impact mass spectrometry (5892A Series II gas chromatograph linked to a 59731A quadrupole mass spectrometer; Hewlett Packard, Bracknell, UK). This was particularly important for 3-hydroxyisobutyric acid which co-elutes with 3-hydroxybutyric acid. To enable comparisons, acids were quantified from their peak areas using n-tetracosane as internal standard. The detection limit for most of the non-polar acids was < 1 mg/mmol of creatinine. Concentrations were related to the urinary creatinine which, although not ideal (6), corrects for differences in water diuresis.

Hypoxanthine was measured by a new method developed in this laboratory using gas chromatography-mass spectrometry with single ion monitoring. Urine with creatinine greater than 1 mmol/L was diluted to this concentration with de-ionised water. Other samples were analysed neat. To an aliquot of urine equivalent to 1 μmol of creatinine was added 60 μL of 0.799 mmol/L of 2,3,7,8 - tetra deuterated hypoxanthine as internal standard (MSD Isotopes Ltd, Croydon, UK). Half of this mixture was applied to 1 mL of cation exchange resin (AG 50W-XB resin, 200-400 mesh, hydrogen form, Bio-Rad Laboratories, Hemel Hempstead, UK) in a disposable 2 mL plastic syringe plugged with cotton wool. After washing twice with 3 mL of water, hypoxanthine was eluted with 3 x 0.5 mL of 5 mol/L ammonium hydroxide and collected into a 1.8 mL glass vial. The pooled eluates were freeze dried. Hypoxanthine was derivatised by heating the residue for 30 min at 60°C with 100 μL of N-(*tert*-butyldimethylsilyl)-N-methyltrifluoroacetamide (MTBSTFA), (Sigma Chemicals Ltd, Dorset, UK) and 10 μL of Analar pyridine as a catalyst. One μL was injected into a gas chromatograph-mass spectrometer (5892A series II gas chromatograph linked to a 59731A quadrupole mass spectrometer, Hewlett Packard, Bracknell, UK), fitted with an SE-30 capillary gas chromatography column, 30 mm x 0.25 mm I.D. film thickness 0.25μm (Econo-Cap, Alltech UK, Carnforth, UK). The split ratio was 13:1. Helium was the carrier gas, flow rate 1 mL min^{-1}. The injector was maintained at 250°C. The column temperature was held at 150°C for 5 min then increased by 5°C min^{-1} to 270°C and held for 10 min. The retention time of hypoxanthine was 22 min. Two of the four original deuterium atoms of the internal standard were replaced

during derivatisation. Quantification of deuterated hypoxanthine and hypoxanthine extracted from urine was achieved by monitoring the ions at m/z 309 and 307, respectively, resulting from loss of a *tert* butyl group, $[M-57]^+$, from the molecular ion $[M]^+$. The concentration of urinary hypoxanthine (μmol $mmol^{-1}$ creatinine) was calculated from the integrated peak areas. The method was sensitive to 0.5μmol $mmol^{-1}$ creatinine and linear to 85 μmol $mmol^{-1}$ creatinine. Recoveries of standard hypoxanthine added to urine were: for 2.9 μmol $mmol^{-1}$ creatinine added, mean 101.5% (range 99.8%-102.7%); for 10.1 μmol $mmol^{-1}$ creatinine 97.3% (96.0-99.9%); for 52.9 μmol $mmol^{-1}$ creatinine 99.8% (99.4%-100.6%) (n=6). Between batch imprecision was low, the coefficient of variation being 3.7% at 8.0 μmol $mmol^{-1}$ creatinine (n=8) and 2.1% at 47.5 μmol $mmol^{-1}$ creatinine (n=6).

Statistical Analysis. The Mann-Whitney U test was used for comparisons.

RESULTS

All the fetuses recovered from the hypoxic challenge. None was excluded from the study.

Blood gases, pH and lactate. During normoxia, arterial blood gases, pH and blood lactate of intact and denervated fetuses were not significantly different. PaO_2 fell from a control value of 2.86 ± 0.12 kPa (mean ± SEM) to 1.55 ± 0.04 kPa ($P<0.001$) during hypoxia. Basal $PaCO_2$ was 6.65 ± 0.20 kPa and levels were maintained during hypoxia (6.50 ± 0.22 kPa after 45 min). pH fell significantly from 7.36 ± 0.01 to 7.22 ± 0.03 (intact; $P<0.01$) and to 7.31 ± 0.02 (denervated; $P<0.01$). Despite rapid recovery of PaO_2 with re-oxygenation, the pH of intact fetuses fell further to 7.18 ± 0.03, 15 min post-hypoxia, when it was significantly lower than for denervated fetuses (7.31 ± 0.02; $P<0.01$), and remained significantly depressed after 45 min (7.20 ± 0.03). Blood lactate of intact fetuses increased from 1.02 ± 0.08 mmol/L basally to 2.98 ± 0.44 mmol/L after 15 min ($P<0.01$) and 6.41 ± 0.51 after 45 min of hypoxia ($P<0.01$). Levels increased further with re-oxygenation to 7.53 ± 1.06 and 7.31 ± 0.75 at 15 and 45 min, respectively. The basal lactate concentration of denervated fetuses was 0.96 ± 0.15 mmol/L. Although levels increased significantly during hypoxia to 1.89 ± 0.19 ($P<0.05$) and 3.77 ± 0.53 ($P<0.01$) at 15 and 45 min, respectively, they did not increase with re-oxygenation (3.77 ± 0.75 after 15 min). Lactate concentrations of intact fetuses were significantly higher than denervated fetuses both during and post-hypoxia ($P<0.05$).

Fetal heart rate and blood pressure. In the intact fetuses FHR fell from 165 ± 6 beats per min (bpm) to 134 ± 4 bpm after 5 min of hypoxia ($P<0.01$). The rate returned to control levels towards the end of the hypoxic period, and tachycardia (204 ± 4 bpm) developed with re-oxygenation, ($P<0.01$ compared with basal). There was a small, but not significant, increase in ABP from 42 ± 2 mm Hg to 52 ± 3 mm Hg with hypoxia. The response of denervated fetuses was similar to that of our earlier study (21). There was no early bradycardia and the heart rate increased, but not significantly, towards the end of hypoxia,

returning to basal post-hypoxia. ABP did not increase significantly from basal levels of 50 ± 1 mm Hg.

Femoral arterial blood flow. In this study, this was monitored only in the intact fetuses. FBF decreased sharply from 47 ± 1 mL min^{-1} to 20 ± 3 mL min^{-1} after 5 min of hypoxia ($P<0.01$) and 12 ± 2 mL min^{-1} after 45 min ($P<0.01$). There was a rebound rise to 60 ± 4 mL min^{-1} 45 min post-hypoxia ($P<0.05$, compared with basal) (Fig. 1). In Fig. 1 we also present for comparison, FBF data for 12 denervated fetuses measured under similar experimental conditions and taken from our previous study (21). In those fetuses, FBF did not change significantly with hypoxia.

Post-mortem findings. There were no differences in body or organ weights between the groups and there was no macroscopic evidence of damage to the kidneys or other organs.

Urinary organic acids and hypoxanthine. One intact fetus became oliguric during hypoxia and there was insufficient urine for analysis. In another, there were problems with the bladder catheter drainage and urine was not collected post-hypoxia.

Urinary organic acids: intact fetuses. In *normoxia* the predominant organic acids were lactic and pyruvic acids, the tricarboxylic acid cycle intermediates, citric, succinic and 2-oxoglutaric acids, a tyrosine metabolite, 4-hydroxybenzoic acid, hippuric acid and glyoxylic acid. There were small amounts of 3-hydroxyisobutyric acid, produced during valine catabolism, but another valine derivative, 2-hydroxyisovaleric acid was not detectable in quantifiable amounts. 2-Hydroxybutyric was detected (in trace amounts) in one sample only.

During hypoxia, the profile changed. Disturbances were apparent by 15 min but were gross and statistically significant by 45 min (Table 1; Figs. 2a, 3a). The striking features were:- markedly increased lactic acid excretion accompanied by raised pyruvic acid, large increases in the tricarboxylic acid cycle intermediates, and in 3-hydroxyisobutyric acid, excretion of 2-hydroxybutyric acid (all fetuses) and of 2-hydroxyisovaleric acid (4 of 6). With re-oxygenation, the organic aciduria intensified. The disturbance was maximal 15 min post-hypoxia, but acid concentrations were not statistically different from those during hypoxia, probably because of the small number of samples and wide inter-individual variation. With mass spectrometry, four fetuses were found to excrete trace amounts of 2-oxoisocaproic acid (from leucine) and two, 2-oxo,3-methylvaleric acid (from isoleucine) during or after hypoxia. Excretion of 4-hydroxybenzoic acid was not altered by hypoxia.

Urinary organic acids: denervated fetuses. In normoxia the urinary organic acid concentrations were not significantly different from those of intact fetuses. Excretion had not increased after 15 min of hypoxia, but by 45 min lactic acid concentrations were significantly higher than basal and smaller, significant, increases were found for pyruvic, succinic, fumaric and 3-hydroxyisobutyric acids. 2-Hydroxybutyric acid was detectable in 5 of 6 samples and 2-hydroxyisovaleric acid in 4 (Table 2; Figs. 2b, 3b). In contrast to intact fetuses, there was no surge in excretion post-hypoxia when there were highly significant differences between the two groups (Table 2).

Urine hypoxanthine. The urinary hypoxanthine of intact animals was similar to basal (11.1; 1.5-25.6 μmol $mmol^{-1}$ creatinine, median and range) after 15 min of hypoxia but by 45 min had increased significantly to 68.1 (30.0-184.4) μmol $mmol^{-1}$ creatinine (Table 1). Fifteen min post-hypoxia excretion was still significantly elevated compared to basal, reaching peak values in 2 fetuses. Levels were declining by 45 min. Basal hypoxanthine concentrations of denervated fetuses were significantly lower than for the intact group ($P<0.05$) (Table 2). They had increased significantly by 45 min of hypoxia to a median concentration of 8.5 (range 2.8-32.8) μmol $mmol^{-1}$ creatinine ($P<0.05$) but at this peak were only within the range found basally for intact fetuses. Concentrations declined post-hypoxia. Figures 4a and 4b show the striking difference between groups.

DISCUSSION

In the late gestation fetal lamb, acute hypoxaemia triggers a rapid chemoreflex response characterised by an initial bradycardia followed by an increase in heart rate and blood pressure with a major redistribution of blood flow (20,21,23-28). This is largely eliminated by bilateral section of the carotid sinus nerves (20,21). The physiological responses of intact and denervated fetal lambs in this study were typical of those reported. Femoral blood flows of denervated animals were not recorded here, but were probably similar to those of our earlier study under the same conditions (21; see Fig. 1), since the heart rate responses were the same indicating complete denervation.

In normoxia, the urinary organic acid excretion of intact and denervated fetuses was not significantly different. However, the intact and denervated fetuses had significantly different responses to hypoxia. In intact fetuses changes in organic acid excretion were apparent by 15 min, statistically significant by 45 min and maximal 15 min after re-oxygenation. In contrast, organic acids of denervated fetuses were only increased significantly after 45 min of hypoxia and there was no surge in excretion post-hypoxia. Because the changes were large in animals in which we documented a dramatic decrease in FBF and barely significant in denervated fetuses in which FBF would decrease more slowly and to a lesser extent (21), it is probable that a major contribution to the organic acid disturbances was from altered metabolism of skeletal muscle, which may become almost ischaemic during the severe vasoconstriction induced by hypoxia in intact fetuses. The further increases post-hypoxia were probably due to a `wash-out' of metabolites during the transient vasodilatation seen with recovery (23), which was evident in the increased FBF of intact fetuses but which does not occur in denervated fetuses. Altered liver and renal tubular metabolism probably also contributed to the organic aciduria. Brain was *not* the source since cerebral blood flow increases in fetal hypoxia (27).

Lactate is the main end-product of anaerobic glucose metabolism. Although 5-10% of blood lactate is cleared by a single passage through the placenta in fetal asphyxia (18), arterial blood lactate increases rapidly and then falls with re-oxygenation. Efflux of lactate from the fetal hind limb was found to increase with moderate hypoxia and was highest

during recovery (18). This probably accounts for much of the increase. Decreased clearance by the liver (28) could also contribute. Blood lactate concentrations increased in both groups of fetuses, but were significantly higher in the intact group. However the difference was small in comparison with the very large differences in their urinary lactate concentrations, explained by cumulative lactate excretion during the sampling periods and indicating considerably greater lactate production by hypoxic, intact, fetuses.

A new observation was that fetal hypoxia has a significant impact on valine metabolism evident from large increases in the intermediate, 3-hydroxyisobutyric acid, and excretion of 2-hydroxyisovaleric acid, not found in normoxia. Peak urine concentrations were much lower in denervated fetuses ($P<0.01$). Skeletal muscle is very active in branched chain amino acid catabolism and has considerable capacity for valine oxidation (29-31). The observed abnormalities can be explained by inhibition of two catabolic enzymes: branched chain oxo-acid dehydrogenase (E.C.1244) and 3-hydroxyisobutyric acid dehydrogenase (E.C.11131) by a high intra-mitochondrial NADH:NAD ratio in hypoxia (29,32,33). 3-Hydroxyisobutyric acid is effectively transported out of muscle (31) and was released during perfusion of rat hind quarters (29). Hypoxic muscle was probably the main source of the valine metabolites found here.

2-Hydroxybutyric acid is produced mainly in the liver by catabolism of the amino acids threonine and methionine to 2-oxobutyric acid. Decarboxylation of this compound by the pyruvate dehydrogenase complex (E.C.1241) is inhibited by a high NADH:NAD ratio in hypoxia (34). 2-Hydroxybutyric acid may have accumulated because of a combination of increased catabolism and reduced liver perfusion (28). The increased tricarboxylic acid cycle acids might have originated in any of the tissues with reduced oxygen delivery, including the kidneys, since these compounds are produced by renal metabolism and secreted by renal tubules (35).

The urinary organic acid excretion profile of fetal sheep in normoxia was similar to that of term human newborns after an uneventful delivery (5), except that the fetuses did not excrete detectable amounts of medium chain dicarboxylic acids or the ketone body 3-hydroxybutyric acid, derived from fat catabolism. Fetal hypoxia caused similar urinary organic acid disturbances to those of severe birth asphyxia, with large increases in lactic and pyruvic acids, and excretion of 2-hydroxybutyric acid. Both hypoxic fetuses and newborns excrete intermediates of branched chain amino acid catabolism, but the profiles differ: fetal excretion of 3-hydroxyisobutyric acid is proportionately higher, and branched chain oxo-acids are excreted infrequently compared with newborns. This might be explained by a species or age difference in the sensitivity of 3-hydroxyisobutyric acid dehydrogenase to inhibition in hypoxia, to greater permeability of fetal tissues to 3-hydroxyisobutyric acid, or to the nature of the insult since our fetuses were only moderately hypoxic, without hypercarbia or severe acidosis . A more notable difference is that, in contrast to fetal hypoxia, excretion of tricarboxylic acid cycle intermediates were not significantly increased after severe birth asphyxia, despite the fact that all affected babies had evidence of renal

damage with haematuria and/or proteinuria. These differences may be due to decreased glomerular filtration following birth asphyxia, or to differences in renal tubular maturation, since much of this occurs late in gestation (36).

The basal urinary hypoxanthine concentration of 5 intact fetuses of 11.1 (1.5-25.6) μmol $mmol^{-1}$ creatinine (median, observed range) was similar to concentrations for active term human newborns on the second day of life (mean 12.0, range 5.3-27.5) μmol $mmol^{-1}$ creatinine (11). In response to hypoxia, excretion by all five fetuses increased markedly and after 45 min the difference from basal was highly significant ($P<0.01$). Concentrations were as high, or higher, after 15 minutes of re-oxygenation, but had clearly fallen by 45 min. Urinary hypoxanthine excretion therefore responded rapidly to changes in tissue oxygen delivery. Our observations are compatible with those of Thiringer et al (19) who found a substantial increase in arterial plasma hypoxanthine of exteriorised fetal lambs during graded hypoxia, and a close correlation with other indices of fetal asphyxia - pH, base deficit, arterial oxygen saturation and blood lactate concentration. Our observations are similar to others who have shown increasing urinary hypoxanthine in moderate to severely hypoxic young pigs (37). Hypoxanthine metabolism differs among species but is probably similar in pigs to man (8).

In our study, carotid sinus denervation had a significant impact on urinary hypoxanthine excretion. Basally, urinary concentrations of denervated fetuses were low compared with intact fetuses ($P<0.05$). To our knowledge, this has not been reported before but cannot be explained by our data. Perhaps intact fetuses excrete more adrenaline basally, with increased ATP degradation through metabolic effects (7). Alternatively, adenosine production may be one mechanism by which chemoreceptors influence vascular tone. Clearly this observation merits further study. Forty five min of hypoxia led to a small increase above normoxic values ($P<0.05$), but at their peak, levels were only as high as the basal concentrations of the intact group and they fell rapidly post-hypoxia. The findings indicate that peripheral vasoconstriction in intact fetuses underlies the normal hypoxanthine response to hypoxia. From the surge in urinary excretion that coincided with restored femoral blood flow, it seems likely that skeletal muscle contributes substantially to the increase post-hypoxia. Thiringer et al, from studies of exteriorised fetal lambs, proposed that *during* hypoxia, the liver is the main source of increased plasma hypoxanthine, and that skeletal muscle releases hypoxanthine mainly during re-oxygenation (18). Their earlier work had shown that brain was an unlikely source (38). The renal tubules may have contributed some hypoxanthine to urine, since renal blood flow decreases in response to acute fetal hypoxia due to increased renal vascular resistance (27,28,39). In contrast, after chemoreceptor denervation (40), or renal denervation (39), the initial response to acute hypoxia is transient vasodilatation with *increased* renal blood flow and renal vasoconstriction is delayed.

The potential value of hypoxanthine as an indicator of perinatal hypoxia has been explored clinically. So far, most investigators have measured hypoxanthine in cord blood

of human newborns. Concentrations were raised in babies with severe birth asphyxia (9,12,13) but only in 52% in one study (10). Levels correlated with blood pH, base deficit and lactate in two studies (12,13), but not in a third (10). However, cord blood hypoxanthine levels of birth asphyxiated babies predicted their two year old developmental outcome poorly (12). The problem with measurements of plasma hypoxanthine is that increases may be transient *in utero*, since the placenta clears hypoxanthine from fetal blood very efficiently (18). Increases are more persistent in urine and its analysis is more likely to detect a previous serious episode. Few have explored this potential so far, probably because of the practical difficulties of collecting the first voidings of newborns, coupled with analytical problems. Manzke et al (15) found raised hypoxanthine in urine during the first 24 h of life in 58% of 45 babies with moderate or severe birth asphyxia. Harkness et al proposed that urine collected on the second day of life would be more helpful, since raised hypoxanthine then could be explained by renal damage and would imply a serious circulatory shut-down (16,17). In their studies, babies who were neurologically abnormal for more than 48 h from birth had the highest excretion but there was no correlation between urinary hypoxanthine and developmental quotient at 1 year of age (17).

Our fetal observations indicate that the organic acid disturbances and increased hypoxanthine excretion are due largely to *peripheral* vasoconstriction, particularly in skeletal muscle. They are transient and resolve when oxygen is restored. They are indicators of overall hypoxia and not of brain hypoxia. Neither do they imply organ damage. Nevertheless, they do indicate a serious hypoxic event, that probably reduced arterial oxygen content to below 1.5 mM (26). However, it should be stressed that often even severe insults *in utero* do not cause brain damage, if self-limiting. The study does not define the duration of hypoxia needed to cause a significant biochemical disturbance. Although we did not observe significant changes until 45 min of hypoxia, they might be apparent after a shorter episode, during the `wash-out' of metabolites associated with re-oxygenation. The findings for lactic acid support the view that an acute antepartum hypoxic event is more likely to be detected by analysis of urine, in which disturbances are cumulative, than of blood in which metabolite changes are minimised by placental clearance and may be transient if hypoxia is relieved before delivery. The same is probably true for hypoxanthine, although plasma levels were not measured. Finally, since the largest disturbance was in lactate excretion, lactate analysis of the first urine samples after birth should be as informative as a full organic acid profile. The value of urinary hypoxanthine for assessing birth asphyxia needs further investigation.

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REFERENCES

1. Nelson KB, Emery ES 1993 Birth asphyxia and the neonatal brain: what do we know and when do we know it. Clinics in Perinatology 20:327-344.
2. Deans AC, Steer PJ 1994 The use of the fetal electrocardiogram in labour. Br J Obstet Gynaecol 101:9-17.
3. Socol ML, Garcia PM, Riter S 1994 Depressed Apgar scores, acid-base status, and neurologic outcome. Am J Obstet Gynecol 170:991-999.
4. Mills GA, Walker V 1988 Organic acids as indicators of the metabolism of premature babies. In: Jones CT (ed) Fetal and Neonatal Development. Perinatal Press, Ithaca, pp 631-634.
5. Walker V, Mills GA 1992 Effects of birth asphyxia on urinary organic acid excretion. Biol Neonate 61:162-172.
6. Rudolphi KA, Schubert P, Parkinson FE, Fredholm BB 1992 Adenosine and brain ischaemia. Cerebrovascular and Brain Metabolism Reviews 4:346-369.
7. Irestedt L, Dahlin I, Hertzberg T, Sollevi A, Lagercrantz H 1989 Adenosine concentration in umbilical cord blood of newborn infants after vaginal delivery and Cesarean section. Pediatr Res 26:106-108.
8. Saugstad OD, Aasen AO, Hetland Ø 1978 Plasma hypoxanthine levels in pigs during acute hypoxemia. Eur Surg Res 10:314-321.
9. Saugstad OD 1975 Hypoxanthine as a measurement of hypoxia. Pediatr Res 9:158-161.
10. Thiringer K 1983 Cord plasma hypoxanthine as a measure of foetal asphyxia. Acta Paediatr Scand 72:231-237.
11. Harkness RA 1988 Hypoxanthine, xanthine and uridine in body fluids, indicators of ATP depletion (Review). J Chromatogr 429:255-278.
12. Ruth VJ, Raivio KO 1989 Purine metabolites as measures of birth asphyxia and predictors of brain damage. Adv Exp Med Biol 253A:339-344.
13. Porter KB, O'Brien WF, Benoit R 1992 Comparison of cord purine metabolites to maternal and neonatal variables of hypoxia. Obstet Gynecol 79:394-397.
14. Harkness RA, Purkiss P, Duffy S, Chalmers RA, Jones M 1988 The effects of fetal energy depletion on amniotic fluid concentrations of amino acids, organic acids and related metabolites. J Inher Metab Dis 11:103-113.
15. Manzke H, Dörner K, Grünitz J 1977 Urinary hypoxanthine, xanthine and uric acid excretion in newborn infants with perinatal complications. Acta Paediatr Scand 66:713-717.
16. Harkness RA, Whitelaw AGL, Simmonds RJ, 1982 Intrapartum hypoxia: the association between neurological assessment of damage and abnormal excretion of ATP metabolites. J Clin Pathol 35: 999-1007.
17. Laing I, Brown JK, Harkness RA 1988 Clinical and biochemical assessments of damage due to perinatal asphyxia: a double blind trial of a quantitative method. J Clin Pathol 41:247-252.
18. Thiringer K, Karlsson K, Rosén KG, Kjellmer I 1984 Contribution of heart muscle, liver, skeletal muscle and placenta to the asphyxial hypoxanthine elevation in the acutely exteriorised fetal lamb. Biol Neonate 45:169-182.

19. Thiringer K, Saugstad OD, Kjellmer I 1980 Plasma hypoxanthine in exteriorized, acutely asphyxiated fetal lambs. Pediatr Res 14:905-909.

20. Itskovitz J, LaGamma EF, Bristow J, Rudolph AM 1991 Cardiovascular responses to hypoxemia in sinoaortic-denervated fetal sheep. Pediatr Res 30:381-385.

21. Giussani DA, Spencer JAD, Moore PJ, Bennet L, Hanson MA 1993 Afferent and efferent components of the cardiovascular reflex responses to acute hypoxia in term fetal sheep. J Physiol 461:431-449.

22. Walker V, Mills GA 1989 Urinary organic acid excretion by babies born before 33 weeks of gestation. Clin Chem 35:1460-1466.

23. Dawes GS, Lewis BV, Milligan JE, Roach MR, Talner NS 1968 Vasomotor responses in the hind limbs of foetal and new-born lambs to asphyxia and aortic chemoreceptor stimulation. J Physiol 195:55-81.

24. Cohn HE, Sacks EJ, Heymann MA, Rudolph AM 1974 Cardiovascular responses to hypoxemia and acidemia in fetal lambs. Am J Obstet Gynecol 120:817-824.

25. Hanson MA 1988 The importance of baro- and chemoflexes in the control of the fetal cardiovascular system. J Dev Physiol 10:491-511.

26. Boyle DW, Hirst K, Zerbe GO, Meschia G, Wilkening RB 1990 Fetal hind limb oxygen consumption and blood flow during acute graded hypoxia. Pediatr Res 28:94-100.

27. Jensen A, Berger R 1991 Fetal circulatory responses to oxygen lack. J Dev Physiol 16:181-207.

28. Iwamoto HS 1993 Cardiovascular effects of acute fetal hypoxia and asphyxia. In: Hanson MA, Spencer JAD, Rodeck CH (eds) Fetus and Neonate Physiological and Clinical Applications. Vol 1. The Circulation, Cambridge University Press, Cambridge, pp 197-214.

29. Harper AE, Miller RH, Block KP 1984 Branched-chain amino acid metabolism. Ann Rev Nutr 4:409-454.

30. Goldberg AL, Chang TW 1978 Regulation and significance of amino acid metabolism in skeletal muscle. Federation Proc 37: 2301-2307.

31. Spydevold Ø 1979 The effect of octanoate and palmitate on the metabolism of valine in perfused hindquarter of rat. Eur J Biochem 97:389-394.

32. Landaas S 1977 Inhibition of branched-chain amino acid degradation by ketone bodies. Scand J Clin Lab Invest 37:411-418.

33. Corkey BE, Martin-Requero A, Walajtys-Rode E, Williams RJ, Williamson JR 1982 Regulation of the branched chain a-ketoacid pathway in liver. J Biol Chem 257:9668-9676.

34. Landaas S, Pettersen JE 1975 Clinical conditions associated with urinary excretion of 2-hydroxybutyric acid. Scand J Clin Lab Invest 35:259-266.

35. Vishwakarma P, Lotspeich WD 1959 The excretion of L-malic acid in relation to the tricarboxylic acid cycle in the kidney. J Clin Invest 38:414-423.

36. Barratt TM 1984 Renal disorders. In: Clayton BE, Round JM (eds) Chemical Pathology and the Sick Child. Blackwell Scientific Publications, Oxford, pp 120-149.

37. Poulsen JP, Øyasaeter S, Sanderud J, Rognum TO, Saugstad OD 1990 Hypoxanthine, xanthine, and uric acid concentrations in the cerebrospinal fluid, plasma, and urine of hypoxemic pigs. Pediatr Res 28:477-481.

38. Thiringer K, Blomstrand S, Hrbek A, Karlsson K, Kjellmer I 1982 Cerebral arterio-venous difference for hypoxanthine and lactate during graded asphyxia in the fetal lamb. Brain Res 239:107-117.

39. Robillard JE, Nakamura KT, Dibona GF 1986 Effects of renal denervation on renal responses to hypoxemia in fetal lambs. Am J Physiol 250:F294-F301.

40. Green LR, Bennet L, Robson S, Hanson MA 1995 Effect of carotid sinus denervation on renal blood flow and urine output during hypoxia in the late gestation fetus. Abstract 17, the 22nd annual meeting of The Society for the Study of Fetal Physiology, Sweden.

Table 1. *Urine organic acids of intact fetuses before, during and after hypoxia*†

Organic acid	Pre-hypoxia	Hypoxia		Post-hypoxia	
		15 min	45 min	15 min	45 min
Lactic	80 18-342	459 112-1058	1556** 813-22436	10690** 3886-11230	7021** 1651-52033
2-Hydroxy-butyric	ND ND-13	7 tr-15	17* 9-214	90** 35-159	55** 25-411
Pyruvic	19 8-42	34 11-162	92 34-699	485** 316-660	314** 248-1359
3-Hydroxy-isobutyric	13 ND-58	42 9-810	102* 39-596	320** 156-410	186** 161-671
2-Hydroxy-isovaleric	ND	tr ND-tr	5* ND-45	27** 9-35	12** 9-67
Succinic	8 ND-19	24 ND-84	72** 28-242	91** 37-223	10 8-25
Fumaric	ND	10 ND-56	48** 42-96	63** 45-144	31** 11-53
2-Oxoglutaric	8 ND-42	45 ND-88	105* 17-113	139** 71-159	94** 74-126
Hypoxanthine	11.1 1.5-25.6	11.9 2.9-45.9	68.1** 30.0-184.4	116.6* 70.2-131.8	77.0 9.9-107.9
Creatinine	0.9 0.4-1.1	1.0 0.3-1.3	0.7 0.2-1.7	0.6 0.4-1.0	0.8 0.3-2.1

† Figures given are μmol $mmol^{-1}$ creatinine median and observed range. Organic acids of six fetuses were measured and hypoxanthine of five. Samples were not analysed from one fetus during hypoxia and one fetus post-hypoxia; ND. not detected; tr. trace; * $P<0.05$; ** $P<0.01$ compared with pre-hypoxia (Mann-Whitney test).

Table 2. *Urine organic acids and hypoxanthine of carotid sinus denervated fetuses before, during and after hypoxia*†

Organic acid	Pre-hypoxia	Hypoxia		Post-hypoxia
		15 min	45 min	15 min
Lactic	49	77†	684**	542**††
	24-111	27-156	87-1883	94-2612
2-Hydroxy-	ND	ND	12*	13*†
butyric	ND	ND-3	ND-24	ND-37
Pyruvic	13	11	37**	48*††
	7-22	8-26	19-109	14-124
3-Hydroxy-	4	6†	38*	52*††
isobutyric	ND-8	3-13	6-122	2-141
2-Hydroxy-	ND	ND	2	2†
isovaleric	ND	ND	ND-5	ND-9
Succinic	5	5	25*	9††
	2-9	ND-8	3-113	3-17
Fumaric	ND	1	17*	4††
	ND	ND-3	ND-76	ND-14

Organic acid	Pre-hypoxia	Hypoxia		Post-hypoxia
		15 min	45 min	15 min
2-Oxoglutaric	7	8	41†	17††
	3-16	1-20	1-54	1-65
Hypoxanthine	1.7†	2.6†	8.5*†	3.5*†
	1.2-3.7	1.8-3.0	2.8-32.8	1.9-16.3
Creatinine	1.4	1.4	1.4	1.6
	0.8-2.3	0.7-2.3	0.5-2.3	0.6-1.9

† Figures given are μmol $mmol^{-1}$ creatinine median and observed range: 6 samples analysed from each collection time; ND. not detected. Samples from 6 fetuses were analysed. * $P<0.05$; ** $P<0.01$ compared with pre-hypoxia; † $P<0.05$; †† $P<0.01$ denervated compared with intact fetuses (values for denervated fetuses were significantly lower).

LEGENDS

Figure 1. Femoral arterial blood flow of intact (°) fetuses during the experimental protocol. Femoral blood flow of twelve carotid sinus denervated fetuses (_) in our earlier study (21) are presented for comparison. Values are means ± SEM.

Figure 2(a) and (b). Urinary lactic acid of fetal sheep before, during and after 60 min of hypoxia: (a) values for 6 intact fetuses. Samples were not analysed from one fetus during hypoxia and one fetus post-hypoxia; (b) values for six carotid sinus denervated fetuses.

Figure 3(a) and (b). Urinary 3-hydroxyisobutyric acid of fetal sheep before, during and after 60 min of hypoxia: (a) values for 6 intact fetuses. Samples were not analysed from one fetus during hypoxia and one fetus post-hypoxia; (b) values for six carotid sinus denervated fetuses.

Figures 4(a) and (b). Urinary hypoxanthine of fetal sheep before, during and after 60 min of hypoxia: (a) value for five intact fetuses. Samples were not analysed from one fetus post-hypoxia; (b) values for six carotid sinus denervated fetuses.

Figure 1

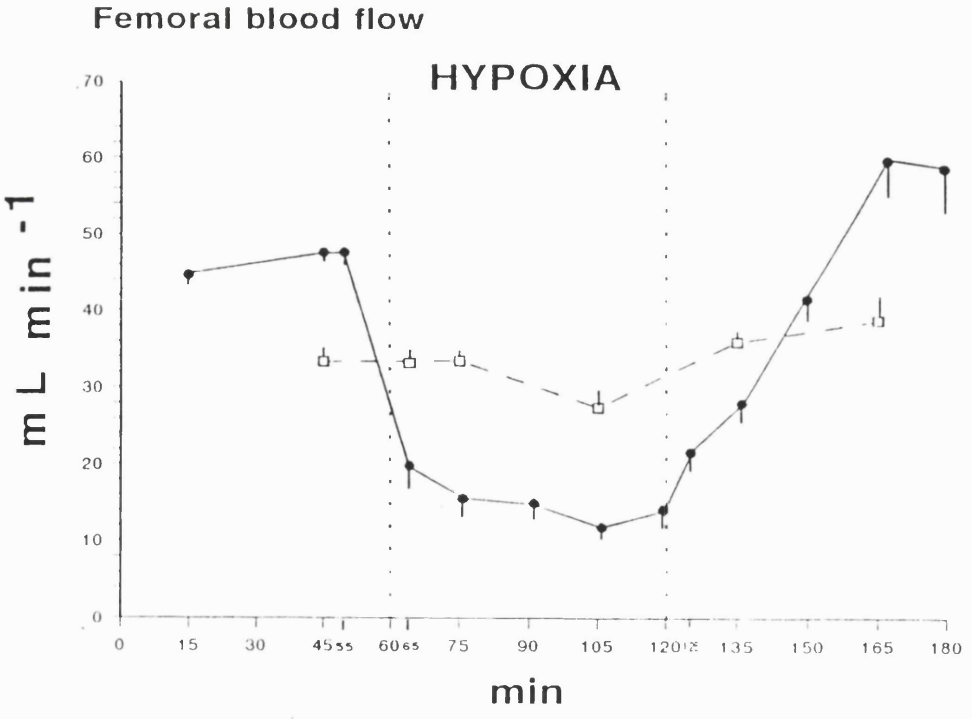
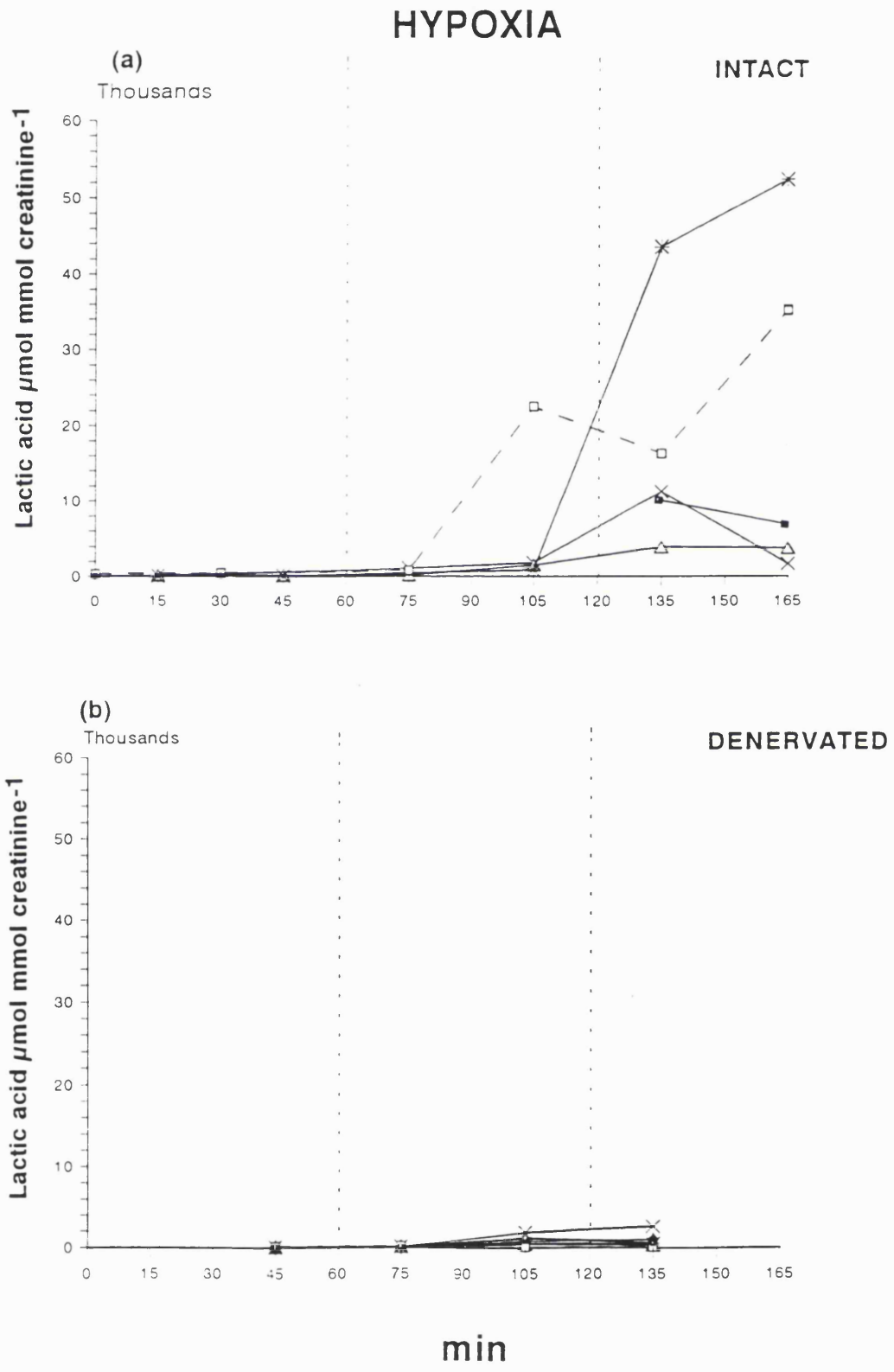


Figure 2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Thousands
INTACT
Lactic acid µmol mmol creatinine-1
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75
90
105
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135
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165
(b)
Thousands
DENERVATED
Lactic acid µmol mmol creatinine-1
60
50
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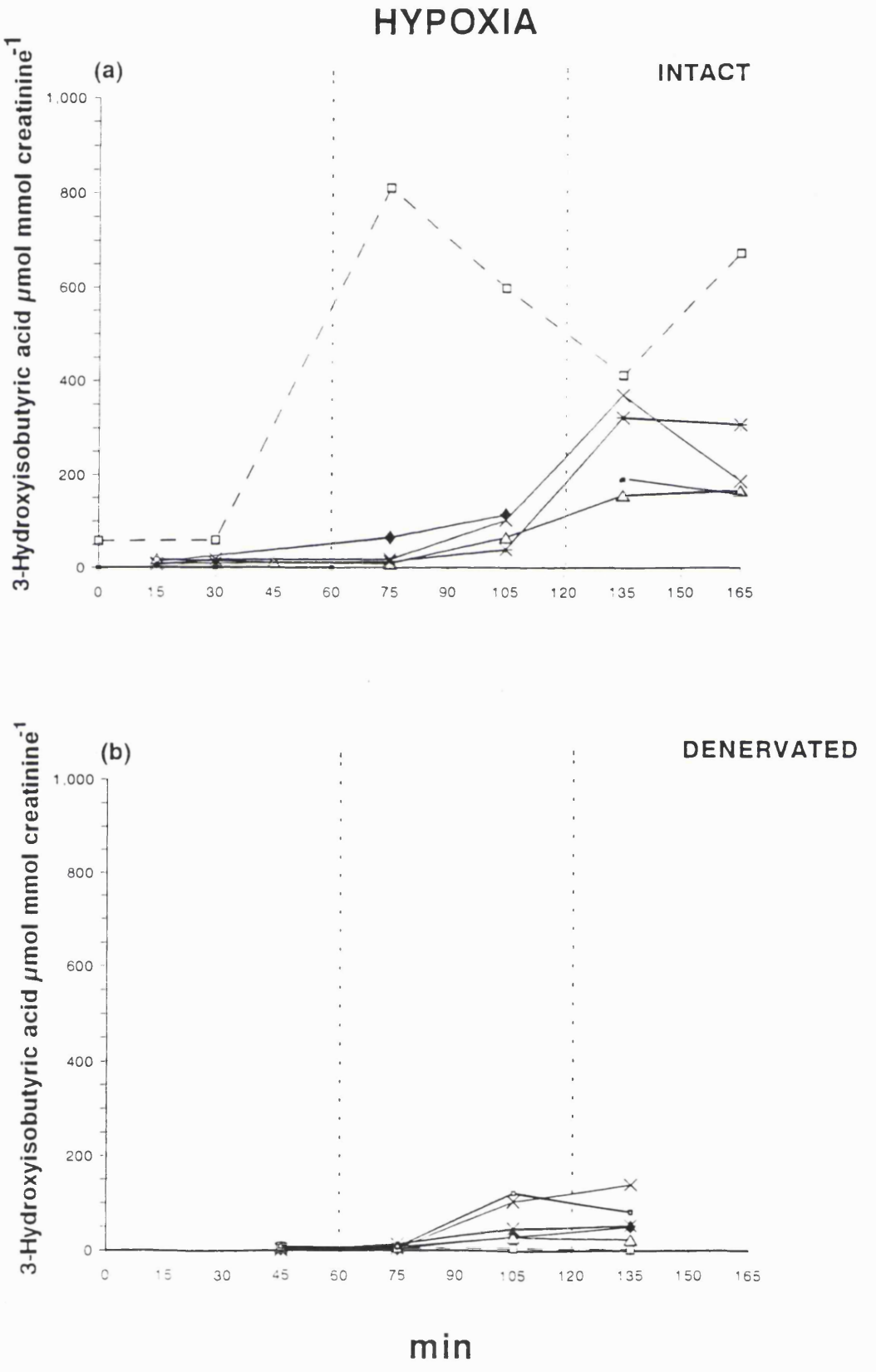
Figure 3


HYPOXIA
(a)
INTACT
3-Hydroxyisobutyric acid μmol mmol creatinine-1
1,000
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0 15 30 45 60 75 90 105 120 135 150 165
(b)
DENERVATED
3-Hydroxyisobutyric acid μmol mmol creatinine-1
1,000
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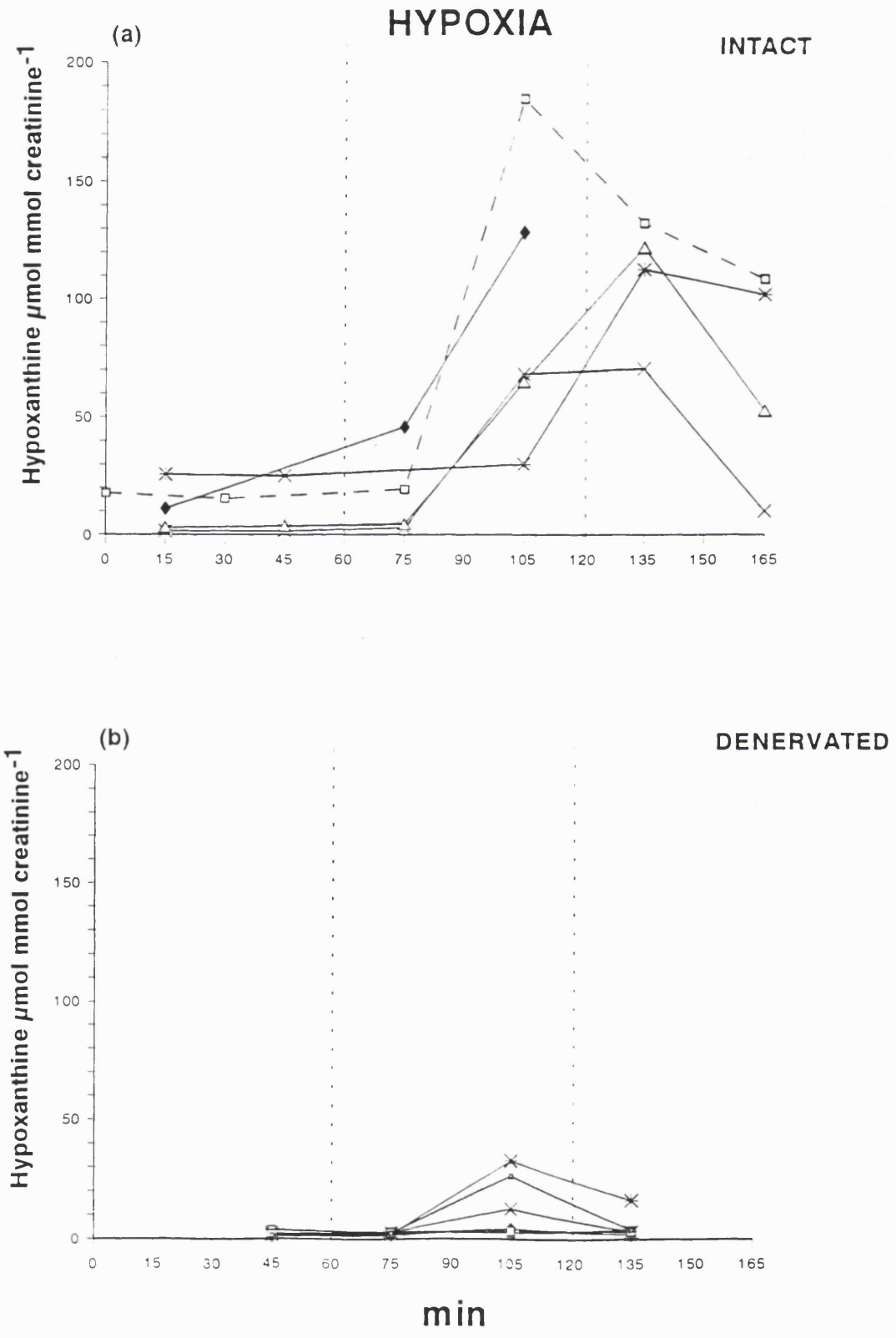
Figure 4


HYPOXIA
(a)
INTACT
Hypoxanthine µmol mmol creatinine-1
200
150
100
50
0
0 15 30 45 60 75 90 105 120 135 150 165
(b)
DENERVATED
Hypoxanthine µmol mmol creatinine-1
200
150
100
50
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0 15 30 45 60 75 90 105 120 135 150 165
min