The role of 5-hydroxytyptamine$_{1A}$ receptors in the control of cardiorespiratory reflexes.

A thesis submitted for the degree of Doctor of Philosophy to the Faculty of Science at the University of London.

by

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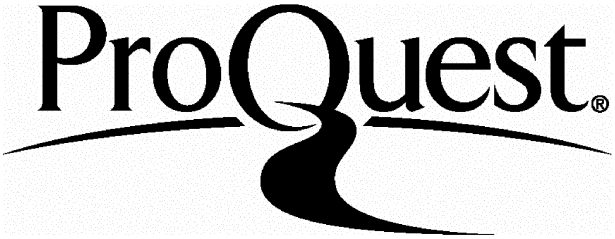
July 1999



Abstract

The work carried out in this thesis examines the modulatory role of central 5-HT_{1A} receptors on the cardiorespiratory responses evoked by stimulating baroreceptor, chemoreceptor and cardiopulmonary receptor afferents. Experiments were performed using spontaneously breathing male rabbits anaesthetised with urethane. The animals were instrumented to allow continuous recordings of arterial blood pressure, heart rate, ECG, renal and phrenic nerve activity. Aortic baroreceptor afferents were activated by electrical stimulation of the left aortic nerve. Carotid chemoreceptor afferents were stimulated by close arterial injection of sodium cyanide into the left lingual artery. Cardiopulmonary afferents were stimulated by bolus injections of phenylbiguanide into the right atrium.

Intracisternal administration of the 5-HT_{1A} receptor partial agonist buspirone had significant effects on baseline cardiorespiratory parameters. In addition, intracisternal administration of buspirone potentiated the reflex vagal bradycardias evoked by stimulating baroreceptor, chemoreceptor and cardiopulmonary afferents. These effects could be attenuated by intravenous pre-treatment with the selective 5-HT_{1A} receptor antagonist WAY-100635. This confirmed that the modulatory effects of buspirone are due to selective agonist effects at 5-HT_{1A} receptors. In most cases, the same dose of buspirone given intravenously caused much smaller potentiating effects, thus suggesting that buspirone was acting at a site within the central nervous system.

Intracisternal administration of WAY-100635 attenuated the reflex bradycardias evoked by stimulating baroreceptor and cardiopulmonary receptor afferents whilst intravenous administration of WAY-100635 had no effect. These results demonstrate that in the urethane-anaesthetised rabbit, central 5-HT_{1A} receptors tonically modulate reflex cardiac vagal outflow.

Experiments in which the dye, Pontamine sky blue, was injected intracisternally suggests that the most likely site of action of the 5-HT_{1A} receptor ligands is at the level of the medulla oblongata.

Acknowledgements

I am indebted to my supervisors Professor David Jordan and Dr Andy Ramage for all the help, advice and encouragement they have given me throughout the course of this study.

I am also grateful to the British Heart Foundation, whose financial support made this research possible.

Finally, I would like to thank my family, friends and colleagues for their continuous support and understanding over the past four years.

Publications

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Abbreviations

ANOVA	analysis of variance
AP-5	2-amino-5-phosphonovalerate
cAMP	cyclic 3',5'-adenosine monophosphate
CNS	central nervous system
CPBG	1-(m-chlorophenyl)-biguanide
CSF	cerebrospinal fluid
CVLM	caudal ventrolateral medulla
CVPN	cardiac vagal preganglionic neurone
DLH	d,l-homocysteic acid
DOB	2,5-dimethoxy-3-bromo-amphetamine
DOI	1-(2,5-dimethoxy-4-iodophenyl)-2 aminopropane
DRG	dorsal respiratory group
DVN	dorsal vagal nucleus
EAA	excitatory amino acid
ECG	electrocardiogram
8-MeO-ClEPAT	8-methoxy-2-(N-2-chloroethyl-N-n-propyl) amino tetralin
8-OH-DPAT	8-hydroxy-2-(di-n-propylamino) tetralin
5-CT	5-carboxamido-tryptamine
5-HT	5-hydroxytryptamine
5-MeODMT	5-methoxy-N,N,dimethyltryptamine
5,7-DHT	5,7-dihydroxytryptamine
GABA	γ-aminobutyric acid
GR-127935	n-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2'-methyl-4'-(5-methyl-1,2,4-oxadiazol-3-yl)[1,1-biphenyl]-4-carboxamide
HRP	horseradish peroxidase
i.c.	intracisternal

i.c.v	intracerebroventricular
IML	intermediolateral cell column
IP_3	inositol triphosphate
IPSP	inhibitory postsynaptic potential
IRNA	integrated renal nerve activity
i.v.	intravenous
LTF	lateral tegmental field
LSD	least significant difference
MABP	mean arterial blood pressure
MDL 72222	3-tropanyl-3,5-dichlorobenzoate
NA	nucleus ambiguus
NaCN	sodium cyanide
NAN-190	1-(2-methoxyphenyl)-4-[4-(2-phthalimido)-butyl]piperazine
NBQX	2,3-dihydroxy-6-nitro-7 sulphamoylbenzo (f)quinoxaline
NMDA	N-methyl-D-aspartate
NTS	nucleus tractus solitarius
PAG	periaqueductal grey matter
PBG	phenylbiguanide
PBR	phrenic burst rate
PDG	phenyldiguanide
RVLM	rostral ventrolateral medulla
RVMM	rostral ventromedial medulla
SPN	sympathetic preganglionic neurone
U-92016A	R-2-cyano-N,N-dipropyl-8-amino-6,7,8,9-tetrahydro-3H-benz[e]indole
VRG	ventral respiratory group
WAY-100135	N-tert-butyl-3-(4-(2-methoxyphenyl)-piperazin 1-yl)-2-phenylpropanamide

WAY-100635	N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]-ethyl]-N-(2-pyridinyl) cyclohexane-carboxamide trihydrochloride
WB4101	2-(2',6'-dimethoxyphenoxyethyl)-aminomethyl-1,4-benzodioxan

1 Introduction

1.1 Discovery of serotonin (5-HT)

Experiments performed as early as 1884 and 1900 provided evidence suggesting the existence of an endogenous vasoconstrictor substance contained in the serum of coagulated blood (Stevens and Lee, 1884, Brodie, 1900). However, it was not until 1948 that this substance was successfully isolated and named serotonin (Rapport et al., 1948). Shortly after its isolation, the active component of serotonin was found to be 5-hydroxytryptamine (5-HT) (Rapport et al., 1949). It was soon realised that this was identical to the substance known as enteramine that had previously been found in enterochromaffin cells (see Page, 1958).

It is now known that the primary site of synthesis of 5-HT is in the enterochromaffin cells which are interspersed with mucosal cells in the stomach and small intestine. These cells contain approximately 90% of the total amount of 5-HT in the body (Rang et al., 1995). From the enterochromaffin cells, 5-HT is secreted into the blood and is rapidly transported into blood platelets where it is stored at high concentrations. As a result, the concentration of 5-HT in the plasma is usually very low (see Martin, 1994). In the event of vascular injury, 5-HT is released from the platelets during aggregation where in conjunction with thromboxane A_2 it causes local vasoconstriction thereby helping to stem the flow of blood (Hindle, 1994).

In 1953, the presence of 5-HT in the human brain was demonstrated suggesting that it may act as a neurotransmitter in the central nervous system (Twarog and Page, 1953). This finding has since been substantiated and although the amount of 5-HT in the brain only accounts for approximately 1% of the total body content, it is thought to play a role in many important physiological processes (see Green, 1985).

1.2 Anatomy of the central serotonergic system

The location of central serotonergic cell bodies and fibres within the mammalian brain have been determined using immunohistochemical techniques (Steinbusch, 1981). 5-HT-containing neurones form a morphologically heterogeneous population of cells which have been subdivided into two divisions: a rostral division, with cell bodies localised in the midbrain and rostral pons which provide ascending projections mainly to the forebrain and a caudal division, located in the medulla oblongata which primarily project to the spinal cord (see Tork, 1990).

The rostral division

The largest group of serotonergic cell bodies are located within the dorsal raphe nucleus which extends from the periventricular grey matter of the rostral pons to the periaqueductal grey matter of the midbrain. Within the rostral division, serotonergic neurones are also found in the median raphe nucleus of the rostral pons and the caudal linear nucleus in the midline of the mesencephalon (see Tork, 1990).

Ascending serotonergic projections from the rostral division are thought to form a dual system (Mulligan and Tork, 1988). Fine varicose axons arise from the dorsal raphe nucleus whereas those arising from the median raphe nucleus are relatively thick and beaded in appearance due to large varicosities (Kosofsky and Molliver, 1987). The latter have been shown to project to and make synaptic contact with neurones in the cortex, septum, hippocampus, pre-optic area and olfactory bulbs (Azmitia and Segal, 1978). The fibres from the dorsal raphe project to the cortex, striatum, thalamus and basal ganglia, however, they appear to make relatively few synaptic connections within these regions (Azmitia and Segal, 1978; Tork, 1990).

The caudal division

Serotonergic cells are located in the raphe magnus nucleus near the junction of the pons and medulla, the raphe pallidus close to the surface of the medulla and the raphe obscurus which runs in the midline of the medulla to the level of the caudal pons (see Tork, 1990). In addition, small clusters of 5-HT-containing cells are present in the nucleus tractus solitarius, area postrema and ventrolateral and ventromedial areas of the rostral medulla (Halliday et al., 1988). Neurones in the caudal division project primarily to the spinal cord (Dahlstrom and Fuxe, 1964), however, along with neurones from the rostral division, they also innervate other areas within the brainstem and also the cerebellum (see Tork, 1990).

1.3 Classification of 5-HT receptors

Gaddum and Picarelli (1957) were the first to suggest that 5-HT could activate different types of receptors. They found that the 5-HT-induced contraction of the guinea pig ileum could be only partially antagonised by either morphine or dibenzyline but could be completely antagonised when the two compounds were given together. Therefore, it was suggested that 5-HT activated two types of receptor, one located at the parasympathetic ganglion causing contraction via acetylcholine release from the postganglionic nerve terminal (the M receptor), the other located on the smooth muscle mediating direct contraction (the D receptor). Although this classification was widely accepted, it soon became clear that some effects evoked by 5-HT, such as vasoconstriction in the canine carotid vascular bed, could not be ascribed to actions at either of the putative receptors (Saxena et al., 1971).

In 1979 Peroutka and Snyder demonstrated, using radioligand binding studies, the existence of two distinct 5-HT receptors in rat brain tissue that they named 5-HT_1 and 5-HT_2. At the 5-HT_1 receptor, 5-HT had high affinity whereas at the 5-HT_2 receptor, spiperone had high affinity and 5-HT had low affinity. Shortly after, further binding affinity studies demonstrated that the 5-HT_1

receptor group could be subdivided into three subtypes, namely the 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1C} receptors (Pedigo et al, 1981., Pazos et al., 1985).

Further investigation showed that the classification systems devised by Gaddum and Peroutka were not compatible, for example, the M receptor was not the same as either the 5-HT_1 or 5-HT_2 receptor. Therefore in 1986, a group of scientists decided to integrate the previous classifications and build upon them taking into account ligand binding affinities of agonists and antagonists and also, to a certain extent, the second messenger systems involved. In this way, 5-HT receptors were classified into three distinct groups; 5-$HT_{1\text{-like}}$ (corresponding to some D receptors and 5-HT_1 binding sites), 5-HT_2 receptors (corresponding to most D receptors and 5-HT_2 binding sites) and 5-HT_3 receptors (corresponding to M receptors) (Bradley et al., 1986).

Since 1986, a large amount of information regarding 5-HT receptors has become available due to advances in molecular biology and the development of more selective 5-HT ligands. Many 5-HT receptor types have been described that fall outside of the classifications proposed by Bradley et al. It therefore became necessary to revise the classification system and in 1994, a group of scientists set about this task. For a 5-HT receptor to be given a particular classification, three fundamental properties now have to be described, these being its drug related-, transductional- and structural-characteristics (Hoyer et al., 1994). Using these criteria, seven distinct groups of 5-HT receptor have now been shown to exist (For reviews, see Hoyer et al., 1994; Martin and Humphrey, 1994; Hoyer & Martin., 1997).

5-HT_1 receptors

This class comprises of five specific subtypes. They all share a common signal transduction mechanism in being negatively coupled to adenylyl cyclase.

5-HT$_{1A}$ receptors

The 5-HT$_{1A}$ receptor subtype is found mainly in the central nervous system (CNS) where it is widely distributed. It is found in high concentrations in areas of the brain concerned with mood and anxiety e.g. the hippocampus, septum and amygdala (Martin and Humphrey, 1994). 5-HT$_{1A}$ receptors have been found to be located presynaptically on serotonergic dorsal raphe neurones (somatodendritic autoreceptors) (Verge et al., 1986). They have also been located postsynaptically to 5-HT nerve terminals on non-serotonergic neurones or glial cells of the hippocampus (Hall et al., 1985). In both cases, receptor activation causes neuronal inhibition (Martin and Humphrey, 1994).

5-HT$_{1B}$ receptors

Initially, it was thought that 5-HT$_{1B}$ receptors were only present in rats and other rodents. It has since been found that the 5-HT$_{1D\beta}$ receptor present in non-rodent species, is in fact structurally homologous to the rodent 5-HT$_{1B}$ receptor (Adham et al., 1992). These two subtypes are therefore simply species variants of the same receptor. The 5-HT$_{1D\beta}$ receptor has therefore now been re-named 5-HT$_{1B}$.

This receptor is found mainly in the CNS but is also present on some peripheral nerves. In the CNS, 5-HT$_{1B}$ receptors are concentrated in the basal ganglia, the striatum and the frontal cortex (Pazos and Palacios, 1985). 5-HT$_{1B}$ receptors are present as terminal autoreceptors controlling 5-HT release in the rat cortex (Engel et al, 1986). However, they are also thought to act as terminal heteroreceptors controlling the release of other neurotransmitters such as acetylcholine and dopamine (Maura and Raiteri, 1986; Galloway et al., 1993).

5-HT$_{1B}$ receptors have also been shown to cause contraction in bovine cerebral arteries (Hamel et al., 1993). Cerebral vessels become dilated during migraine (Villalon et al., 1997a). Many believe that the anti-migraine properties of the drug sumatriptan (a 5-HT$_{1B/1D}$ receptor agonist) are due to its actions on 5-HT$_{1B}$ receptors in the cerebral vasculature.

5-HT_{1D} receptors

The 5-HT_{1D} receptor (previously known as the 5-$HT_{1D\alpha}$ receptor), has been found in the rat brain predominantly in the raphe nuclei, striatum, nucleus accumbens, hippocampus and olfactory tubercle (Hamblin et al., 1992; Bach et al., 1993). 5-HT_{1D} receptor mRNA has been found in human and guinea pig trigeminal ganglia. In the rat, the plasma extravasation evoked by stimulation of this ganglia is inhibited by 5-HT_{1D} receptors (Buzzi et al., 1991, Matsubara et al., 1991). It is possible that the therapeutic effects of the anti-migraine drug sumatriptan may be due to inhibition of trigeminal neurones via activation of 5-HT_{1D} receptors (Moskowitz, 1992).

5-ht_{1E} receptors

The gene encoding this putative receptor has been cloned and sites expressing the mRNA have been located in the cortex of rodent and human brains (Barone et al., 1993, 1994). The lack of any selective ligands for this receptor subtype has however precluded the identification of a functional role for this receptor.

5-ht_{1F} receptors

A fifth 5-HT_1 receptor has been cloned (Adham et al., 1993). Radioligand binding studies have revealed a unique distribution for this receptor. In the CNS, it is found in the pyramidal cells of the cortex, the dorsal raphe and hippocampus (Adham et al., 1993) and in the periphery, it has been located in the mesentery and the uterus (Hoyer et al., 1994). A functional role for this receptor has yet to be established, although it has been suggested that 5-ht_{1F} receptors may be the targets for drugs with anti-migraine properties based on the fact that sumatriptan has almost equal affinity for 5-ht_{1F} receptors as for 5-$HT_{1B/1D}$ receptors (Villalon et al., 1997a).

5-HT$_2$ receptors

The 5-HT$_2$ receptor class consists of three subtypes, all of which are preferentially linked to the phospholipase C pathway. Activation leads to stimulation of phosphotidyl inositol metabolism thus increasing IP_3 production (see Martin and Humphrey, 1994).

5-HT$_{2A}$ receptors

5-HT$_{2A}$ receptors are widely distributed in peripheral tissues and cause contraction of many vascular and non-vascular smooth muscle preparations when activated (see Bradley et al., 1986). In addition, they have also been shown to cause platelet aggregation (De Clerck et al., 1984). Centrally, they are present in highest concentrations in the cortex and to a lesser degree in the hippocampus and caudate nuclei (Hoyer et al., 1986). The precise role of central 5-HT$_{2A}$ receptors is not clear however, in the rat nucleus accumbens, 5-HT$_{2A}$ receptors have been shown to mediate neuronal excitation (North and Uchimura, 1989).

5-HT$_{2B}$ receptors

These receptors were isolated and cloned from the rat stomach fundus where they were found to mediate contraction (Foguet et al., 1992). They were identified subsequently as 5-HT$_{2B}$ receptors (Baxter et al., 1994). 5-HT$_{2B}$ receptor mRNA has been shown to be present in the rat heart, lung and gastro-intestinal tract (Foguet et al., 1992). In the isolated rat jugular vein, activation of 5-HT$_{2B}$ receptors mediates endothelium dependent relaxation (Ellis et al., 1995).

5-HT$_{2C}$ receptors

The 5-HT$_{2C}$ receptor (previously named 5-HT$_{1C}$) has a widespread central distribution but there is no evidence of a receptor role in peripheral tissues. Centrally it occurs at highest concentrations in the choroid plexus epithelial cells

suggesting a role in CSF production (Hartig, 1989). It is also present to a lesser degree in the limbic system, basal ganglia, hypothalamus and hippocampus (Molineaux et al., 1989). Other studies have suggested a role of 5-HT_{2C} receptors in locomotion and feeding (Curzon and Kennet, 1990).

5-HT_3 receptors

5-HT_3 receptors are structurally distinct from other 5-HT receptor subtypes in that they form ligand-gated ion channels (Derkach et al., 1989). They are present on both central and peripheral neurones where receptor activation leads to depolarisation. This is due to the rapid opening of the transmembrane channel causing an increased Na^+/K^+ conductance. This response can rapidly desensitise (Yakel et al., 1991).

In the periphery, these receptors are located exclusively on sensory and enteric neurones and on pre- and postganglionic autonomic neurones (Wallis, 1989). Activation of 5-HT_3 receptors can cause pain and sensitisation of nociceptive neurones (Richardson et al., 1985), affect intestinal tone and induce nausea and vomiting (Costall and Naylor, 1990; Andrews et al., 1988) and can have profound effects on the cardiovascular system (Saxena and Villalon, 1990). 5-HT_3 receptors are also found centrally and are present at highest densities in nuclei of the lower brainstem e.g. the area postrema, nucleus tractus solitarius, the dorsal vagal nuclei and the spinal trigeminal nucleus. In addition, they can also be found in the substantia gelatinosa of the spinal cord (Pratt et al., 1990).

5-HT_4 receptors

Using selective, high-affinity ligands, the distribution of 5-HT_4 receptors has been mapped in the rat and guinea pig brain (Grossman et al., 1993). They are present at high densities in various areas of the limbic system including the olfactory tubercles, nucleus accumbens, corpus striatum, hippocampus and substantia nigra. 5-HT_4 receptors are positively coupled to adenylyl cyclase, the

resulting elevation of cAMP mediating the various cellular responses (see Martin and Humphrey, 1994). In rat hippocampal and mouse colliculi neurones, activation of 5-HT_4 receptors has been shown to increase neuronal excitability via the inhibition of Ca^{2+}-activated- and voltage-gated K^+ channels respectively (Torres et al., 1995; Fagni et al., 1992). In the periphery, 5-HT_4 receptors mediate contraction of gastrointestinal smooth muscle via release of ACh from enteric ganglia (Tonini et al., 1989). 5-HT_4 receptors can also act directly on smooth muscle causing relaxation (Baxter et al., 1991). 5-HT_4 receptors have been located in human right atrium where they mediate an increase in contractile force (Kaumann et al., 1991). It is thought that the activation of these receptors may be involved in facilitating atrial fibrillation (Kaumann, 1994).

5-ht$_5$ receptors

The genes encoding two putative 5-HT receptors have been isolated from a mouse cDNA library and shown to possess 88% structural homology (Matthes et al., 1993). The two receptors are termed 5-ht_{5A} and 5-ht_{5B}. *In situ* hybridisation studies have shown that 5-ht_{5A} receptor mRNA is present in the cerebral cortex, hippocampus, habenula, olfactory bulb and granular layer of the cerebellum (Plassat et al., 1992). Studies using receptor specific antisera suggest that 5-ht_{5A} receptors are predominantly expressed in astrocytes (Carson et al., 1996). The 5-ht_{5B} receptor appears to be more sparsely distributed, it has been found only in the habenula and hippocampus (Matthes et al., 1993). In cells expressing the cloned 5-ht_{5A} receptor, the receptor was found to be negatively linked to adenylyl cyclase (Carson et al., 1996).

5-ht$_6$ receptors

The 5-ht_6 receptor has been cloned from a rat cDNA library (Ruat et al., 1993a). It is positively coupled to adenylyl cyclase via the Gs G protein. Rat and human mRNA has been found in the striatum, amygdala, nucleus accumbens, hippocampus, cortex and olfactory tubercle (Kohen et al., 1996). There are no

selective ligands for this receptor, thus it has been difficult to determine its functional role. However, in a study in which repeated intracerebroventricular (i.c.v.) injections of 5-ht_6 receptor antisense oligonucleotides were given, a behavioural syndrome of yawning, stretching and chewing was evoked (Bourson et al., 1995).

5-HT_7 receptors

The most recently characterised receptor is the 5-HT_7 receptor. The gene has been cloned from cDNA libraries of a number of species including the rat (Ruat et al., 1993b) and the mouse (Plassat et al., 1993). In each case, the receptor has been shown to be positively coupled to adenylyl cyclase.

In situ hybridisation studies reveal expression of 5-HT_7 receptor mRNA in the rat brain. Binding sites were observed in the medial thalamic nuclei, areas of the limbic system, substantia nigra, hypothalamus and dorsal raphe nuclei (Gustafson et al., 1996), The presence of 5-HT_7 receptor mRNA in the suprachiasmatic nucleus has suggested that the receptor may be involved in regulating circadian rhythms (Lovenberg et al., 1993).

The 5-HT_7 receptor has a wide vascular distribution and has been found in rat renal artery, vena cava, portal, femoral and jugular vein (Ullmer et al., 1995). Activation of vascular 5-HT_7 receptors has been shown to cause dilatation (Villalon et al., 1997b).

1.4 Effects of 5-HT on the cardiovascular system

Intravenous (i.v.) injection of 5-HT elicits complex changes in the cardiovascular system (Salmoiraghi et al., 1956). The cardiovascular response can usually be subdivided into three distinct phases (see below), although it can vary depending on the species of animal studied and the dose of 5-HT administered (Saxena and Villalon, 1990). Previous studies suggested that the complex nature of the response is due to the activation of at least three different 5-HT receptor subtypes (Kalkman et al., 1984; Dalton et al., 1986).

With the recent discovery of novel receptor subtypes and specific ligands, these findings have since been extended and it now appears that at least five classes of receptor may mediate the cardiovascular effects of 5-HT (Villalon et al., 1997a).

Phase 1

The initial response to intravenous administration of 5-HT is usually a transient fall in heart rate and a hypotension. This is a reflex response evoked by stimulation of 5-HT_3 receptors on vagal sensory afferent nerve endings located in the lungs and the heart (McQueen, 1990)(see section 1.11). When this reflex is suppressed such as during deep anaesthesia or following vagotomy, 5-HT can actually cause an increase in heart rate by acting directly on cardiac 5-HT receptors. In the pig, the tachycardia is mediated by sinoatrial 5-HT_4 receptors (Kaumann, 1990), whereas in the cat, tachycardia appears to be due to activation of 5-HT_7 receptors (Villalon et al., 1997c). In the rabbit, 5-HT can also cause tachycardia indirectly by acting on 5-HT_3 receptors located on the postganglionic cardiac sympathetic nerves thus causing the release of noradrenaline (Fozard, 1984).

Phase 2

After the initial reflex effects, 5-HT evokes a pressor response. This appears to be due in part to the vasoconstrictor effects of 5-HT_{2A} receptors located on many arteries and veins (Leff and Martin, 1986). In the dog however, the pressor effects of 5-HT appear to be mediated indirectly via the release of catecholamines from the adrenal medulla (Fenuik et al., 1981). This effect is also mediated via 5-HT_{2A} receptors (Martin, 1994).

The pressor response is also thought to be due to activation of central 5-HT_2 receptors which when activated, cause an increase in sympathetic nerve discharge (McCall et al., 1987, Ramage et al., 1993, Ramage and Daly, 1998), possibly via direct effects on the sympathoexcitatory neurones of the rostral

ventrolateral medulla (Vayssettes-Courchay et al., 1991). Activation of central 5-HT_2/5-HT_{1C} receptors, in the subfornical region may also contribute to the pressor response by promoting the release of vasopressin (Anderson et al., 1992; Pergola et al., 1993).

An increase in arterial blood pressure and sympathoexcitation can also be evoked by activation of forebrain 5-HT_{1A} receptors (Deodglu and Fisher, 1991; Anderson et al., 1992). The site at which these effects are mediated is unclear, but it is thought to be located somewhere close to the third ventricle. Possibilities include the anterior hypothalamus/pre-optic area as microinjections of 5-HT and the 5-HT_{1A} receptor agonist 8-OH-DPAT into these areas have been shown to cause similar effects (Smits and Struyker-Boudier, 1976; Szabo et al., 1996).

Phase 3

The intermediate pressor response evoked by 5-HT is usually followed by a more prolonged depressor phase that may be due to actions at a number of peripheral sites. Firstly, 5-HT has been found to inhibit the release of noradrenaline from sympathetic nerve terminals innervating vascular tissues. This is thought to be due to activation of pre-junctional 5-$HT_{1B/1D}$ receptors (Villalon et al., 1994; Molderings et al., 1996).

5-HT can also elicit vasodilatation by stimulating the release of nitric oxide from the endothelium (Cocks and Angus, 1983). The endothelial receptors mediating this response may vary between species. In the guinea pig jugular vein, this effect of 5-HT appears to be due to activation of 5-HT_{1D} receptors (Gupta, 1992), whereas in the same vessels of the rat, the effect is due to 5-HT_{2B} receptors.(Ellis et al., 1995).

Recently, it has been suggested that activation of 5-HT_7 receptors on the vascular smooth muscle of resistance vessels may also mediate vasodilatation, thus contributing to the long lasting depressor response evoked by 5-HT (Terron, 1997; Villalon et al., 1997b).

The depressor response is also due to activation of central 5-HT receptors within the brainstem (Coote et al., 1987). The receptors involved are of the 5-HT_{1A} subtype and are discussed in more detail in the following section.

1.5 5-HT_{1A} receptors and cardiorespiratory control

Effects of 5-HT_{1A} receptor agonists

The effects of 5-HT_{1A} receptors on the cardiovascular system have been most thoroughly investigated using either 8-OH-DPAT or flesinoxan. These two compounds possess high affinity and selectivity for the 5-HT_{1A} receptor subtype. Intravenous administration of 8-OH-DPAT or flesinoxan cause dose-dependent decreases in arterial blood pressure and heart rate in rats (Fozard et al., 1987), cats (Ramage and Fozard, 1987; McCall et al., 1987), rabbits (Hof and Fozard., 1989; Shepheard et al., 1990) and dogs (Di Francesco et al., 1988). The cardiovascular effects of these drugs have been studied due to their possible therapeutic use in the treatment of hypertension.

Activation of central 5-HT_{1A} receptors

The depressor response evoked by 8-OH-DPAT and flesinoxan does not appear to be due to stimulation of cardiovascular afferents. The response persists following bilateral vagotomy which would eliminate the cardiopulmonary afferents (Ramage and Fozard, 1987). In addition, the carotid sinus nerve activity has been found to decrease along with blood pressure in the anaesthetised cat suggesting that baroreceptor afferents are not involved (Ramage and Fozard, 1987; Ramage et al., 1988).

When either 8-OH-DPAT or flesinoxan is administered directly into the central nervous system via the vertebral artery or an intracisternal (i.c.) injection, the hypotensive effects of the drugs are markedly increased (Doods et al., 1988; Wouters et al., 1988). The response to i.v. 8-OH-DPAT has also been blocked by prior i.c. administration of the 5-HT_{1A} receptor antagonist 8-MeO-ClEPAT whereas the same dose of antagonist given i.v. was found to be

ineffective (Fozard et al., 1987). 8-OH-DPAT has no effect on blood pressure or heart rate in pithed rat preparations and does not affect peripheral sympathetic nervous transmission (Fozard et al., 1987). Therefore, taken together, these findings suggest that the prolonged hypotension and bradycardia produced by the 5-HT_{1A} receptor agonists are due to actions in the CNS. The effects are most likely mediated at the level of the brainstem as the response is unaffected following mid-collicular transection (Clement and McCall, 1990).

That the cardiovascular effects of flesinoxan and 8-OH-DPAT are due to activation of 5-HT_{1A} receptors is supported by that fact that many other structurally distinct 5-HT_{1A} receptor agonists produce similar cardiovascular effects, these include buspirone (Romero et al., 1993), urapidil (Ramage, 1991), U-92016A (McCall et al., 1994a) and ipsapirone (Ramage and Fozard, 1987).

Involvement of α_2-adrenoceptors

In the rat, depletion of 5-HT stores using parachlorophenylalanine did not affect the cardiovascular response produced by 8-OH-DPAT suggesting a postsynaptic site of action (Fozard et al., 1987). In the same study, it was suggested that the mechanism of action of 8-OH-DPAT may involve an indirect catecholaminergic component as the effects of 8-OH-DPAT could be reduced by the α_2-adrenoceptor antagonist idazoxan. Similar experiments performed in the cat showed that idazoxan caused only a slight reversal of the hypotension produced by 8-OH-DPAT whereas the hypotension evoked by the α_2-adrenoceptor agonist clonidine was completely reversed (Ramage and Fozard., 1987). This suggests that in the cat, the hypotensive action of 8-OH-DPAT does not involve stimulation of α_2-adrenoceptors.

Effects on the sympathetic nervous system

Ongoing sympathetic nerve activity recorded from thoracic preganglionic, cardiac, splanchnic and renal nerves is dose-dependently reduced following

administration of 8-OH-DPAT (Ramage and Fozard, 1987; Ramage and Wilkinson, 1989). Activity is reduced in both pre- and postganglionic nerves and it has been suggested that the fall in arterial blood pressure evoked by the 5-HT_{1A} receptor agonists is due to a centrally-mediated inhibition of sympathetic nerve activity (McCall and Clement, 1994). In the cat, the cardiac sympathoinhibition evoked by 8-OH-DPAT was significantly attenuated by pre-treatment with the 5-HT_{1A} receptor antagonists spiperone and WAY-100135 (Escandon et al., 1994).

Simultaneous recordings from more than one sympathetic nerve have shown that different sympathetic outflows vary in their sensitivity to the sympathoinhibitory action of 8-OH-DPAT and flesinoxan (Ramage and Wilkinson, 1989). In the cat, the renal nerve activity was found to be far more sensitive to the inhibitory effects of 8-OH-DPAT and flesinoxan compared to the other sympathetic outflows (Ramage et al., 1988, Ramage and Wilkinson, 1989). It was therefore concluded that 5-HT_{1A} receptor activation may be more important in the control of renal sympathetic outflow than other areas (Ramage, 1990).

Effects on heart rate

In the cat, the fall in heart rate evoked by i.v. 8-OH-DPAT can be substantially blocked by atropine methionitrate or bilateral vagotomy indicating that it is primarily due to an increase in vagal tone (Ramage and Fozard, 1987). In anaesthetised rabbits however, the bradycardia was found to be mainly mediated via a reduction in cardiac sympathetic nerve activity (Shepheard et al., 1990).

Effects on respiration

The effects of 5-HT_{1A} receptor agonists on respiratory activity have been less well characterised. In conscious dogs, the hypotensive effects of 8-OH-DPAT are associated with an increase in respiratory rate (Di Francesco et al.,

1988). In anaesthetised cats, i.v. administration of 8-OH-DPAT causes an increase in respiratory frequency and a fall in tidal volume (Gillis et al., 1989). A similar increase in respiratory frequency was observed in cats in which phrenic nerve activity was measured as an indication of central respiratory drive (Lalley et al., 1994). This effect was however only observed following low doses of 8-OH-DPAT, higher doses causing phrenic nerve depression or silencing (Lalley et al., 1994). In artificially ventilated rabbits anaesthetised with urethane or pentobarbitone, i.v. 8-OH-DPAT had no effect on phrenic burst rate, however under pentobarbitone anaesthesia, 8-OH-DPAT caused an increase in the number of action potentials in individual phrenic bursts (Shepheard et al., 1990).

1.6 Sympathetic control of the cardiovascular system

Sympathetic preganglionic neurones

The motor outflow of the sympathetic nervous system originates from the sympathetic preganglionic neurones (SPNs) located in the thoracic and upper lumber segments of the spinal cord. From here, SPNs project via the ventral roots to a number of topographically arranged sympathetic ganglia or to the adrenal chromaffin cells. Within the ganglia, they make synaptic contact with sympathetic postganglionic neurones that supply various organs including the heart and blood vessels (see Coote, 1988; Loewy, 1990).

SPNs are found in four main areas within the spinal cord. The majority are located in the intermediolateral cell column (IML) in the lateral horn of the grey matter, however, SPNs are also found in the lateral funiculus. In addition, groups of SPNs exist in an area between the IML and the central canal, (the intercalated cell group) and also dorsolateral to the central canal, (the central autonomic nucleus)(see Cabot, 1990).

The ongoing activity of an individual SPN is dependent on the inputs that it receives from supraspinal neural structures and from spinal afferents arising from skin, skeletal muscle and viscera (see Dampney, 1994). SPNs receive

both excitatory and inhibitory inputs from afferent projections, however, the ongoing activity in SPNs has been found to be mainly dependent on excitatory inputs (Dembowsky et al, 1985). The predominant excitatory drive to SPNs, especially those with a vasomotor function, is from supraspinal neural structures. In animals spinally transected at the cervical level, both resting sympathetic nerve activity and arterial blood pressure are markedly reduced (Alexander, 1946). However, even after loss of supraspinal inputs, many sympathetic preganglionic neurones maintain their tonic activity and blood pressure gradually normalises suggesting that inputs from spinal afferents and intrinsic spinal circuits are capable of exerting some compensatory control of sympathetic outflow (Weaver and Polosa, 1997).

A variety of neurochemicals have been identified within terminals making synaptic contact with SPNs. These include monoamines such as adrenaline, noradrenaline and 5-HT; neuropeptides such as substance P, vasopressin, oxytocin and neuropeptide Y and amino acids including glycine, glutamate and GABA (see Coote, 1988). Most of these substances have been shown to influence the excitability of SPNs when applied directly onto the neurones suggesting that they may act tonically to control the level of excitability of SPNs (see Coote, 1988).

A recent study has shown that either GABA or glutamate is present in virtually all inputs to SPNs that project to the adrenal medulla and superior cervical ganglia (Llewellyn-Smith et al., 1998). It has therefore been hypothesised that all nerve fibres innervating SPNs contain either GABA or glutamate and that fast synaptic transmission is mediated predominantly by amino acids. The variety of other neurochemicals also located in the IML are presumably co-localised with GABA or glutamate and may serve to modulate the effects of the amino acids (Llewellyn-Smith et al., 1998).

5-HT_{1A} receptors in the spinal cord

The IML of the spinal cord receives a dense input from 5-HT-containing neurones of the medullary raphe and brainstem reticular formation (Dahlstrom and Fuxe, 1965; Loewy, 1981; Loewy and McKeller, 1981). Administration of 5-HT receptor antagonists in intact but not in spinal animals reduces the spontaneous firing of SPNs suggesting that descending serotonergic inputs provide a tonic excitation (McCall, 1983). In agreement with this, iontophoretically applied 5-HT has been found to excite the majority of SPNs (DeGroat and Ryall 1967; Coote et al, 1981; McCall, 1983). This excitatory effect is thought to be mediated by 5-HT_2 receptors (Lewis et al., 1993) which have been located in the IML of the spinal cord (Dashwood et al 1988a; Thor et al., 1993). 5-HT can also have inhibitory effects on SPNs which appear to be due to actions on glyginergic inhibitory interneurones since the IPSPs evoked can be blocked by application of strychnine (Lewis et al., 1993).

5-HT_{1A} receptors are also found in the IML although they occur at much lower densities than 5-HT_2 receptors (Dashwood et al., 1988a). Iontophoretic application of the 5-HT_{1A} receptor agonist 8-OH-DPAT failed to affect the firing rate of SPNs (Clement and McCall, 1990). Also, intrathecal administration of 8-OH-DPAT failed to cause any changes in blood pressure or renal sympathetic nerve activity (Yusof and Coote, 1988). Therefore, spinal 5-HT_{1A} receptors probably play a minor role in the control of SPNs and the sympathoinhibitory response evoked by intravenous administration of 8-OH-DPAT is unlikely to be due to actions within the spinal cord.

Sympathetic premotor neurones

The supraspinal cell groups that provide synaptic inputs to SPNs have been termed 'sympathetic premotor neurones' (see Dampney, 1994). Trans-synaptic retrograde labelling studies using the pseudorabies virus have revealed that five main groups of premotor neurones innervate the SPNs that project to the adrenal medulla and the major sympathetic ganglia. These are

located in the medullary raphe nuclei, the rostral ventrolateral medulla, the rostral ventromedial medulla, the A5 noradrenergic cell group and the paraventricular hypothalamic nucleus (Strack et al., 1989).

The medullary raphe nuclei

The medullary raphe nuclei (raphe pallidus, obscurus and magnus) are located in the midline of the medulla and contain many neurones that project directly to the IML of the spinal cord (Loewy, 1981). The role of the medullary raphe nuclei in the control of the cardiovascular system is unclear as electrical or chemical stimulation within various areas of nuclei produces either excitation of sympathetic nerve activity and pressor responses (McCall, 1984; Pilowsky et al., 1986; Dreteler et al., 1991) or inhibition of sympathetic nerve activity and depressor responses (Gilbey et al., 1981; McCall, 1984; McCall and Humphrey, 1985; Coleman and Dampney, 1995). Furthermore, stimulation at sites within the raphe nuclei have been shown to cause differential effects on sympathetic activity. For example, stimulation in various areas of the raphe obscurus causes an inhibition of renal and cardiac sympathetic nerve activities with a concomitant increase in the activity of sympathetic vasoconstrictor fibres to skeletal muscle (Futuro-Neto and Coote, 1982).

Two distinct populations of neurones have been identified within the raphe with activity temporally related to sympathetic nerve discharge (Morrison and Gebber, 1984). While the transmitter content of these neurones is not known, the majority are excited by baroreceptor stimulation and thus are presumably sympathoinhibitory in function. Approximately one third of these neurones terminate in the IML where they may mediate spinal sympathoinhibition (Morrison and Gebber, 1984, 1985). The remaining sympathoinhibitory neurones that are not antidromically activated from the IML may mediate sympathoinhibition at supraspinal levels. In this respect, stimulation of depressor sites within the raphe has been shown to inhibit the discharge of sympathoexcitatory neurones in the rostral ventrolateral medulla (RVLM)

(McCall, 1988). This is thought to be due to an inhibitory GABAergic pathway from the raphe to the RVLM as the effect could be blocked by microiontophoretic application of bicuculline onto the sympathoexcitatory neurones of the RVLM (McCall, 1988). The paucity of GABAergic cell bodies in the medullary raphe nuclei (Ruggiero et al., 1985) and the inability to antidromically activate any medullary raphe neurones by stimulation in the RVLM (McCall, 1988) suggests this pathway is probably multisynaptic possibly involving neurones of the caudal ventrolateral medulla (Coleman and Dampney, 1998). A smaller group of neurones in the medullary raphe nuclei are inhibited by baroreceptor activation and are therefore assumed to be sympathoexcitatory (Morrison and Gebber, 1984). These neurones could be antidromically activated from the dorsolateral funiculus but not the IML. Therefore, whether they serve a true sympathoexcitatory function is questionable. Medullary raphe neurones do not appear to be critical in the control of the baroreceptor reflex as it is unaffected by midline medullary lesions (McCall and Harris, 1987). The presence of both sympathoexcitatory and sympathoinhibitory neurones within the nuclei may however provide an explanation for the variable cardiovascular responses obtained by chemical and electrical stimulation.

The medullary raphe nuclei also contain a population of IML-projecting neurones that are distinct from the two populations described by Gebber and colleagues. They display regular firing patterns unrelated to sympathetic nerve discharge and are not affected by baroreceptor inputs (McCall and Clement, 1989). It has been suggested that these medullary raphe neurones may be serotonergic, based on their electrophysiological and pharmacological similarities to dorsal raphe 5-HT-containing neurones and that they provide tonic excitatory inputs to SPNs in the spinal cord (McCall, 1984; McCall and Clement, 1989).

5-HT_{1A} receptors in the medullary raphe

Anatomical studies have confirmed the existence of spinally-projecting serotonergic neurones in the nucleus raphe magnus, pallidus and obscurus (Loewy and McKellar, 1981). A high density of 5-HT_{1A} receptors have also been located within the raphe (Thor et al., 1992a). Receptor binding is dramatically reduced following pre-treatment with the serotonergic neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) suggesting that these receptors are in fact autoreceptors located presynaptically on serotonergic neurones. Activation of central 5-HT_{1A} receptors is known to cause an inhibition of neuronal activity (Colino and Halliwell, 1987; Sprouse and Aghajanian, 1987) and in the serotonergic cells of the dorsal raphe this is thought to be mediated by activation of somatodendritic autoreceptors (Verge et al., 1985).

Microinjection of 8-OH-DPAT into the raphe pallidus/obscurus evokes a hypotension and sympathoinhibition (Nosjean and Guyenet, 1991; Helke et al, 1993). Taking the previous findings into consideration it was suggested that the effects of 8-OH-DPAT may be due to an inhibition of firing of sympathoexcitatory serotonergic neurones within the raphe via activation of the inhibitory 5-HT_{1A} autoreceptors, i.e. disfacilitation (McCall and Clement, 1994). This may explain the well-characterised hypotensive response evoked by intravenous administration of 5-HT_{1A} agonists such as flesinoxan and 8-OH-DPAT. Putative serotonergic raphe neurones antidromically activated from the spinal cord are indeed inhibited by iontophoretic application or intravenous administration of 5-HT or 8-OH-DPAT (McCall and Clement. 1989). However, low doses of 8-OH-DPAT completely inhibited the activity of these medullary 5-HT neurones whilst at the same time had little effect on the activity of cardiac sympathetic nerve activity (McCall et al., 1989). A better correlation was observed when the effects of 8-OH-DPAT on medullary serotonergic neurones and renal sympathetic nerve activity were compared. However, even then, total inhibition of the neurones occurred, whilst the renal nerve activity was reduced by only 80% (Ramage et al., 1992). In addition, in animals in which the majority

of medullospinal serotonergic neurones in the raphe obscurus, pallidus and magnus were destroyed by electrolytic lesions, the sympathoinhibitory effects of intravenous 8-OH-DPAT were not significantly affected, suggesting that the effects of 8-OH-DPAT must be due, at least in part, to actions at other central sympathetic neurones (McCall et al., 1989).

The rostral ventromedial medulla

The rostral ventromedial medulla (RVMM) is situated just lateral to the pyramidal tracts in the medulla and is also known as the parapyramidal region or nucleus interfascicularis hypoglossi. Neurones within the RVMM have been described as sympathetic premotor neurones although very little is known about their actual involvement in sympathetic control. Functional studies have produced conflicting results as microinjections of glutamate into this region have caused both pressor (Minson et al., 1987) and depressor responses (Aicher et al., 1994).

5-HT_{1A} receptors in the RVMM

The RVMM contains a high concentration of serotonergic neurones (Loewy and McKeller, 1981). Autoradiographic studies have revealed that high densities of 5-HT_{1A} receptors occur within this region and that 5-HT_{1A} receptor binding is dramatically reduced following pre-treatment with the serotonergic neurotoxin 5,7-DHT (Thor et al., 1992a). This suggests that within the RVMM, most of the 5-HT_{1A} receptors also occur as autoreceptors on serotonergic neurones.

Microinjection of 8-OH-DPAT into the RVMM causes a marked fall in blood pressure (Helke et al., 1993). Assuming that the RVMM provides excitatory inputs to the SPNs, the effects of i.v. 8-OH-DPAT may be due to inhibition of the RVMM neurones via activation of the 5-HT_{1A} autoreceptor.

The rostral ventrolateral medulla

The term rostral ventrolateral medulla (RVLM) describes an area of the medullary reticular formation situated rostral to the lateral reticular nucleus and caudal to the facial nucleus. It extends dorsally from the ventral surface of the medulla to the rostral part of the nucleus ambiguus. (Guyenet, 1990).

Electrical or chemical lesions within the RVLM produce marked falls in blood pressure similar to those observed following spinal transection (Guertzenstein and Silver, 1974; Feldberg, 1976), suggesting that this area plays an important role in the control of resting vasomotor tone. Microinjections of the EAA glutamate into discrete areas of the RVLM have also been shown to evoke large pressor responses due to activation of sympathetic vasoconstrictor fibres in various vascular beds (Bachelard et al., 1990; Dampney and McAllen, 1988).

The RVLM is functionally and anatomically heterogeneous containing a variety of neuronal groups that are not rigidly defined by clear cytoarchitectonic borders. These include the C1 catecholaminergic cell group, respiratory neurones of the Botzinger complex (see section 1.8) and respiratory motoneurones of the rostral nucleus ambiguus (Lipski et al., 1996). The RVLM has also been shown to contain a group of neurones that can be antidromically activated by electrical stimulation within the IML (Brown and Guyenet, 1985; Barman and Gebber, 1985; Terui et al., 1986). They are located in a relatively compact ventromedial region of the RVLM (Kanjhan et al., 1995) which, in the cat, is often referred to as the subretrofacial nucleus (Dampney and McAllen, 1988).

Single-unit recordings reveal that the ongoing discharge of the IML-projecting RVLM neurones is highly pulse-synchronous due to a powerful inhibitory input from arterial baroreceptors suggesting that the neurones are sympathoexcitatory in function (Sun and Spyer, 1991). Ongoing discharge is also modulated by respiratory activity. Some neurones show peak activity during inspiration while in others, the activity is markedly depressed during the

inspiratory period (Haselton and Guyenet, 1989a). The resulting pattern of neuronal discharge is highly correlated with lumber sympathetic nerve activity (Sun and Guyenet, 1986).

Two classes of sympathoexcitatory neurones have been identified in the RVLM. One group have relatively fast-conducting axons, a high discharge rate and have been shown *in vivo* to exhibit intrinsic pacemaker properties, generating regular spontaneous activity in the absence of synaptic inputs (Sun et al., 1988a). The second group do not possess pacemaker properties, have slower conduction velocities and generally discharge at a lower rate (Guyenet, 1990).

Combined electrophysiological and anatomical studies have shown that there is considerable overlap between the areas of the RVLM containing C1 adrenergic neurones and those containing sympathoexcitatory RVLM neurones (Kanjhan et al., 1995). This has led to the suggestion that sympathoexcitatory neurones may possess an adrenergic phenotype. Further studies have shown that whilst the majority of neurones with pacemaker activity are non-catecholaminergic (Sun et al., 1988b), some non-pacemaker sympathoexcitatory neurones are indeed immunoreactive for catecholamine-synthesizing enzymes and therefore appear to be C1 cells (Haselton and Guyenet, 1989b). It is unlikely however that these sympathoexcitatory neurones utilise adrenaline as the primary neurotransmitter at the level of the spinal cord as it has been found to have predominantly inhibitory effects on SPNs (Coote, 1988).

Discrete microinjections of EAAs into the sympathoexcitatory area of the RVLM have revealed that distinct but overlapping regions exist that exert preferential effects on the blood flow to individual vascular beds. For example, in the cat, neuronal pools controlling the renal vasculature are located more rostrally than those involved in the control of hindlimb and mesenteric vascular beds (Lovick, 1987). In a similar study, discrete microinjections of glutamate into medial areas of the RVLM preferentially excited cutaneous vasoconstrictor

fibres, whereas stimulation of more lateral regions had greater effects on vasoconstrictor fibres supplying skeletal muscle (Dampney and McAllen, 1988). From these studies and others, it appears that sympathoexcitatory neurones of the RVLM are arranged topographically depending on the vascular bed they are controlling.

It is now accepted that ongoing activity of sympathoexcitatory neurones of the RVLM contribute significantly to the maintenance of resting vasomotor tone, however, the specific mechanism by which tonic activity in the RVLM is generated remains unknown. One theory is that the neurones within the RVLM are chemosensitive and tonically excited at normal blood pH, pCO_2 and pO_2 values (Dampney and Moon. 1980). Another group has suggested that the RVLM pacemaker neurones may be responsible for generating basal activity (Guyenet, 1990). Finally, it has also been proposed that basal sympathetic activity may not be generated within the RVLM itself, rather it arises from a group or groups of neurones known as the network oscillator and is then relayed to the RVLM premotor neurones (Barman and Gebber, 1989).

5-HT_{1A} receptors in the RVLM

Immunohistochemical studies have shown that the RVLM is rich in 5-HT-immunoreactive terminals and some serotonergic varicosities have been observed in contiguity with bulbospinal C1 neurones (Steinbusch, 1981; Nicholas and Hancock; 1988). Although the sources of the serotonergic input have not been clearly defined, they are thought to include the medullary raphe nuclei and the serotonergic neurones of the RVMM (Nicholas and Hancock; 1988). The RVLM has also been shown to contain a small number of 5-HT-immunoreactive cell bodies (Steinbusch, 1981). Autoradiographic studies reveal that a high density of 5-HT_{1A} receptor binding is found within the RVLM particularly in regions containing C1 catecholaminergic neurones (Thor et al., 1992a).

In the rat, bilateral microinjection of 8-OH-DPAT into the rostral ventrolateral medulla causes a marked decrease in blood pressure and heart rate and an increase in hindlimb conductance (Lovick, 1989). In the dog, a similar depressor response is obtained, associated with a decrease in renal sympathetic nerve activity (Laubie et al., 1989). In the latter study, the effects of 8-OH-DPAT were prevented by prior microinjection of the non-selective 5-HT antagonist methiothepin.

The above studies suggest that the rostral ventrolateral medulla may be necessary in mediating the sympathoinhibitory effects of i.v. 8-OH-DPAT. In agreement with this, intravenous administration of 8-OH-DPAT in the cat has been shown to cause a dose-dependent decrease in the firing rate of sympathoexcitatory neurones in the RVLM (Clement and McCall, 1990). In this study, there was a very close relationship between the inhibition of RVLM neurones and the sympathoinhibitory effects on the inferior cardiac nerve. Similar findings have also been observed in the rat, the inhibition of RVLM neurone firing being similar to the inhibition of the lumber sympathetic nerve (Nosjean and Guyenet, 1991). The RVLM receives inputs from other brain areas, therefore it is possible that 8-OH-DPAT may be acting indirectly to inhibit neuronal activity. In the case of the rat, this seems unlikely however, as microiontophoresis of spiperone onto sympathoexcitatory RVLM neurones reverses the inhibitory effect of i.v. 8-OH-DPAT (Kubo et al., 1995). Therefore, the hypotensive effects of intravenous 8-OH-DPAT seem to be due at least in part to a direct inhibition of RVLM sympathoexcitatory neurones. In this respect, 8-OH-DPAT and other 5-HT_{1A} receptor agonists including flesinoxan and buspirone, have all been shown to inhibit the spontaneous and amino acid-evoked activity of most neurones in the RVLM of the rat when applied iontophoretically. In many cases, these effects could be blocked by administration of spiperone. Some of the neurones tested were barosensitive sympathoexcitatory neurones.(Wang and Lovick, 1992).

In the cat however, iontophoretic application of 8-OH-DPAT had no effect on RVLM neurones although i.v. administration of 8-OH-DPAT was able to inhibit the same neurones (Clement and McCall, 1990). It was therefore suggested that in this species, 8-OH-DPAT may either act on distal dendrites of the RVLM neurones, or it may be acting on central sympathetic neurones that lie antecedent to the RVLM.

The lateral tegmental field

One such possible area is the lateral tegmental field (LTF). This region has been implicated in the generation of sympathetic tone and contains both sympathoinhibitory and sympathoexcitatory neurones (Gebber and Barman, 1985). These neurones are not spinally projecting but are thought to project to the medullary raphe nuclei and RVLM respectively (Gebber, 1990). It has been suggested that the sympathoexcitatory LTF neurones provide a tonic excitatory input to sympathetic pre-motor neurones of the RVLM (Gebber and Barman, 1985).

5-HT_{1A} receptors in the LTF

I.v. administration of 8-OH-DPAT produces a dose dependent inhibition of sympathoexcitatory neurones in the LTF which correlates highly with the inhibition of the inferior cardiac nerve and renal nerve activity (Clement and McCall, 1992; Vayssettes-Courchay, 1993a). Bilateral microinjection of 8-OH-DPAT into the LTF also caused a decrease in heart rate, blood pressure and renal sympathetic nerve activity. Direct application of 5-HT or 8-OH-DPAT by iontophoresis inhibited the spontaneous discharge of the majority of sympathoexcitatory neurones thus suggesting a role for these neurones in the sympatholytic actions of 8-OH-DPAT (Clement and McCall, 1992). It was suggested that if 5-HT_{1A} receptors on these neurones are present on the terminals of axons projecting to the RVLM it may account for the hypotension produced by microinjection of 8-OH-DPAT into the RVLM.

Chemical lesions in the LTF using the neurotoxin kainic acid have been shown to abolish the hypotensive, bradycardic and sympathoinhibitory response to 8-OH-DPAT (Vayssettes-Courchay et al., 1993a; Clement and McCall, 1993). However, it has been suggested that the lesions produced using this method maybe more extensive than first thought and are unlikely to be restricted to the site at which the agent is microinjected. Studies in which the neurotransmission in the LTF was blocked by bilateral microinjection of muscimol showed that the sympatholytic response to 8-OH-DPAT was still abolished confirming a role for the lateral tegmental field (Vayssettes-Courchay et al., 1995). It should however be noted that the resting sympathetic tone was significantly decreased following treatment with muscimol.

1.7 Parasympathetic control of the heart

Location of cardiac vagal preganglionic neurones

The location of cardiac vagal preganglionic neurones (CVPNs) has been investigated in a number of studies using the neuroanatomical tracer horseradish peroxidase (HRP). Studies in the rat and cat, in which HRP was injected into the cardiac branch of the vagus nerve or directly into the myocardium, have revealed that the majority of vagal preganglionic neurones that innervate the heart originate from the nucleus ambiguus, with smaller populations located in the dorsal vagal nucleus and in an intermediate zone in between these two nuclei (Nosaka et al., 1979a; Geis et al., 1981; Stuesse, 1982; Izzo et al., 1993).

The nucleus ambiguus

The nucleus ambiguus (NA) is situated in the ventrolateral portion of the medullary reticular formation and has been shown to comprise of two main longitudinal regions; the dorsal and the ventral divisions (see Hopkins et al., 1996). The dorsal or principal division, which can be further subdivided into the compact, semicompact and loose formations, has been shown to contain the

special visceral efferent motoneurones that supply the musculature of the oesophagus, pharynx, larynx and soft palate (Lawn, 1966; Bieger and Hopkins, 1987). The cardiac vagal preganglionic neurones are located in the ventral division of the NA, which is also known as the external formation (Bieger and Hopkins, 1987; Nosaka et al., 1979a). This region also contains preganglionic neurones that project to the lungs and subdiaphragmatic viscera via the vagal, glossopharyngeal and laryngeal nerves (Hopkins et al., 1996).

The dorsal vagal nucleus

The dorsal vagal nucleus (DVN) is located dorsomedially in the caudal medulla and is the main source of vagal preganglionic neurones that project to the gastrointestinal system. It also contains neurones that innervate cervical and thoracic viscera (Kalia, 1981). Studies suggest that the CVPNs within this nucleus are localised within lateral areas (Nosaka et al., 1979a; Izzo et al., 1993).

Electrophysiological studies

The location of CVPNs within the brainstem has also been investigated using electrophysiological techniques (McAllen and Spyer, 1976; Nosaka et al., 1982). By stimulating the cardiac branch of the vagus nerve and recording the evoked antidromic potentials of medullary neurones, these studies have also found CVPNs to be located within the NA, the DVN and in an intermediate zone in between these nuclei. By measuring the antidromic latencies and estimating the axonal conduction velocities, it appears that two main types of CVPN project from these nuclei, some with small myelinated axons (B-fibres) and others with unmyelinated axons (C-fibres) (Izzo and Spyer, 1997).

CVPNs with B-fibre axons

Antidromically identified CVPNs with B-fibre axons have been studied most extensively in the cat. In this anaesthetised preparation, the majority of

these neurones are silent, however those that display spontaneous activity have been shown to fire with both pulse- and respiratory-related rhythms (McAllen and Spyer, 1978a; Gilbey et al., 1984). If silent CVPNs are induced to fire by iontophoretic application of DLH, similar patterns of ongoing discharge are observed often in association with small decreases in heart rate thus confirming the neurones are cardioinhibitory in function (McAllen and Spyer, 1978a).

The pulse-related discharge of the CVPNs with B-fibre axons has been shown to be due to excitatory inputs from arterial baroreceptors since it was reversibly abolished by carotid occlusion in cats in which the aortic nerves were sectioned. In addition, the CVPNs were excited by electrical or natural stimulation of baroreceptor afferents (McAllen and Spyer, 1978b). The inputs from the baroreceptor afferents are thought to be relayed to the CVPNs by both direct and indirect pathways from the nucleus tractus solitarius (NTS) (McAllen and Spyer, 1978b). Consequently, the CVPNs are maximally active in phase with the systolic rise in blood pressure.

Respiratory modulation results in the neurones being active during expiration but inhibited throughout inspiration. Intracellular recordings of CVPNs in the cat show that the cell membrane is depolarised during post-inspiration, has a variable level of excitation during expiration and is hyperpolarised throughout inspiration (Gilbey et al., 1984). The hyperpolarisation of these neurones during inspiration is great enough to abolish the excitatory effects produced by the baroreceptor inputs (Gilbey et al., 1984). The origin of this respiratory modulation remains uncertain although it probably involves a cholinergic muscarinic action as iontophoresis of atropine blocks the inspiratory related inhibition (Gilbey et al., 1984). This inhibitory input may arise from neighbouring inspiratory neurones within the NA. Excitatory inputs may also be received from post-inspiratory neurones which show a similar firing pattern to CVPNs. Alternatively, both CVPNs and post-inspiratory neurones may share a tonic excitation (see Spyer, 1996).

Iontophoretic application of bicuculline has been shown to increase the discharge rate of CVPNs in the NA, suggesting that they receive a tonic GABAergic inhibitory input (Gilbey et al., 1985). This input does not however appear to be involved in the respiratory-related modulation of their activity (Jordan and Spyer, 1987).

In the cat and rat, CVPNs with B-fibre axons have only been found in the nucleus ambiguus (McAllen and Spyer, 1976; Nosaka et al., 1982). In these species, CVPNs found in the dorsal vagal nucleus have C-fibre axons (see below). In contrast to the rat and cat, electrophysiological studies in the rabbit have shown that presumed CVPNs with B-fibre axons are located in both the NA and the DVN (Jordan et al., 1982; Ellenberger et al., 1983). In both these studies, the identified neurones were antidromically activated by stimulation of the cervical vagus nerve. Despite the fact that the neurones were not shown to project to the cardiac branch of the vagus, they displayed properties similar to CVPNs with B-fibre axons previously described in the cat, including a pulse- and respiratory-related modulation in their ongoing activity, and an excitatory input from baroreceptor afferents. In addition, iontophoretic application of DLH caused excitation of the neurones and a concomitant bradycardia (Jordan et al., 1982). Thus it appears that in rabbits a species difference exists regarding the brainstem location of CVPNs with B-fibre axons.

CVPNs with C-fibre axons

In the cat, rat and rabbit CVPNs with C-fibre axons are located predominantly in the DVN (McAllen and Spyer, 1976; Nosaka et al., 1982; Jordan et al., 1982). In contrast to B-fibres, the spontaneous or DLH-induced activity of these neurones is not modulated by inputs from baroreceptors and does not exhibit any respiratory-related modulation (Ford et al., 1990; Jones et al., 1998). Another characteristic of C-fibres within the DVN is that they receive a powerful inhibitory input from vagal afferents which can be reduced by the

GABA receptor antagonist bicuculline (Wang et al., 1995a; Jordan and Wang, 1997).

It was previously suggested that CVPNs with B-fibre axons were solely responsible for the negative chronotropic effects of vagus nerve activation (Geis and Wurster, 1980; Middleton et al., 1950) and that the C-fibre population may have inotropic effects or be involved in controlling coronary blood flow (Feigl, 1969; Geis and Wurster, 1980). Subsequent studies have shown however, that selective stimulation of CVPNs with C-fibre axons can also evoke a bradycardia (Nosaka et al., 1979b; Wooley et al., 1987; Jones et al., 1995). In the rat, rabbit and cat, the bradycardia produced by selective C-fibre stimulation is smaller in magnitude and less rapid in onset than that produced by B-fibre stimulation and it has been suggested that differences may exist between the ganglionic neurotransmission of the B- and C-fibre responses (Jones et al., 1995).

5-HT_{1A} receptors in the nucleus ambiguus and dorsal vagal nucleus

Anatomical studies have shown that both the NA and DVN contain nerve terminals immunoreactive for 5-HT (Steinbusch, 1981). More specifically, 5-HT-immunoreactive boutons have been shown to make synaptic contact with retrogradely labelled CVPNs in the NA suggesting that serotonergic neurones may be involved in regulating the parasympathetic supply to the heart (Izzo et al., 1993). In this respect, intracerebroventricular administration of 5-HT evokes a bradycardia which is due in part to an increase in vagal tone (Dalton, 1986). The origin of the serotonergic inputs to the CVPNs has not been clearly defined by anatomical studies. The medullary raphe nuclei are obvious candidates and stimulation the raphe obscurus has been shown to increase the extracellular levels of 5-HT in the DVN (Brodin et al., 1990).

Intravenous administration of 5-HT_{1A} receptor agonists also evoke a bradycardia. This is due in part to the well documented sympathoinhibition, but is also due to an increase in vagal activity to the heart (Gradin et al., 1985a, b; Ramage and Fozard, 1987; Ramage et al., 1988). This specifically implicates 5-

HT_{1A} receptors in mediating the excitatory effects of 5-HT on cardiac vagal drive. In support of this, 5-HT_{1A} receptor binding sites have been located in both the NA and the DVN (Pazos and Palacios, 1985; Dashwood et al., 1988b; Manaker and Verderame, 1990). In the anaesthetised cat, microinjections of 5-HT or 8-OH-DPAT into the NA evoked a vagal bradycardia (Izzo et al., 1988). Similar effects were produced in the rat when either 5-HT or 8-OH-DPAT were microinjected into the DVN (Sporton et al., 1991). In the latter study, microinjections of the 5-HT_2 or 5-HT_3 receptor agonists DOI and PBG had no effect on cardiovascular variables.

Iontophoretic application of 5-HT onto neurones within the DVN can evoke both excitatory and inhibitory responses (Wang et al., 1995b). The excitatory responses appear to be due in part to the activation of 5-HT_3 receptors since the responses can be partially antagonised by the 5-HT_3 receptor antagonist granisetron (Wang et al., 1996). *In vitro* studies also suggest that the excitatory effects of 5-HT may involve activation of postsynaptic 5-HT_{2A} receptors (Albert et al., 1996). However, the excitatory effects of 5-HT also appear to be due to the activation of 5-HT_{1A} receptors as the response is attenuated by the 5-HT_{1A} receptor antagonists WAY-100635 or pindolol (Wang et al., 1995b). The location of the 5-HT_{1A} receptors is not known, however the fact that activation of this receptor subtype in the raphe and hippocampus results in neuronal inhibition suggests that the effects on CVPNs may be mediated indirectly via inhibitory interneurones (Ramage, 1990).

1.8 Respiratory control

The respiratory cycle

The normal respiratory cycle consists of three distinct phases, inspiration, post-inspiration and expiration. These phases are evident in the motor outflows to inspiratory or expiratory muscles (Richter, 1996). The motor innervation to the diaphragm is supplied by the phrenic nerves, the activity of which is often measured to monitor the central respiratory cycle. Phrenic nerve activity

increases throughout inspiration, decreases during post-inspiration and is absent during expiration.

Central respiratory neurones have been recorded intracellularly and classified by their discharge profiles in relation to phrenic nerve activity. In this way, six types of respiratory neurones that display different activity during the three-phase respiratory cycle have been identified *in vivo*: pre-inspiratory, early-inspiratory, throughout-inspiratory, late-inspiratory, post-inspiratory and expiratory neurones (see Richter, 1982, 1996; Bianchi et al., 1995).

Location of respiratory neurones

Two distinct groups of respiratory neurones are located in the medulla, namely the dorsal respiratory group (DRG) and the ventral respiratory group (VRG). The DRG is situated within the ventrolateral nucleus of the solitary tract and consists largely of inspiratory neurones, which in the cat, have been shown to project to the spinal cord (Ezure, 1990). The VRG exists as a longitudinal column of cells in the vicinity of the nucleus ambiguus extending rostrally from retroambigual to retrofacial levels (Ezure, 1990; Richter and Spyer, 1990). Within the VRG, bulbospinal inspiratory neurones are concentrated in intermediate areas just rostral to the obex. Bulbospinal expiratory neurones are found in caudal retroambigual areas and are also clustered at the rostral end of the VRG forming the Botzinger complex (Ezure, 1990). Expiratory neurones in the Botzinger complex have been shown to project to and inhibit several subpopulations of inspiratory neurones in both the DRG and VRG during expiration (Merrill et al., 1983; Jiang and Lipski, 1990). Some bulbospinal Botzinger neurones are known to project to cervical regions of the spinal cord where they monosynaptically inhibit phrenic motoneurones (Merrill and Fedorko, 1984). An additional group of respiratory neurones are present in the medial parabrachial and Kolliker-Fuse nuclei of the pons and are known collectively as the pontine respiratory group (Richter, 1996).

Generation of respiratory rhythm

The precise mechanism by which respiratory rhythm is generated has been the subject of much investigation (see Funk and Feldman, 1995). Studies *in vivo* suggest that rhythm may be produced by a network of reciprocally-connected respiratory neurones, whereas those performed *in vitro* tend to suggest that rhythm is produced by a population of neurones with pacemaker properties (Funk and Feldman, 1995; Richter, 1996). There is now a growing consensus that both pacemaker and network properties are important in respiratory rhythm generation. It is possible that a group of neurones with pacemaker properties may provide a basic oscillatory drive to a network of respiratory neurones which synchronise, modify and transmit rhythmic activity to respiratory motoneurones (Ramirez and Richter, 1996).

Location of respiratory rhythm generator

Whilst the exact mechanism of respiratory rhythmogenesis remains ambiguous, recent progress has been made regarding the location of the putative rhythm generator. Evidence suggests that the essential neurones involved in the generation of respiratory rhythm are located within a discrete area of the VRG. Using the *in vitro* neonatal rat brainstem-spinal cord preparation, Smith et al., (1991) found that respiratory rhythm was only eliminated when transverse microsections were made within a 200 μm thick area at the caudal end of the retrofacial nucleus. This critical area, located just caudal to the Botzinger complex was termed the pre-Botzinger complex (Smith et al., 1991).

Medullary slices containing the pre-Botzinger complex have been shown to generate respiratory rhythm similar to that generated by the whole brainstem *in vitro* (Smith et al., 1991; Ramirez et al., 1996). In the anaesthetised rat, bilateral microinjection of the $GABA_A$ receptor agonist muscimol into the pre-Botzinger area eliminates respiratory activity (Koshiya and Guyenet, 1996). Also, in the anaesthetised cat, selective lesioning of the pre-Botzinger complex

by local microinjection of the sodium channel blocker tetrodotoxin eliminated rhythmic respiratory activity, causing a central apnoea (Ramirez et al., 1998). All classes of respiratory neurones purported to be involved in the generation of respiratory rhythm are present in the pre-Botzinger complex, providing further evidence of the critical role for this medullary area in respiratory rhythmogenesis (Schwarzer et al., 1995).

The respiratory rhythm is ultimately transmitted to motoneurones in the spinal cord thereby evoking rhythmic contractions of the diaphragm and intercostal and abdominal muscles. In addition, glossopharyngeal and vagal motoneurones innervating the upper airways also receive respiratory input. The majority of neurones in the pre-Botzinger complex however, can not be antidromically activated by spinal cord or cranial nerve stimulation (Smith et al., 1991; Schwarzer et al., 1995). It therefore seems likely that neurones from the pre-Botzinger complex provide the excitatory drive to bulbospinal neurones and pre-motoneurones situated in other areas of the respiratory nuclei.

5-HT_{1A} receptors and respiratory control

Synaptic interactions between neurones of the respiratory network appear to be mediated mainly by excitatory and inhibitory amino acids. However, there is a growing body of evidence to suggest that respiratory rhythm is influenced by a number of neuromodulators including 5-HT (see Bonham, 1995).

Serotonergic nerve terminals have been identified in the vicinity of the dorsal and ventral respiratory groups (Maley and Elde, 1982; Connelly et al., 1989; Voss et al., 1990) and also in close apposition to phrenic motoneurones (Pilowsky et al., 1990). This serotonergic innervation is thought to arise mainly from the medullary raphe nuclei (Connelly et al., 1989; Holtman et al., 1990). Electrical stimulation of the raphe nuclei has been shown to cause both excitation and inhibition of respiration (Holtman et al., 1986, Lalley, 1986; Lalley et al., 1997). The inhibition of phrenic nerve discharge and expiratory neuronal activity evoked by electrical stimulation of the raphe obscurus was partially

attenuated by prior administration of the 5-HT_{1A} receptor antagonist NAN-190 (Lalley et al., 1997).

The effects of exogenously applied 5-HT on respiratory activity are complex. In the neonatal rat brainstem-spinal cord preparation, 5-HT has a biphasic effect on the respiratory rhythm causing a transient increase, followed by secondary decrease in respiratory frequency. In addition, it also evokes tonic activity of the phrenic motoneurones (Di Pasquale et al., 1997; Onimaru et al., 1998). Similar results have been obtained *in vivo* following administration of 5-HT into the fourth ventricle of the cat (Rose et al., 1995).

The excitatory effects of 5-HT on phrenic motoneurone discharge have been shown to be due to actions at spinal postsynaptic 5-HT_{2A} receptors (Di Pasquale et al., 1997). It is less clear exactly which receptor subtypes mediate the biphasic effects of 5-HT on respiratory rhythm since agonist and antagonist studies have yet to produce conclusive findings. The secondary decrease in respiratory frequency appears to be due to activation of 5-HT_2 receptors (possibly 5-HT_{2C}) (Rose et al., 1995; Onimaru et al., 1998), whilst the initial increase in respiratory frequency has been suggested to be mediated by 5-HT_{2A} (Onimaru et al., 1998), 5-HT_1 (Morin et al., 1990) and 5-HT_{1A} receptors (Rose et al., 1995). Further evidence implicating 5-HT_{1A} receptors has been provided by studies in cats, as intravenous administration of low doses of the 5-HT_{1A} receptor agonists 8-OH-DPAT or 5-MeODMT evokes an increase in respiratory frequency (Lalley et al., 1994; Lalley, 1994). It is still not known however where in the respiratory network 5-HT_{1A} receptor agonists act to cause the observed changes in respiratory frequency

Larger intravenous doses of 5-HT_{1A} receptor agonists have clear depressant effect on all classes of respiratory neurones, causing hyperpolarisation and reducing or abolishing rhythmic neuronal discharge. In addition, phrenic nerve activity is usually depressed or completely abolished (Lalley et al., 1994; Lalley 1994). These effects can be reversed by i.v. administration of the 5-HT_{1A} receptor antagonist NAN-190 (Lalley et al., 1994).

Iontophoretic application of 5-HT_{1A} receptor agonists has similar effects on respiratory neurone excitability suggesting that the effects of systemically administered agonists on these neurones may be due, at least in part, to a direct post-synaptic inhibition (Lalley et al., 1994).

1.9 The nucleus tractus solitarius

The nucleus tractus solitarius (NTS) is the principal site of termination of cranial sensory afferents arising from the gastrointestinal, respiratory and cardiovascular systems. This nucleus also receives sensory information from gustatory afferent nerves and plays an important role in many homeostatic processes (see Loewy, 1990). The NTS is located in the dorsomedial medulla oblongata and extends rostrally from the cervical spinal cord to caudal edge of the facial motonucleus. The more caudal region exists as a single nucleus located along the midline however, at the level of the obex, the nucleus splits and becomes bilaterally located either side of the fourth ventricle (Lawrence and Jarrott, 1996).

The structure of the NTS is fairly complex comprising of a number of different cytoarchitectonic subnuclei. Histological and neurophysiological studies have shown that within the NTS there exists a certain degree of viscerotopic organisation, in that functionally different afferents terminate in different subnuclei (Jordan and Spyer, 1986). For example, afferents arising from the respiratory system project mainly to the ventral and ventrolateral NTS, cardiovascular afferents are found predominantly in the dorsolateral and dorsomedial regions, whereas gastric afferents are found in the parvocellular NTS (Kalia and Mesulam, 1980; Loewy, 1990; Sun, 1995). Some overlap does occur, particularly in a subregion known as the commissural NTS which receives inputs from all the major visceral afferents (Kalia and Mesulam, 1980; Loewy, 1990).

The NTS plays an important role in various cardiovascular and respiratory reflexes relaying the afferent inputs to autonomic effector nuclei to evoke the

appropriate reflex adjustments (see sections 1.10-1.12). The NTS is however far more than a simple relay station, and it is thought that significant amounts of synaptic integration occurs here. Integration may occur in part at the level of the afferent input as various studies have shown that some NTS neurones receive synaptic inputs from more than one afferent nerve (Donoghue et al., 1985; Mifflin et al., 1988a). In most cases, the different afferents produce similar effects on the NTS neurone suggesting that the converging afferent nerves may be of a similar type or that the NTS neurone is functionally dedicated (Mifflin and Felder, 1990).

The importance of the NTS as a centre of cardiovascular integration is also highlighted by the large number of afferent projections it receives from brain nuclei known to influence the cardiovascular system. These include the medullary raphe nuclei (Thor and Helke, 1987), the caudal ventrolateral medulla (Roder and Ciriello, 1992), the rostral ventrolateral medulla (Wang and Li, 1988), the area postrema (Hay and Bishop, 1991), the midbrain periaquiductal grey matter (Bandler and Tork, 1987), the parabrachial nucleus (Felder and Mifflin, 1988), the perifornical region of the hypothalamus (Mifflin et al., 1988b) and the amygdala (Schwaber et al., 1982; Cox et al., 1986). Many of these inputs have been shown to modulate the reflexly evoked inputs to the NTS.

Information is relayed from the NTS to other areas of the brainstem and spinal cord via a multitude of efferent projections. Areas receiving projections from the NTS include the IML cell column (Loewy and Burton, 1978), the RVLM, the caudal ventrolateral medulla (Ross et al., 1985), the medullary raphe nuclei (Loewy and Burton, 1978), the vagal preganglionic nuclei (Ross et al., 1985; Deuchars and Izzo, 1991), the hypothalamus and other forebrain areas (Ricardo and Koh, 1978).

5-HT in the NTS

Serotonergic nerve terminals have been located within most subdivisions of the NTS using immunohistochemical techniques (Steinbusch, 1981; Maley and Elde, 1982). Much of this serotonergic innervation originates from neurones within the medullary raphe nuclei (Thor and Helke, 1987; Schaffar et al., 1988) however, intrinsic serotonergic neurones are also present within the medial subdivision of this nucleus (Calza et al., 1985). In addition, 5-HT has been localised within the cell bodies and central terminals of vagal sensory afferent nerves that project to the NTS (Nosjean et al., 1990; Sykes et al., 1994).

Microinjection of 5-HT into the caudal NTS of rats has variable effects on the cardiovascular system. Low doses of 5-HT (pmol) evoke a fall in blood pressure and heart rate (Laguzzi et al., 1984; Shvaloff and Laguzzi, 1986) whereas injections of higher doses (nmol) have been shown to cause increases in blood pressure with variable effects on heart rate (Wolf et al., 1981). The NTS contains binding sites for many 5-HT receptor subtypes including 5-HT_{1A}, 5-HT_{1B}, 5-HT_2 and 5-HT_3 receptors (Manaker and Verderame, 1990; Thor et al., 1992b; Dashwood et al., 1988b; Pratt and Bowery, 1989). Therefore, the opposing effects are thought to be due to the actions of 5-HT at multiple receptor subtypes.

The hypotensive response is seemingly due to activation of 5-HT_2 receptors as it can be blocked by pre-treatment with the 5-HT_2 receptor antagonist ketanserin (Shvaloff and Laguzzi, 1986; Callera et al, 1997a) and is mimicked by microinjection of the 5-HT_2 receptor agonist DOB (Merahi et al., 1992a; Merahi and Laguzzi, 1995). The 5-HT_2 receptors in question are not thought to be located on the vagal afferent terminals within the NTS as the response is unaffected by ganglionectomy (Merahi et al., 1992b). The hypotension evoked by microinjection of the 5-HT_2 receptor agonist DOI into the NTS can be blocked by prior microinjection of kynurenic acid into the caudal ventrolateral medulla or bicuculline into the RVLM (Sevoz et al., 1996a). This

suggests that the neural mechanisms involved may be similar to those that mediate the baroreceptor reflex (see section 1.10).

The increase in blood pressure evoked by higher doses of 5-HT is thought to be mediated by 5-HT_3 receptors as it can be mimicked by administration of the 5-HT_3 receptor agonists 2-methyl-5-HT or PBG and attenuated by the 5-HT_3 receptor antagonist ondansetron (Merahi et al., 1992b; Merahi and Laguzzi, 1995). This effect can also be blocked by prior microinjection of bicuculline into the NTS (Merahi et al., 1992b), suggesting that 5-HT_3 receptors may activate inhibitory GABAergic pathways which have been reported to exist in the NTS (Meeley et al., 1985; Izzo et al., 1992). However, the pressor effect evoked by 5-HT_3 receptor stimulation is considerably reduced following ganglionectomy indicating that actions on vagal afferent nerve terminals within the NTS are required for full expression of this effect (Merahi et al., 1992b). This finding is compatible with the results of receptor binding studies which have shown that the majority of 5-HT_3 receptors in the NTS are located presynaptically on vagal afferent terminals (Pratt and Bowery, 1989). In the rat, the sympathoexcitation and increase in blood pressure evoked by microinjection of the 5-HT_3 receptor agonist CPBG into the NTS appears to be due to the activation of the slow-conducting, clonidine-sensitive sympathoexcitatory neurones of the RVLM (Sevoz et al., 1998).

The involvement of 5-HT_1 receptors in the depressor response evoked by microinjection of 5-HT into the NTS has been investigated in a number of studies and has produced conflicting results. One study in rats suggests that the effect may involve the activation of 5-HT_{1B} receptors (Feldman and Galiano, 1995), whereas a similar study has shown that the effect can be blocked by the 5-HT_{1A} receptor antagonist WB4101 (Itoh and Bunag, 1991). In the latter study, microinjection of the 5-HT_{1A} receptor agonist 8-OH-DPAT into the NTS surprisingly had no effect on arterial blood pressure or heart rate. Other studies in the rat, cat and dog, have also shown that 8-OH-DPAT has no effect on baseline cardiovascular variables when microinjected in the NTS

(Shvaloff and Laguzzi, 1986; Vayssettes-Courchay et al., 1993b; Laubie et al., 1989), thus casting doubt on the involvement of 5-HT_{1A} receptors in mediating this 5-HT-evoked depressor response. These studies also suggest that the well characterised depressor response evoked by i.v. 8-OH-DPAT is not due to actions at the level of the NTS.

Iontophoretic studies *in vitro* and *in vivo* reveal that 5-HT can cause both excitation and inhibition of NTS neurones (Feldman, 1994; Wang et al., 1997). In the latter study, iontophoresis of 5-HT_{1A} and 5-HT_2 receptor agonists evoked both excitatory and inhibitory responses, while application of 5-HT_3 receptor agonists predominantly evoked excitation. The variability of some of these responses is probably due to the fact that the neurones tested were not a physiologically homogenous group. In this respect, neurones that received inputs from cardiac vagal afferents showed more consistent excitatory responses to 5-HT_{1A} receptor ligands (Wang et al., 1997).

1.10 The baroreceptor reflex

The baroreceptor reflex is the principal mechanism by which the CNS regulates short term variations in arterial blood pressure. Fluctuations in pressure are detected by sensory afferents in the aortic arch and carotid sinus which are stimulated when increased pressure stretches the vessel wall. The afferent input in response to an increase in pressure is relayed to the CNS and via a multisynaptic pathway causes changes in autonomic outflow to the heart and blood vessels that serve to reduce arterial pressure to within normal values.

Experimental stimulation of baroreceptor afferents

Various methods exist for stimulating baroreceptor afferents. In many early studies, the 'blind-sac' preparation was used. In this preparation, a balloon-tipped catheter is inserted into the vascularly isolated carotid sinus to enable direct mechanical distension of the sinus (see Heymans and Neil, 1958).

Similar catheters may also be placed in the descending aorta which, when distended, occlude aortic blood flow and provide an increased pressure stimulus to the aortic and carotid baroreceptors (Korner et al., 1972).

Small chemically-evoked increases in arterial blood pressure have also been used to stimulate arterial baroreceptors. Intravenous injection of the vasoconstrictor drug phenylephrine is a method used frequently. The resulting increase in pulse interval in relation to the evoked increase in pressure is measured as an indication of baroreflex sensitivity (Smyth et al., 1969). One potential problem when using this method is that the effectiveness of constant pressor stimuli may be altered by any changes in baseline arterial blood pressure that occur during the experimental protocol.

In a number of studies, the baroreceptor reflex has been elicited by direct electrical stimulation of the aortic nerves (see below). This method is not used particularly frequently in the cat and dog, as in these species the aortic nerves contain both barosensory and chemosensory afferents and electrical stimulation can cause either depressor or pressor responses depending on the stimulus parameters or anaesthetic used (Neil et al, 1949; Edis and Shepheard, 1971). Whilst aortic nerves in the rabbit and rat the may also contain some chemoreceptor fibres (Aars, 1971; Brophy et al., 1999), electrical stimulation of the aortic nerves in these species does not produce a pressor response or a hyperpnoea, but consistently evokes a depressor response, suggesting that they consist mainly of barosensory neurones (Sapru et al., 1981; Neil et al., 1949; Chalmers et al., 1967). In these species, stimulation of the aortic nerve is therefore often a useful method of mimicking arterial baroreceptor stimulation.

Neural pathway of the baroreceptor reflex

Baroreceptor afferents from the carotid sinus project via the carotid sinus nerve into the glossopharyngeal nerve. Those afferents arising from the aortic arch project via the aortic nerve into the superior laryngeal nerve and then join the main trunk of the vagus nerve. Via the glossopharyngeal and vagus nerves,

the baroreceptor afferents project to the CNS where they terminate primarily in the dorsal areas of the medial and lateral NTS at sites generally rostral to the obex (Donoghue et al., 1982, 1984; Ciriello, 1983).

The identity of the neurotransmitters involved in mediating the baroreceptor reflex within the NTS has been the subject of much study. It is now generally accepted that the primary neurotransmitter is glutamate as microinjection of glutamate itself or its analogues into the NTS mimics the baroreceptor reflex whereas microinjection of the glutamate receptor antagonist kynurenic acid blocks both the vagal and sympathetic component of the reflex (Talman et al., 1981; Guyenet et al., 1987; Talman, 1989). Blockade of the non-NMDA receptor subtype within the NTS of rats also abolishes the baroreceptor reflex evoked by aortic nerve stimulation (Gordon and Leone, 1991) and abolishes the short latency excitatory post synaptic potentials evoked by stimulating the solitary tract (Andresen and Yang, 1990). Selective blockade of the NMDA receptors within the NTS however, only partially attenuates the reflex responses evoked by stimulation of the aortic nerve at high frequencies (Kubo and Kihara, 1988a; Gordon and Leone, 1991). This suggests that glutamate mediates the baroreceptor reflex by synaptic activation of non-NMDA receptors within the NTS although NMDA receptor activation may play a modulatory role particularly during conditions of high intensity input. In addition, metabotropic glutamate receptors are also present in the NTS and have been shown to modulate excitatory transmission (Glaum and Miller, 1992).

Sympathetic component of the baroreceptor reflex

Activation of arterial baroreceptors causes a fall in sympathetic nerve activity and blood pressure via the inhibition of tonically active sympathetic premotor neurones in the RVLM (Agarwal et al., 1990). This inhibition may be due in part to the disfacilitation of excitatory inputs (Granata and Kitai, 1992), however, it appears to be mainly mediated via a postsynaptic inhibitory input (Dampney et al., 1988). It was originally thought this was due to a direct

inhibitory projection from the NTS to the sympathoexcitatory region of the RVLM, however, evidence now suggests that baroreceptor inputs are conveyed from the NTS to the RVLM indirectly (Sved and Gordon, 1994).

Neurones from the NTS project to a group of vasodepressor neurones located in the caudal ventrolateral medulla (CVLM)(Ross et al., 1985) where they mediate excitation via the release of glutamate onto NMDA receptors (Gordon, 1987; Agarwal et al., 1990). Anatomical studies reveal that the CVLM contains GABAergic neurones that make synaptic contact with neurones in the RVLM (Blessing and Li, 1989). It has therefore been suggested that during the baroreceptor reflex, the CVLM inhibits the sympathoexcitatory neurones of the RVLM via release of GABA (Blessing, 1991). In support of this, microinjection of the GABA antagonist bicuculline into the sympathoexcitatory region of the RVLM blocks the inhibition of RVLM neurones evoked by baroreceptor stimulation and also causes a dose-dependent blockade of the vasodepressor response elicited by stimulation of the CVLM (Sun and Guyenet, 1985; Blessing and Li, 1989).

Chemical lesions within the rostral regions of the CVLM using the excitotoxic agent kainic acid eliminates the baroreceptor-evoked sympathoinhibition and consequently increases resting blood pressure (Cravo et al., 1991; Masuda et al., 1991). In addition, neurones within the caudal regions of the CVLM provide a tonic inhibition of RVLM neurones that is independent of the baroreceptor reflex (Cravo et al., 1991).

The CVLM also contains the A1 group of catecholaminergic neurones which are not involved in the reflex pathway described above, but may, via direct connections to the hypothalamus, mediate the increase in vasopressin release that accompanies baroreceptor unloading (Dampney, 1994).

Vagal component of the baroreceptor reflex

Activation of arterial baroreceptors causes a bradycardia and decrease in cardiac contractility. This is due in part to a reduction of cardiac sympathetic

activity but is mainly caused by an increase in the discharge of vagal cardioinhibitory neurones (Loewy and Spyer, 1990).

Numerous anatomical studies have provided evidence of direct connections between NTS and vagal preganglionic neurones in the NA and DVN (Loewy and Burton, 1978; Ross et al., 1985; Deuchers and Izzo, 1991). Identified cardiac vagal preganglionic neurones have also been shown to be excited at short latency by stimulation within the NTS (Agarwal and Calaresu, 1992). These findings suggest that the activation of CVPNs by baroreceptor stimulation may be mediated via direct projections from second order neurones in the NTS. Other studies have shown however that the central delay between stimulation of baroreceptor afferents and activation of CVPNs is considerably longer than would be expected if a disynaptic pathway existed. The existence of multisynaptic pathways between the NTS and vagal nuclei has therefore also been proposed (McAllen and Spyer, 1978b).

Respiratory component of the baroreceptor reflex

Baroreceptor inputs also reflexly affect respiration. Increases in arterial pressure reduce ventilation or evoke an apnoea, whereas a fall in systemic pressure increases respiratory activity (see Heymans and Neil, 1958). Intracellular recordings of respiratory neurones in the VRG have shown that baroreceptor activation hyperpolarises inspiratory neurones throughout the respiratory cycle, inhibiting their neuronal discharge. This may be the result of a direct inhibitory connection from baroreceptor afferents to inspiratory neurones, or, alternatively baroreceptor activation may inhibit neurones that tonically excite medullary respiratory neurones (Richter and Seller, 1975). Stimulation of baroreceptors also depolarises expiratory neurones during inspiration (Richter and Seller, 1975) This is thought to be due to a disinhibition of expiratory neurones due to the inhibitory effects on inspiratory neurones.

1.11 The cardiopulmonary reflex

Cardiac vagal C-fibres

In the heart, non-myelinated vagal afferent nerves are present in both the atria and ventricles. There is a preponderance of these vagal C-fibres in the walls of the left ventricle at varying depths. In contrast, the walls of the right ventricle contain very few C-fibre afferent nerves (Hainsworth, 1991). Most vagal afferents are either mechanosensitive or chemosensitive and many respond to both types of stimuli causing a reflex bradycardia, hypotension and in some cases, a reflex apnoea or tachypnoea (Thoren, 1979; Evans et al., 1990).

The physiological roles of atrial and ventricular receptors are still uncertain. Mechanosensitive ventricular C-fibres respond to both increased ventricular pressure and contractility and roles in the regulation of arterial pressure and blood volume have been proposed. Chemosensitive afferents may be stimulated by substances released during myocardial ischaemia (Thoren, 1979; Hainsworth, 1991).

Pulmonary and bronchial vagal C-fibres

In the lungs, non-myelinated vagal afferents are present in the interstitial tissue between the alveoli the pulmonary capillaries (pulmonary C-fibres or J-receptors) and also in the conducting airways (bronchial C-fibres) (Paintal, 1973a; Coleridge and Coleridge, 1984). Pulmonary C-fibre afferents are supplied by the pulmonary circulation, whereas bronchial C-fibres are supplied by the systemic circulation (Coleridge and Coleridge, 1984). Stimulation of both types of afferent nerve evoke qualitatively similar reflex responses which include tachypnoea or apnoea, bronchoconstriction, bradycardia and hypotension (Coleridge and Coleridge, 1984).

Pulmonary and bronchial vagal C-fibre afferents are involved in airway defence reflexes evoked by inhalation of irritants into the lower airways.

Pulmonary C-fibre afferents are also stimulated by oedema in the alveolar interstitium (Coleridge and Coleridge, 1984).

Experimental stimulation of 'cardiopulmonary' afferents

Many chemicals can be used experimentally to stimulate vagal C-fibre afferents in the heart and lungs. Examples include acetylcholine, histamine, capsaicin, 5-HT and the structural 5-HT analogues phenyldiguanide (PDG) and phenylbiguanide (PBG) (Paintal, 1973b).

If PBG is injected into the right atrium or pulmonary artery and the evoked responses are measured within the pulmonary circulation time (which in the cat is approximately 5 s), then the effects produced are generally assumed to be due to stimulation of pulmonary C-fibres exclusively (Heymans and Neil, 1958; Daly, 1991). After this time period, stimulation of cardiac and bronchial C-fibres afferents accessible from the coronary and systemic circulations may also contribute to the reflex responses. However, as cardiac C-fibre endings are present in the right atrium and since the circulation times are relatively short in smaller animals, it is often more convenient to refer to the afferent fibres stimulated by right atrial injection of PBG as the 'cardiopulmonary C-fibre afferents' (Verberne and Guyenet, 1992; Vardhan et al., 1993a; Wilson et al., 1996). The pattern of cardiovascular and respiratory responses evoked by collective stimulation of these afferents is therefore described as the cardiopulmonary reflex.

Stimulation of cardiopulmonary C-fibres with PBG can be blocked by pre-treatment with the 5-HT_3 receptor antagonist MDL 72222 indicating that the effects are mediated via 5-HT_3 receptors located on vagal afferent terminals (Kay and Armstrong, 1990).

Neural pathway of the cardiopulmonary reflex

Non-myelinated cardiopulmonary C-fibre afferents project via the vagus nerves to the NTS. Antidromic mapping studies in the cat have shown that

these afferent nerves terminate within medial regions along the border of the parvocellular subnucleus at sites rostral to obex and caudal to obex in the dorsal portion of the commissural subnucleus (Kubin et al., 1991). Chemical lesion of neurones within this area have been shown to impair the reflex responses evoked by stimulating cardiopulmonary afferents (Bonham and Joad, 1991). Immunohistochemical localisation of Fos, a DNA-binding protein expressed by active neurones, have confirmed the above finding in rabbits. Following repetitive injections of PBG, Fos-positive neurones in the NTS were restricted to medial and commissural regions (Gieroba et al., 1995).

Neurotransmission of cardiopulmonary inputs within the NTS is presumably glutamatergic as the reflex is blocked by microinjection of kynurenic acid into the NTS (Verberne and Guyenet, 1992; Vardhan et al., 1993a; Vayssettes-Courchay et al., 1997). One study in anaesthetised rats suggests that the excitation of NTS neurones by cardiopulmonary afferents is mainly mediated by non-NMDA receptors (Wilson et al., 1996). Studies in conscious rats however produce contrasting results implicating NMDA receptors (Chianca and Machado, 1996), while others have suggested that both NMDA and non-NMDA receptors are involved (Vardhan et al., 1993a).

Bilateral microinjection of kynurenic acid into the CVLM or bicuculline into the RVLM attenuate the sympathoinhibition and subsequent depressor response evoked by stimulating cardiopulmonary afferents (Verberne and Guyenet, 1992). This suggests that as with the baroreceptor reflex, the sympathoinhibition evoked by the cardiopulmonary reflex involves excitatory glutamatergic projections from the NTS to the CVLM which in turn inhibits the sympathoexcitatory premotor neurones of the RVLM via the release of GABA (Verberne and Guyenet, 1992). In this respect, Fos-positive neurones of the CVLM were shown to project to the sympathoexcitatory region of the RVLM (Gieroba et al., 1995).

The vagal bradycardia evoked by the cardiopulmonary reflex is presumably due to an NTS-mediated excitation of CVPNs in the NA and DVN.

In this respect, antidromically activated CVPNs in both nuclei have been shown to be excited following right atrial injection of PBG (Jones et al., 1994a, 1998).

Respiratory component of the cardiopulmonary reflex

Stimulation of the cardiopulmonary C-fibres can elicit variable respiratory responses depending upon the species of animal studied, or the intensity of stimulation. The two common responses are an apnoea, where respiration temporarily ceases in the post-inspiratory phase, or a pattern of rapid shallow breathing in which the expiratory interval is shortened (Spyer, 1996; Richter, 1996). Modelling studies of the respiratory network predict that both of these effects can result from variable increases in activity of post-inspiratory neurones (Richter, 1982). Extracellular recordings from medullary respiratory neurones have shown that during cardiopulmonary stimulation, post-inspiratory neurones are indeed tonically excited, whereas inspiratory and expiratory neurones are generally inhibited (Jones and Jordan, 1993; Wilson and Bonham, 1997).

1.12 The chemoreceptor reflex

The peripheral arterial chemoreceptors are located in the carotid and aortic bodies and in concert with the central chemoreceptors in the ventral medulla, they act to maintain arterial blood pO_2, pCO_2 and pH within normal physiological levels (Daly, 1983). Carotid bodies, located bilaterally at the bifurcation of the common carotid artery, have been found in all mammals investigated (see Marshall, 1994). Aortic bodies are situated adjacent to the walls of the aortic arch, but do not appear to be present in all species. Studies in the cat and dog suggest the presence of functional aortic bodies (Neil et al., 1949; Edis and Shepheard, 1971), whilst in the rat and rabbit, they are generally assumed to be absent or to play no significant role in hypoxic chemoreception (Sapru et al., 1981; Chalmers et al., 1967). In the case of the rat, this assumption may need revising in light of recent findings which demonstrate that chemoreceptor glomus tissue is present in the vicinity of the

aortic arch and that the aortic nerve contains afferent nerves of putative chemoreceptor function (Brophy et al., 1999). In certain species including mice and rats, chemoreceptor tissue has also been found in areas of the abdomen (see Marshall, 1994).

Carotid body chemoreceptor afferents are excited by falls in arterial blood pO_2 via an ill-defined transduction mechanism (Heymans and Neil, 1958). Afferents arising from the aortic bodies are also stimulated although they are far less sensitive to hypoxic stimuli than those arising from the carotid bodies (Lahiri et al., 1981). Carotid body chemoreceptors are also excited by increases in arterial blood pCO_2 and H^+ (see Marshall, 1994).

Stimulation of chemoreceptor afferents evokes a reflex increase in ventilation proportional to the stimulus intensity (Heymans and Neil, 1958). The cardiovascular responses produced are largely dependent on the degree of the simultaneously evoked hyperventilation. If the increase in ventilation is modest or if the ventilation is held constant, then chemoreceptor stimulation produces a bradycardia and increase in total peripheral resistance due to peripheral vasoconstriction. In freely breathing animals however, chemoreceptor stimulation usually evokes a tachycardia and vasodilatation. These are secondary effects to the evoked hyperventilation due to increased pulmonary stretch receptor input, the incidental hypocapnia and alterations in the activity of central inspiratory neurones (Daly and Scott, 1962).

Under certain conditions, stimulation of chemoreceptors has also been shown to evoke the alerting stage of the defence response, comprising of a rise in blood pressure, tachycardia, renal, mesenteric and cutaneous vasoconstriction, muscle vasodilatation, pupillary dilatation and retraction of the nictitating membranes (see Marshall, 1994).

Experimental stimulation of chemoreceptor afferents

Carotid body chemoreceptors can be stimulated experimentally using a variety of techniques (see Marshall, 1994). Close arterial injection of metabolic

inhibitors such as sodium cyanide is a method that has been used extensively. The stimulatory effects of sodium cyanide are due to its ability to produce histotoxic hypoxia by blocking the cytochrome oxidase pathway, however, it may also affect other cellular processes within the carotid body (Gonzales et al., 1992).

Neural pathway of the chemoreceptor reflex

Both myelinated and non-myelinated chemoreceptor afferents arising from the carotid body project via the sinus nerve into the glossopharyngeal nerve and terminate mainly in the NTS. The sites of chemoreceptor afferent termination within the NTS have been examined by injecting neuronal anterograde tracers into the carotid body (Finley and Katz, 1992). These have revealed that chemoreceptor afferents terminate predominantly in the commissural nucleus at the level of and caudal to the obex and also in the medial subnuclei. Similar findings have been obtained from antidromic mapping experiments (Donoghue et al., 1984). Chemical lesions within the region of the commisural NTS markedly attenuated the respiratory response to hypoxia, whereas lesions in more rostral areas had little effect (Housley and Sinclair, 1988).

As with the baroreceptor and cardiopulmonary reflexes, synaptic transmission of chemoreceptor input within the NTS is thought to be glutamatergic as it can be blocked by microinjection of kynurenic acid (Sevoz et al, 1997). Effects on both NMDA and non-NMDA receptors are involved as blockade of both of these receptor subtypes is necessary to inhibit the chemoreceptor reflex (Vardhan et al., 1993b).

Chemoreceptor stimulation evokes an increase in arterial pressure due to increased sympathetic outflow to the vasculature. These effects can be blocked by microinjection of kynurenic acid into the RVLM suggesting that they are mediated by a glutamatergic activation of sympathoexcitatory RVLM neurones (Koshiya et al., 1993; Sun and Reis, 1995). This has been substantiated in

experiments in rats in which extracellular recording were made of RVLM neurones excited by chemoreceptor inputs, the excitation being mainly mediated by actions on NMDA receptors (Kubo et al, 1993; Sun and Reis, 1995).

Neither kynurenic acid or bicuculline affected the chemoreceptor-evoked sympathoexcitation when microinjected bilaterally into the CVLM (Koshiya et al., 1993). Thus, in contrast to the baroreceptor and cardiopulmonary receptor reflex pathways, the CVLM appears to play no role in the sympathetic component of the chemoreceptor reflex. Projections from the commissural NTS may directly transmit the excitatory chemoreceptor inputs to the RVLM (Urbanski and Sapru, 1988; Otake et al., 1993). In addition, sympathoexcitatory neurones of the RVLM may also be activated via descending pathways from the hypothalamus or amygdala which have been shown to receive chemoreceptor inputs (Thomas and Calaresu, 1972; Cechetto and Calaresu, 1984) and are involved in mediating the defence response (see Jordan, 1990).

Respiratory component of the chemoreceptor reflex

Brief stimulations of carotid chemoreceptor afferents evoke increases in tidal volume and phrenic nerve amplitude when the stimuli is given during inspiration (Eldridge, 1972). The same stimuli given during expiration may prolong the expiratory pause or enhance the expiratory effort, but there is no effect on subsequent inhalations (Eldridge, 1972; Haymet and McCloskey, 1975). Intracellular recordings from respiratory neurones of the DRG have shown that inspiratory neurones are only excited by brief chemoreceptor afferent stimulation if the stimulus is applied during inspiration (Lipski et al., 1977a). If the inspiratory neurones are made to fire during expiration and a brief chemoreceptor stimulus is given, the neurones are actually inhibited. This is thought to be due to an inhibitory input from expiratory neurones of the Botzinger complex, which are known to be excited by chemoreceptor

stimulation and can also be antidromically activated from the NTS region (Lipski et al., 1984).

More prolonged stimulations of peripheral chemoreceptors causes an increase in the amplitude of phrenic nerve firing, presumably reflecting an increased tidal volume. Respiratory rate is also usually increased largely resulting from a decreased inspiration time (Matsumoto, 1982; Morris et al., 1996).

The enhanced respiratory motor movements following chemoreceptor afferent stimulation are due to excitatory effects on medullary respiratory neurones. Prolonged chemoreceptor stimulation has been shown to increase the activity of bulbospinal pre-inspiratory, post-inspiratory and expiratory neurones during their respective bursting phases (Lawson et al., 1989; Dogas et al., 1995; Sun and Reis, 1996). In rats, the chemoreceptor-evoked excitation of pre-inspiratory and expiratory neurones was abolished by iontophoresis of the EAA antagonist kynurenic acid (Sun and Reis, 1996). In the dog, the chemoreceptor-evoked excitation of expiratory neurones can be blocked by pressure microinjection of AP-5 but not NBQX suggesting that it is mediated via the actions of EAAs on NMDA receptors (Dogas et al., 1995).

The mechanism by which chemoreceptor stimulation evokes the decrease in inspiratory time is not well known, however one group has provided preliminary data to suggest it may be due to an inhibitory effect on inspiratory neurones in the pre-Botzinger region (Morris et al., 1996). These neurones send excitatory projections to more caudal inspiratory neurones and chemoreceptor stimulation decreases their bursting duration.

1.13 Modulation of cardiorespiratory reflexes

Respiratory modulation of cardiac vagal activity

The ongoing activity of small myelinated CVPNs is powerfully modulated by respiration. Neurones fire predominantly in the expiratory phase whereas during inspiration, CVPNs are thought to receive direct inhibitory inputs,

presumably from central inspiratory neurones (see section 1.7). The excitability of CVPNs therefore varies throughout the respiratory cycle and, as a consequence, their sensitivity to excitatory inputs will also show respiratory modulation. In this respect, the reflex vagal cardioinhibition evoked by stimulation of arterial baroreceptors or chemoreceptors has been shown to vary with respect to the respiratory cycle (Davidson et al., 1976). Whilst brief stimulation of these cardiovascular afferents causes a fall in heart rate when delivered during the expiratory phase of the central respiratory cycle, the same stimuli are relatively ineffective during the inspiratory phase (Gandevia et al., 1978; Potter, 1981; Daly, 1991). In these experiments, inputs from the lungs were eliminated by temporarily interrupting artificial respiration, therefore, the respiratory modulation can be assumed to be due primarily to the inhibitory effects of central inspiratory activity on cardiac vagal outflow.

Inputs from slowly adapting pulmonary stretch receptors also contribute to the respiratory modulation of reflex cardiac vagal outflow. During periods of phrenic silence, the reflex bradycardias evoked by baroreceptor or chemoreceptor stimulations have been shown to be markedly reduced by inflation of the lungs (Gandevia et al., 1978; Potter, 1981; Daly and Kirkman, 1989). Thus in the normal animal, input from the pulmonary stretch receptors during inspiration would act to reinforce the inhibitory effects of central inspiratory drive on cardiac vagal outflow. Indirect evidence suggests that the inhibitory effects of pulmonary stretch receptors are not mediated at the level of the CVPNs (Potter, 1981), and neither central inspiratory activity or lung inflation affect synaptic transmission of baroreceptor inputs in the NTS (Mifflin et al., 1988a). The precise site at which the pulmonary stretch receptors exert their modulatory effect on reflex vagal outflow therefore remains obscure, however it is likely to be at a point in the reflex pathway between the NTS and CVPNs.

Respiration exerts similar modulatory effects on the reflex bradycardia evoked by stimulation of the cardiac C-fibre afferents, however the vagal

cardioinhibition evoked by pulmonary C-fibre stimulation is relatively unaffected by central inspiratory activity or pulmonary stretch receptors (Daly and Kirkman, 1988, 1989; Daly, 1991). To explain these findings, is has been suggested that the chronotropic effects of the pulmonary C-fibre reflex are mediated via vagal cardioinhibitory neurones which are not modulated by respiratory activity (Daly and Kirkman, 1989; Daly, 1991). Such neurones exist in the DVN and are indeed excited by stimulation of pulmonary C-fibre afferents, however, it appears that activation of these neurones alone could not produce the degree of cardioinhibition evoked by this reflex (Jones et al., 1998).

Respiratory modulation of sympathetic activity

The ongoing activity in sympathetic nerves is also modulated by respiration (Adrian et al., 1932). This modulation is due in part to the oscillations in arterial blood pressure that accompany ventilation which result in respiratory-related changes in baroreceptor and efferent sympathetic nerve activity (Pilowsky, 1995). However, since respiratory modulation of sympathetic activity is still present in vagotomised, paralysed, artificially ventilated animals, it is clear that additional mechanisms are involved (Miyawaki et al., 1995).

Whole nerve recordings have shown that different patterns of respiratory modulation are present in different sympathetic nerves. In the rat for example, cardiac, splanchnic and renal nerves show peak activity during early inspiration with a post-inspiratory or early expiratory dip, whereas nerves in the cervical or lumber sympathetic trunk have a post-inspiratory activation (Numao et al., 1987). In a number of studies, the discharge patterns of individual sympathetic preganglionic neurones have been analysed in relation to phrenic nerve activity and have been shown to display a variety of respiratory-related rhythmicities (Gilbey et al., 1986; Zhou and Gilbey, 1992). The peak firing of individual SPNs was found to occur either during inspiration, post-inspiration or expiration (Zhou and Gilbey, 1992). In addition, some SPNs had a firing pattern unrelated to phrenic nerve activity. Whilst it has been postulated that the activity of SPNs is

influenced by direct descending inputs from medullary respiratory neurones (Gilbey et al., 1986), there is as yet little direct evidence to support this. It has been suggested that the respiratory modulation of ongoing sympathetic nerve activity is generated at the brainstem level since sympathetic premotor neurones in the rostral ventrolateral medulla have been shown to display similar respiratory-related patterns of activity to those seen in SPNs. At least five different types of respiratory modulation have been described in bulbospinal sympathoexcitatory neurones of the RVLM: inspiratory depression, expiratory depression, early inspiratory activation, post-inspiratory activation and no modulation (see Pilowsky, 1995; McAllen 1987; Haselton and Guyenet, 1989a).

It has been suggested that a common cardiorespiratory oscillator is responsible for generating the rhythmic discharges in respiratory neurones, sympathetic neurones, and cardiac vagal preganglionic neurones (Richter and Spyer, 1990). Studies have shown however that whilst hyperventilation causes quiescence of phrenic nerve activity, it does not abolish the respiratory-related rhythms in the sympathetic nerve activity (Barman and Gebber, 1976). In addition, rhythmic sympathetic discharges have been recorded with frequencies different to that of the central respiratory rhythm (Johnson and Gilbey, 1996). These findings therefore suggest that respiratory and sympathetic activity are generated independently but, under normal conditions, the sympathetic oscillator is entrained by the respiratory network (see Habler et al., 1994). Coupling between the respiratory and sympathetic networks may take place at the RVLM. In this respect, anatomical studies have shown that inspiratory neurones of the VRG and expiratory neurones of the Botzinger complex project to neurones within the RVLM region (Pilowsky et al., 1994; Sun et al., 1997).

The sympathetically mediated responses evoked by brief baroreceptor stimulation have also been shown to vary with the phase of respiration. In vagotomised dogs, the evoked lumber sympathoinhibition and skeletal muscle vasodilatation were more pronounced during expiration (Seller et al 1968). In a similar preparation, the bradycardia evoked by baroreceptor or chemoreceptor

stimulation was also more marked during the expiratory phase (Davis et al., 1977). The exact mechanism for the inspiratory suppression of the reflex cardiac and vascular sympathoinhibition is not known, however, it may simply be due to the simultaneous excitatory effects of central inspiratory drive on the background sympathetic nerve activity.

In animals in which the vagus nerves remain intact, stimulation of slowly adapting pulmonary stretch receptors during lung inflation has been shown to reflexly inhibit sympathetic pre-ganglionic neuronal activity (Lipski et al., 1977b). The vasoconstriction evoked by chemoreceptor stimulation is attenuated by increased pulmonary stretch receptor input, however the vasodilatation or vasoconstriction evoked by stimulating or unloading the arterial baroreceptors is unaffected (Daly et al., 1986). The exact mechanism by which pulmonary stretch receptor afferents modulates sympathetic activity remains unknown.

Modulation of cardiorespiratory reflexes from higher brain areas

Cardiovascular reflexes are susceptible to modulation from many higher brain regions, however, the modulatory effects of the hypothalamus and amygdala have been most commonly studied (see Jordan, 1990). Stimulation of the perifornical region of the hypothalamus produces a pattern of autonomic responses referred to as the 'defence reaction', since they resemble the responses to threatening or aversive stimuli in conscious animals (see Spyer, 1996). Both the baroreceptor and pulmonary C-fibre reflexes are powerfully inhibited during the defence response (Coote et al., 1979; Jones et al., 1994b). In the case of the baroreceptor reflex, this is due in part to interactions at the level of the NTS. Stimulation within the hypothalamus inhibits NTS neurones receiving baroreceptor inputs via a GABAergic mechanism (Mifflin et al., 1988b; Jordan et al., 1988). NTS neurones receiving chemoreceptor inputs are however excited by electrical stimulation of the hypothalamus confirming suggestions that the pressor response evoked by the defence reaction may be

due in part to facilitation of the arterial chemoreceptor reflex (Hilton and Joels, 1965; Silva-Carvalho et al., 1993).

In rabbits, exposure to aversive stimuli can evoke the 'playing dead response', characterised by a fall in blood pressure and heart rate accompanied by changes in respiration, most commonly an increase in respiratory frequency and decrease in tidal volume (Applegate et al., 1983). These responses appear to be due in part to a facilitatory effect of neurones from the central nucleus of the amygdala on NTS neurones receiving baroreceptor inputs (Cox et al., 1986). In addition, the bradycardia may also be due to direct descending inputs to CVPNs within the dorsal vagal nucleus (Schwaber et al., 1982; Cox et al., 1986).

Serotonergic modulation of cardiorespiratory reflexes

The modulatory effects of 5-HT on cardiorespiratory reflexes has been demonstrated in a number of studies. In anaesthetised rats, raising central levels of 5-HT by intracerebroventricular administration of 5-HT or specific serotonin reuptake inhibitors attenuates the reflex bradycardia evoked by i.v. adrenaline (Lin et al., 1980). Conversely, in rats and rabbits, depletion of endogenous 5-HT within the NTS or brainstem regions potentiates the reflex bradycardia and sympathoinhibition evoked by stimulation of baroreceptor afferents (Itoh et al., 1992; Head and Korner, 1982). These findings suggest that while serotonergic neurones may not be integral parts of the neural pathway, they may exert inhibitory influences on baroreceptor reflex control.

The inhibitory effects of 5-HT on the baroreceptor reflex may be mediated via the activation of 5-HT_3 receptors. In both anaesthetised and conscious rats, microinjection of 5-HT or 5-HT_3 receptor agonists into the NTS significantly reduces the baroreceptor reflex-evoked bradycardia (Merahi et al., 1992b; Callera et al., 1997b). This effect is thought to be due to activation of presynaptic 5-HT_3 receptors located on vagal afferent terminals which, via release of glutamate, activates a local inhibitory GABAergic system. In this

respect, 5-HT_3 receptor activation has been shown to cause the release of glutamate within the NTS (Ashworth-Preece et al., 1995) and the inhibitory effects of 5-HT_3 receptor agonists can be attenuated by nodose ganglionectomy or by prior microinjection of bicuculline (Merahi et al., 1992b).

Presumably acting via a similar mechanism, microinjection of 5-HT_3 receptor agonists into the NTS also inhibits the reflex vagal bradycardia evoked by stimulation of cardiopulmonary and chemoreceptor afferents in anaesthetised rats (Sevoz et al., 1996b, 1997). Microinjection of 5-HT_3 receptor agonists into the NTS did not however affect the sympathoinhibition evoked by stimulation of baroreceptor of cardiopulmonary afferents (Nosjean et al., 1995; Sevoz et al., 1996b) and also had no effect on the sympathoexcitatory or respiratory responses evoked by stimulating chemoreceptor afferents (Sevoz et al., 1997).

In the previous studies, microinjection of 5-HT_3 receptor antagonists alone into the NTS had no effect on any of the cardiorespiratory reflexes evoked by stimulating chemoreceptor or cardiopulmonary receptor afferents, suggesting that these receptor subtypes are not tonically activated (Sevoz et al., 1996b, 1997). More recent studies have however provided contrasting data. In a similar preparation, intracisternal injection, or local microinjection into the NTS of the 5-HT_3 receptor antagonist granisetron attenuates the vagal bradycardia evoked by stimulating cardiopulmonary afferents (Pires et al., 1998). In addition, the reflex effects can be potentiated by i.c. injection of the 5-HT_3 receptor agonist PBG. Blockade of central 5-HT_3 receptors has also been shown to attenuate the reflex bradycardia evoked by stimulation of upper airway afferents in anaesthetised rabbits (Dando et al., 1995). Thus, in contrast to the previous findings, these results suggest that activation of 5-HT_3 receptors plays a facilitatory role in the reflex activation of cardiac vagal outflow.

Activation of 5-HT_1 receptors has also been shown to modulate cardiorespiratory reflexes. In the anaesthetised rat, i.c. injection of 5-HT_{1A} receptor antagonists including methiothepin and pindolol attenuate the vagal

bradycardia evoked by stimulation of cardiopulmonary afferents (Bogle et al., 1990). In rabbits, the vagal bradycardia evoked by stimulation of upper airway afferents can be attenuated by i.c. injection of the 5-HT_{1A} receptor antagonist WAY-100635 and potentiated by the 5-HT_{1A} receptor partial agonist buspirone (Futuro-Neto et al., 1993, Dando et al., 1998). Surprisingly, the 5-HT_{1A} receptor agonist 8-OH-DPAT inhibited the vagal bradycardia evoked by upper airway afferent stimulation in rabbits (Futuro-Neto et al., 1993). This effect has subsequently been found to be due to actions of 8-OH-DPAT on 5-$HT_{1B/1D}$ receptors as it can be blocked by prior administration of the 5-$HT_{1B/1D}$ receptor antagonist GR-127935 and mimicked by the 5-$HT_{1B/1D}$ receptor agonist sumatriptan (Dando et al., 1998). Therefore it appears that in rats and rabbits, 5-HT_{1A} receptor pathways play a facilitatory role in the reflex activation of cardiac vagal outflow whereas activation of 5-$HT_{1B/1D}$ receptors has inhibitory effects. Activation of 5-HT_{1A} and 5-$HT_{1B/1D}$ receptors has also been shown to have similar facilitatory and inhibitory effects on the reflex vagal bronchoconstriction evoked by inhaled capsaicin in anaesthetised guinea pigs (Bootle et al., 1998).

1.14 Aims of the present study

Previous studies in rabbits have shown that buspirone can also potentiate the vagal bradycardia evoked by stimulating cardiopulmonary afferents (Dando et al., 1994). In the present study we have sought to determine whether these modulatory effects of buspirone are indeed due to selective actions at central 5-HT_{1A} receptors. In addition, experiments have been performed in anaesthetised rabbits to determine if the cardiorespiratory responses evoked by stimulating baroreceptor and chemoreceptor afferents are similarly modulated by central 5-HT_{1A} receptor activation. Finally, studies have been performed to determine whether 5-HT_{1A} receptor-mediated mechanisms are tonically involved in the neural pathways controlling the cardiopulmonary, baroreceptor and chemoreceptor reflexes.

2 Methods

2.1 Surgical procedures

Anaesthesia

Experiments were performed on male New Zealand White rabbits (2.25 - 3.55 kg). The animals were anaesthetised using 1.5 g kg^{-1} urethane (25 % w/v; dissolved in distilled water) given intravenously via an ear vein over a period of approximately 15 minutes. Throughout the experiment, the level of anaesthesia was assessed by continuously monitoring the stability of arterial blood pressure, heart rate, phrenic nerve activity and also the absence of limb withdrawal in response to a paw pinch. Supplementary doses of urethane (0.15 g kg^{-1}) were given intravenously if required.

Body temperature was monitored using a rectal temperature probe and maintained between 37 and 38°C using a homeothermic blanket system (Harvard Apparatus).

Tracheal cannulation

The trachea was cannulated below the larynx using a polythene tube (4 mm external diameter, 3 mm internal diameter). This was attached to a Y-shaped adapter to enable connection to a ventilator (Harvard Apparatus) if required. Under normal experimental conditions, oxygen was passed across the face of the adapter so that the animal was allowed to breathe oxygen-enriched air spontaneously. The rate of oxygen delivery was controlled using a flow gauge (Platon).

Cannulation of brachial blood vessels

The right brachial artery was cannulated (Portex non-sterile tubing, 0.96 mm external diameter, 0.58 mm internal diameter) and connected to a pressure transducer (Gould Statham) to allow measurement of arterial blood pressure. The blood pressure signal was amplified (Grass Instruments 7P1) and

continuously displayed on a chart recorder (Grass Instruments RPS 7C8 Polygraph). Heart rate was derived electronically from the blood pressure wave using a tachometer (Grass Instruments 7P4) and was also displayed on the chart recorder.

The left brachial artery was cannulated (Portex non-sterile tubing 0.96 mm external diameter, 0.58 mm internal diameter) to allow arterial blood samples to be taken. 60 μl samples were collected in heparinised capillary tubes (Hawksley & Sons Ltd) and the blood gas content and pH were measured at regular intervals (Ciba-corning 238 pH/Blood Gas Analyser). Arterial blood gases were kept within the ranges PaO_2 100-140 mmHg, $PaCO_2$ 25-40 mmHg, $[HCO_3^-]$ 12-20 mM l^{-1}, pH 7.3-7.45 by adjusting the oxygen flow rate across the face of the tracheal cannula and by slow intravenous infusions of sodium bicarbonate (1.0 M).

The right and left brachial veins were cannulated (Portex non-sterile tubing, 0.96 mm external diameter, 0.58 mm internal diameter) to allow injection of drugs and an infusion respectively. The infusion solution consisted of 50 ml distilled water and 50 ml gelofusin and contained 0.84% (w/v) sodium bicarbonate and 0.2% (w/v) glucose. This solution, which helped to prevent non-respiratory acidosis and maintain blood volume, was administered at a rate of 6 ml kg^{-1} hr^{-1} using an infusion pump (Watson Marlow 502S).

Cannulation of other blood vessels

A cannula (Portex non-sterile tubing, 0.8 mm external diameter, 0.4 mm internal diameter) was inserted into the right external jugular vein and advanced 7 cm so that its tip lay within or close to the right atrium. A small cannula (Portex non-sterile tubing, 0.61 mm external diameter, 0.28 mm internal diameter) was also inserted into the left lingual artery and advanced 2.5 cm so that the tip lay close to the carotid bifurcation. In a number of animals, the correct positioning of the cannulae was confirmed by post-mortem examination.

Cannulation of the bladder

To gain access to the bladder an incision was made in the lower abdomen. The bladder was emptied using a syringe and 19 gauge needle. A cannula (polythene tubing, external diameter 35 mm, internal diameter 25 mm) was inserted so that urine could drain freely thus preventing any reflexes arising from this organ. The abdomen wall was closed using 4/0 suture, and covered with saline-moistened cotton wool.

Exposure of atlanto-occipital membrane

Using a pedal-operated drill (Citenco), small indentations were made in the left and right zygomatic arches. This enabled the head of the animal to be positioned in a head holder (made by RFH medical engineering) and held securely. A midline incision was then made in the skin from the skull to the lower cervical vertebrae. The underlying muscle was removed by blunt dissection and cautery so as to expose the atlanto-occipital membrane. During the experimental protocol, the membrane was pierced with a 25 gauge needle connected to a Hamilton syringe by a length of polythene tubing (Portex non sterile tubing, external diameter 0.8 mm, internal diameter 0.4 mm). This enabled drugs to be administered intracisternally (i.c.) in a volume of 20 μl over a period of 20 s.

Intracisternal injection of dye

The extent of drug diffusion within the cerebrospinal fluid following intracisternal administration was analysed in a number of animals by injecting 20 μl of Pontamine sky blue dye i.c. (20 mg ml^{-1}, dissolved in 0.5M sodium acetate). After 50 minutes, the animals were killed by anaesthetic overdose and the brain removed and fixed in 10% formal saline.

Exposure of the phrenic nerve

By retracting the overlying muscles, the left phrenic nerve was exposed low in the neck using a lateral approach. The nerve was dissected from the surrounding medium, cleaned of excess connective tissue and then placed on a bipolar silver wire recording electrode and crushed peripherally to eliminate the possibility of recording activity from any afferent fibres. The nerve and electrode were covered with President light body dental polyvinylsiloxane (Coltene UK) which solidified around the arrangement. This prevented the nerve from drying out or excess fluid accumulating and short-circuiting the electrode. Phrenic nerve activity was amplified (Digitimer NL 104; gain 10-20 K), filtered (Digitimer NL 125; 200-10,000 Hz) and in some experiments integrated using an E.M.G. integrator (Digitimer NL 703) which rectified and smoothed the signal using a 20 ms time constant.

Exposure of the aortic nerve

The left aortic nerve was also exposed low in the neck and dissected away from the vago-sympathetic trunk. The identity of the aortic nerve was confirmed by recording its ongoing activity using a bipolar electrode. The nerve was then placed on a bipolar stimulating electrode and covered with President light body dental polyvinylsiloxane.

Exposure of the renal nerve

The left kidney was exposed retroperitoneally through a left flank incision. The kidney was deflected laterally to reveal the renal vein and artery and the renal nerves that run between these vessels. A nerve was dissected free of the surrounding tissue, placed on a bipolar silver wire recording electrode and crushed peripherally. As with the other nerves, the renal nerve and electrode were covered with President light body dental polyvinylsiloxane. Renal nerve activity was amplified (Digitimer NL 104; gain 10-20 K), filtered (Digitimer NL

125; 100-1500 Hz) and integrated using an E.M.G. integrator (Digitimer NL 703) which rectified and smoothed the signal using a 20 ms time constant.

Electrocardiogram recording

The electrocardiogram (ECG) was recorded as lead II using needle electrodes placed into the right fore foot, left hind foot and an earth attached to muscle in the neck. The signal was amplified (Digitimer NL 104; gain 10-20 K) and filtered (Digitimer NL 115; 10-100 Hz) and a digital pulse was produced at each R wave using an amplitude discriminator (Digitimer NL 201).

2.2 Stimulation of cardiorespiratory reflexes

Stimulation of cardiopulmonary afferents using phenylbiguanide

The cannula inserted in the left external jugular vein was filled with a solution of phenylbiguanide (PBG) (1 mg ml^{-1}) and connected to a 100 μl Hamilton syringe. This enabled the stimulation of the cardiopulmonary afferents by bolus injections of PBG into the right atrium. Doses of PBG were selected that evoked sub-maximal bradycardias (7-40 μg kg^{-1}). In all experiments, at least seven and a half minutes were allowed to elapse between successive PBG injections in order to prevent tachyphylaxis.

Activation of baroreceptor afferents by electrical stimulation of the aortic nerve

Baroreceptor afferents arising from the aortic arch were activated by electrically stimulating the left aortic nerve. Rectangular pulses of 1 ms duration were delivered to the stimulating electrode by an isolated stimulator box (Digitimer DS2) which was triggered using a pulse generator (Digitimer D4030). In each animal, using test stimuli of fixed frequency (40 Hz), the stimulus intensity sufficient to evoke the maximal reflex bradycardia was determined. Thereafter, the intensity was set at a supramaximal level (5-8 V).

With the pulse duration and intensity held constant, the aortic nerve was stimulated at two minute intervals, for periods of 5 seconds, at increasing

frequencies from threshold levels to those causing maximal responses (5, 10, 20, 40, 80 and 160 Hz). In this way, the effects of drugs on both maximal and submaximal baroreceptor stimulation could be examined.

Stimulation of chemoreceptor afferents using sodium cyanide

The carotid body chemoreceptor afferents were stimulated by bolus injections of sodium cyanide (NaCN) solution (0.075-0.5%) through the catheter inserted in the left lingual artery using a 100 μl Hamilton syringe. Doses of NaCN were selected that evoked sub-maximal bradycardias (8-189 $\mu g\ kg^{-1}$). Small volumes of NaCN solution were injected so as to avoid stimulating carotid sinus baroreceptors. Ten minutes were allowed to elapse between successive chemoreceptor stimulations so as to prevent any desensitisation.

2.3 Data analysis

Data collection

Arterial blood pressure, E.C.G. and derived R wave pulses, phrenic and renal nerve activities (raw and integrated) were recorded onto a computer hard disk (Viglen IV/33) and optical disk (Panasonic LF7010) using a CED 1401plus interface and commercially available data collection software (CED Spike 2). In addition, data was recorded on to video tape via a digital interface (Instrutech VR-100A).

When a drug was injected or a reflex stimulus applied, a reference mark was made using a foot pedal. These marks were recorded onto hard disk along with the other variables and were used to determine the exact timing of a stimulus when performing the subsequent off-line analysis. Measurements of baseline and reflex responses were made using Spike 2 data analysis scripts specifically written for each variable (see Appendix sections 5.1 - 5.4).

Analysis of blood pressure

Systolic and diastolic pressures were measured 2 seconds before each reflex stimuli was applied (baseline) and then at the maximum reflex response. Mean arterial blood pressure (MABP) was calculated as diastolic pressure + (systolic pressure - diastolic pressure)/3. For each reflex response, changes in MABP (maximum response MABP - baseline MABP) were calculated.

Analysis of R-R interval

Using the R-wave pulses and a specifically written Spike 2 script, a plot of R-R intervals against time was obtained. Using this script, both the R-R interval 2 seconds before the reflex (baseline) and the maximum R-R interval attained during the reflex were measured. From these two measurements, the reflex change in R-R interval was obtained (maximum R-R interval - baseline R-R interval).

Analysis of phrenic nerve activity

Using either the raw or integrated phrenic nerve activity trace, the number of phrenic bursts was counted for 30 seconds before a stimulus mark to obtain the baseline phrenic burst rate (bursts min^{-1}). For the cardiopulmonary and chemoreceptor reflex, a count of the phrenic bursts was also made for 30 seconds after the stimulus mark to obtain the reflex phrenic burst rate. Any reflex changes in phrenic burst rate could then be calculated (reflex burst rate - baseline phrenic burst rate). In the case of the baroreceptor reflex a similar procedure was carried out, however, due to the shorter nature of the reflex responses, the phrenic bursts were counted for 10 seconds before and after the stimulus mark.

Analysis of renal nerve activity

Using a Spike 2 script, the level of integrated renal nerve activity (IRNA) was measured for 30 seconds directly before a stimulus and averaged to give a

mean baseline value of IRNA. This was also performed for the 30 seconds following the reflex stimulus. From these measurements, reflex changes in renal nerve activity were calculated (mean reflex IRNA - mean baseline IRNA). In the case of the baroreceptor reflex a similar procedure was carried out however, due to the shorter nature of the reflex responses, averages were made over 10 second periods.

Since the absolute values of IRNA varied substantially between animals, in each experiment, the mean control values for baseline IRNA and the reflex change in IRNA were normalised to 100%. Any subsequent changes in these variables were expressed as percentages of this value.

2.4 Experimental protocols

After completion of the surgery, animals were allowed to stabilise for at least 30 minutes. Experiments were only performed in animals in which the baseline variables were stable. Only those experiments in which stable control reflexes were obtained have been included in these studies.

In all experiments, the sympathetic activity to the heart was blocked by pre-treatment with the selective β_1 adrenoceptor antagonist atenolol (1 mg kg^{-1} i.v.). Atenolol was used since it does not bind to 5-HT receptors (Middlemiss et al., 1977) and poorly penetrates the central nervous system (Street et al., 1979). In the presence of such a sympathetic blockade, changes in R-R interval are indicative of changes in cardiac vagal activity. To confirm that this dose of atenolol was adequate to block the sympathetic drive to the heart throughout the duration of the experiments, atropine methylnitrate was given at the end of the longest protocol (107.5 minutes, protocol 1b) and the reflex stimulations were repeated. After treatment with atropine, the reflex increases in R-R interval evoked by stimulating baroreceptor or cardiopulmonary afferents were abolished.

All i.c injections of test drugs and vehicle were given in a volume of 20 μl over a period of 20 seconds. Test drugs injected i.v. were given in a volume of 1 ml also over a period of 20 seconds.

Schematic diagrams of the different protocols used are shown in figures 2.1-2.4.

Protocol 1

In protocol 1 (figure 2.1 a), the effects of the 5-HT_{1A} receptor ligand buspirone were examined on the reflex responses evoked by stimulating cardiopulmonary afferents with PBG and baroreceptor afferents by electrical stimulation of the aortic nerve. Since the cardiopulmonary reflex has been previously shown to be modulated by buspirone (Dando et al., 1994), stimulation of these afferents in this protocol served as a positive control.

5 minutes after administration of atenolol, alternate PBG and aortic nerve stimulations were carried out to elicit the cardiopulmonary and baroreceptor reflexes respectively. 2.5 minutes after a PBG stimulation, the aortic nerve was stimulated at six frequencies (5, 10, 20, 40, 80 and 160 Hz at 2 minute intervals). 2.5 minutes after the last aortic nerve stimulation, another PBG stimulus was given and the cycle repeated.

After a control period consisting of three PBG stimulations and two sets of aortic nerve stimulations, the test drug or vehicle was administered and 5 minutes later, the alternate PBG and aortic nerve stimulations were resumed for a period of 45 minutes. In these experiments, the test drug was the 5-HT_{1A} receptor ligand buspirone administered either i.v. or i.c. and the vehicle control was saline administered i.c.

To determine whether the effects of buspirone were due to selective agonist actions at 5-HT_{1A} receptors, the above protocol was altered slightly in some experiments. In these experiments, i.c. injection of buspirone was performed 20 minutes after i.v. injection of the selective 5-HT_{1A} receptor antagonist WAY-100635 (figure 2.1 b).

Figure 2.1

Protocol 1

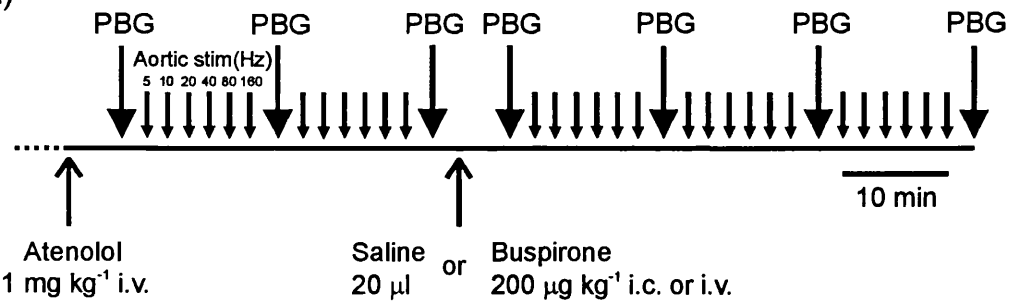
Schematic diagram illustrating the experimental protocol used to investigate:-

a) the effect of vehicle (saline) or buspirone applied i.c. or i.v. on the reflex responses evoked by stimulating cardiopulmonary and baroreceptor afferents.

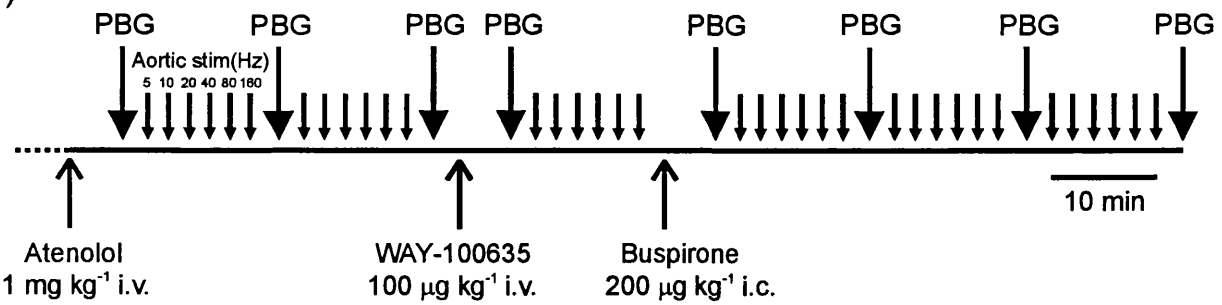
b) the effect of buspirone applied i.c. on the reflex responses evoked by stimulating cardiopulmonary and baroreceptor afferents following i.v. pre-treatment with WAY-100635.

Cardiopulmonary afferents were stimulated by right atrial injection of phenylbiguanide (PBG).
Baroreceptor afferents were activated by electrical stimulation of the left aortic nerve at 5, 10, 20, 40, 80 and 160 Hz.

a)



b)



Protocol 2

In protocol 2, the effects of the 5-HT_{1A} receptor ligand buspirone were examined on the reflex responses evoked by stimulating carotid chemoreceptor afferents using NaCN. Again the cardiopulmonary afferents were also stimulated in this protocol so as to provide a positive control.

5 minutes after the administration of atenolol, alternate PBG and NaCN stimulations were performed at 5 minutes intervals to elicit the cardiopulmonary and chemoreceptor reflexes respectively (figure 2.2 a).

After a control period consisting of three PBG and NaCN stimulations, the test drug or vehicle was administered and 5 minutes later, the alternate PBG and NaCN stimulations were resumed for a period of 35 minutes. As with protocol 1 a, the test drug was the 5-HT_{1A} receptor ligand buspirone administered either i.v. or i.c. and the vehicle control was saline administered i.c.

The above protocol was also modified in some experiments to determine whether the effects of buspirone were due to selective agonist actions at 5-HT_{1A} receptors. In these experiments, i.c. injection of buspirone was performed 20 minutes after i.v. injection of the 5-HT_{1A} receptor antagonist WAY-100635 (figure 2.2 b).

Figure 2.2

Protocol 2

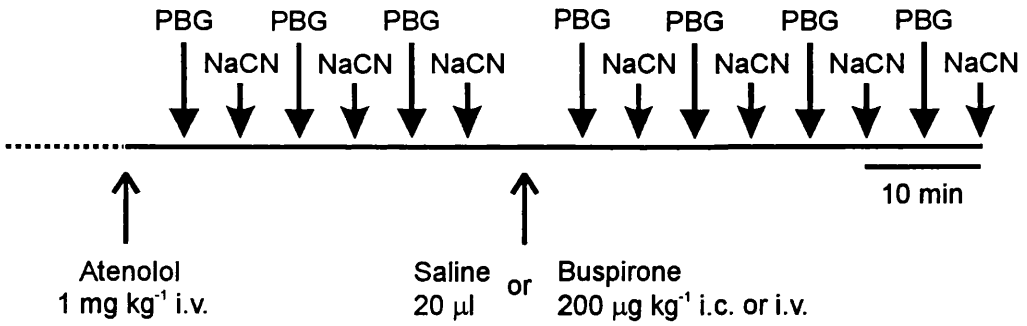
Schematic diagram illustrating the experimental protocol used to investigate:-

a) the effect of vehicle (saline) or buspirone applied i.c. or i.v. on the reflex responses evoked by stimulating cardiopulmonary and chemoreceptor afferents.

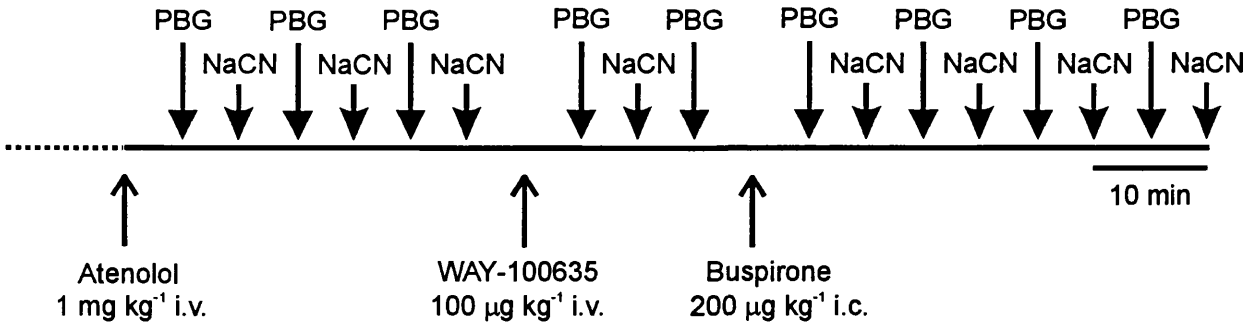
b) the effect of buspirone applied i.c. on the reflex responses evoked by stimulating cardiopulmonary and chemoreceptor afferents following i.v. pre-treatment with WAY-100635.

Cardiopulmonary afferents were stimulated by right atrial injection of phenylbiguanide (PBG).
Chemoreceptor afferents were stimulated by injection of sodium cyanide (NaCN) into the left lingual artery.

a)



b)



In protocols 3 and 4, the effects of intracisternal administration of the 5-HT_{1A} receptor antagonist WAY-100635 was examined on the three reflexes. Different protocols were required as pilot experiments suggested that the duration of action of WAY-100635 was much shorter than that of buspirone.

Protocol 3

In this protocol, alternate aortic nerve and NaCN stimulations were performed to evoke the baroreceptor and chemoreceptor reflexes respectively.

1.5 minutes after atenolol, the aortic nerve was stimulated at 80 Hz then 2 min later at 160 Hz. After a further 1.5 minutes a NaCN stimulation was carried out. This pattern of stimulations was repeated 10 minutes later, beginning 11.5 minutes after atenolol.

After a control period consisting of three sets of aortic nerve and NaCN stimulations, the 5-HT_{1A} receptor antagonist WAY-100635 was administered i.c. 1.5 minutes later, the same pattern of alternate aortic nerve and NaCN stimulations were repeated for a further 23.5 minutes (figure 2.3).

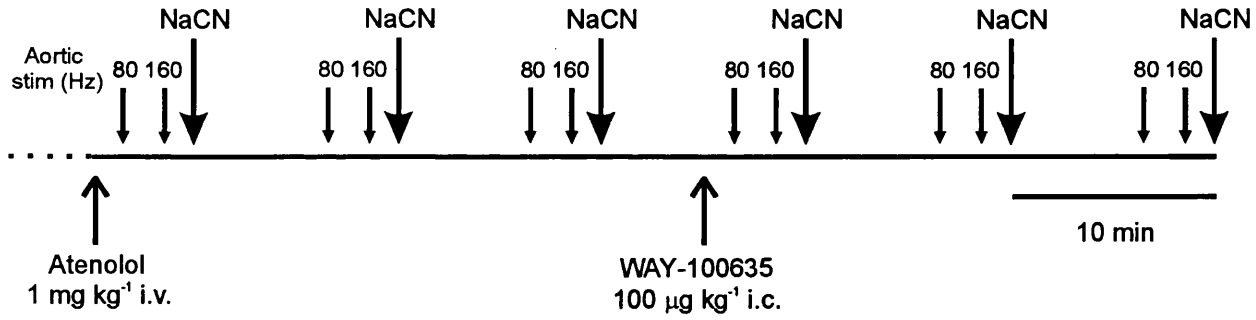
Figure 2.3

Protocol 3

Schematic diagram illustrating the experimental protocol used to investigate the effect of WAY-100635 applied i.c. on the reflex responses evoked by stimulating baroreceptor and chemoreceptor afferents.

Baroreceptor afferents were activated by electrical stimulation of the left aortic nerve at 80 and 160 Hz.

Chemoreceptor afferents were stimulated by injection of sodium cyanide (NaCN) into the left lingual artery.



Protocol 4

In this protocol, the effects of WAY-100635 on the cardiopulmonary reflex were examined.

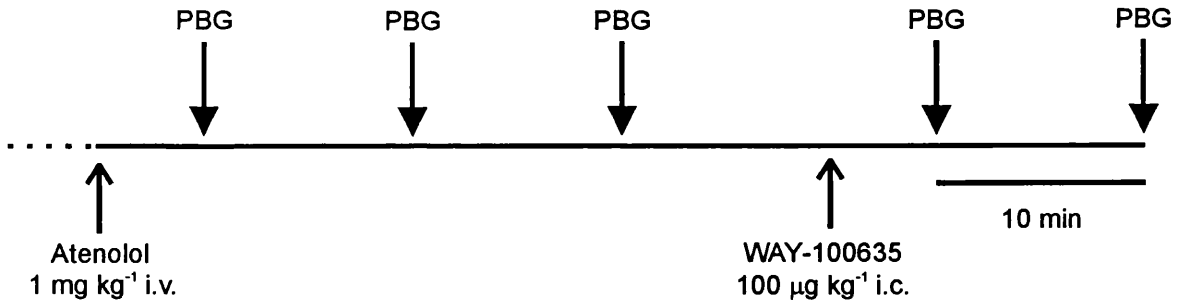
5 minutes after administration of atenolol, the cardiopulmonary afferents were stimulated with an injection of PBG. This was repeated at ten minute intervals and after a control period consisting of three PBG stimulations, WAY-100635 was administered i.c. PBG stimulations were then repeated 5 and 15 minutes after administration of the drug (figure 2.4).

Figure 2.4

Protocol 4

Schematic diagram illustrating the experimental protocol used to investigate the effect of WAY-100635 applied i.c. on the reflex responses evoked by stimulating cardiopulmonary afferents.

Cardiopulmonary afferents were stimulated by right atrial injection of phenylbiguanide (PBG).



2.5 Statistical analysis

In experimental protocols 1, 2 and 3 the two different reflexes tested were analysed separately, (in protocol 4 only one reflex was tested). The reflexes evoked during the control period of each protocol were averaged to give mean control responses for each reflex. The same was also carried out for the baseline values measured immediately before each reflex.

For each variable, drug-induced changes in baseline or reflex response size could then be calculated by subtracting the mean control values before the drug from the values at any time point after treatment with the drug. These changes, rather than the actual values, are shown in the graphs in the next section.

Protocol 1 & 2

In protocols 1 & 2, the changes in the baseline variables caused by the test drugs were compared with those caused by the vehicle control (saline) at matched time intervals using 2 way analysis of variance (ANOVA) and the least significant difference (LSD) test for comparisons between means (Sokal and Rohlf, 1967). The changes in the cardiopulmonary and chemoreceptor reflex responses caused by the test drugs were also compared with those caused by saline at the same time points using two-way ANOVA followed by the LSD test. In the case of the baroreceptor reflex responses, to account for the different frequencies of stimulation used, time-matched comparisons of drug-induced changes were made using three-way ANOVA followed by the LSD test.

This type of analysis allowed the effects of buspirone administered either intracisternally, intravenously or after pre-treatment with WAY-100635 to be compared with those caused by saline at the same time points after their administration. In the same way, the effects of buspirone administered intravenously or in the presence of the antagonist WAY-100635 have also be compared with those caused by intracisternal administration of buspirone alone.

Protocol 3

The changes in baseline variables and baroreceptor reflex responses caused by intracisternal administration of WAY-100635 have been compared with those caused by saline using the saline control data from protocol 1. Saline was found to have no significant effect on the baseline variables or baroreceptor reflex responses evoked over the period of the experiment in protocol 1 (Students paired t-test). Thus the comparisons between these two groups using three-way ANOVA and the LSD test were considered valid although not perfectly time matched.

The changes in baseline variables and chemoreceptor reflex responses caused by intracisternal administration of WAY-100635 have been compared with those caused by saline using the saline control data from protocol 2. Comparisons were made using two-way ANOVA and the LSD test. Again, the comparisons were not perfectly time matched but this was considered valid, as in protocol 2, saline was found to have no significant effect on any of the baseline or chemoreceptor reflex responses evoked over the period of the experiment (Students paired t-test).

Protocol 4

The changes in baseline variables and cardiopulmonary reflex responses caused by intracisternal administration of WAY-100635 were analysed using two-way ANOVA and the LSD test. Time matched comparisons were made with the effects of saline using the saline control data from protocol 2.

All values are expressed as mean ± standard error mean. Differences in the mean were taken as significant when $p<0.05$.

2.6 Drugs

The following drugs were dissolved in distilled water:-

Urethane (Sigma Chemicals Ltd., Poole, U.K.)

Sodium bicarbonate (BDH Ltd., Poole, U.K.)

D-Glucose (BDH Ltd., Poole, U.K.)

Sodium cyanide (BDH Ltd., Poole, U.K.)

Sodium acetate (BDH Ltd., Poole, U.K.)

The following drugs were dissolved in 0.9% saline:-

Atenolol (Sigma Chemicals Ltd., Poole, U.K.)

Atropine methylnitrate (Sigma Chemicals Ltd., Poole, U.K.)

Buspirone (8-(4-(4-(2-pyrimidinyl)-1-piperazinyl)butyl)-8-azaspirol (4,5)decane-7,9-dione hydrochloride; Sigma Chemicals Ltd., Poole, U.K.)

Phenylbiguanide (Sigma Chemicals Ltd., Poole, U.K.)

WAY-100635 (n-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-n-(2-pyrinidinyl)cyclohexanecarboxamide 3HCl; Research Biochemicals Inc., Semat, St. Albans, U.K.)

Other drugs:-

Gelofusin (B. Braun Medical Ltd., Aylesbury, U.K.)

Pontamine sky blue 6BX (BDH Ltd., Poole, U.K.)

3 Results

The four protocols used in the present studies have examined the effects of 5-HT_{1A} receptor ligands on the reflex responses evoked by stimulating cardiopulmonary, baroreceptor and chemoreceptor afferents. In the protocols in which two different reflexes were evoked in the same animal (protocols 1, 2 and 3), a sufficient time interval was allowed to elapse between successive reflex stimulations so as to enable cardiorespiratory parameters time to return to baseline levels. It is therefore unlikely that any interactions occurred between the different reflexes evoked and the effects of the drugs on the different reflex responses have therefore been analysed separately.

The effects of the 5-HT_{1A} receptor ligands on the different reflexes are described separately in the following sections.

In section 3.2, the effects of the ligands on the reflex responses evoked by stimulating cardiopulmonary afferents are summarised. This section contains data from protocol 1, in which the effects of saline i.c. and buspirone (i.c., i.v. and after pre-treatment with WAY-100635) were examined and data from protocol 4, in which the effects of i.c. WAY-100635 were investigated.

Section 3.3 summarises the effects of the ligands on the reflex responses evoked by baroreceptor afferent stimulation. This section contains data obtained from protocol 1, in which the effects of saline i.c. and buspirone (i.c., i.v. and after pre-treatment with WAY-100635) were examined and data from protocol 3, in which the effects of i.c. WAY-100635 were investigated.

In section 3.4, the effects of the ligands on the reflex responses evoked by stimulating carotid chemoreceptor afferents are described. This section contains data obtained from protocol 2, in which the effects of saline i.c. and buspirone (i.c., i.v. and after pre-treatment with WAY-100635) were studied and also data from protocol 3, in which the effects of i.c. WAY-100635 were examined.

In each protocol, baseline variables were also measured immediately prior to the stimulation of the different afferents thus enabling the effects of the drugs on

baseline cardiovascular and respiratory parameters to be analysed. A general description of the effects of the 5-HT_{1A} receptor ligands on baseline cardiorespiratory variables is given in the following section (3.1) using sample data from the four protocols. However, sections 3.2 - 3.4, also contain graphs illustrating the effects of the drugs on the baseline variables immediately prior to each reflex stimulation.

Tables of results showing the effects of the 5-HT_{1A} receptor ligands on baseline cardiorespiratory variables and reflex responses are presented in Appendix 5.5.

3.1 Effects of 5-HT_{1A} receptor ligands on baseline variables

The effects of buspirone administered intracisternally (i.c.) and intravenously (i.v.) on baseline cardiorespiratory variables were examined in protocols 1 and 2. In both protocols, the effects of i.c. saline and i.c. buspirone after i.v. pre-treatment with WAY-100635 were also investigated. Since the results of the above experiments were virtually identical in protocols 1 and 2, only data from protocol 1 is described below. For brevity, only the effects on baseline values 5, 20, 35 and 50 minutes after the drug are shown.

In protocols 3 and 4, WAY-100635 was administered intracisternally. In both protocols it had minimal effects on baseline parameters, however, only in protocol 3 did any effects reach significance and thus the results from these experiments are described below. The effects of WAY-100635 on the baseline values 1.5, 11.5 and 21.5 minutes after administration of the drug are shown.

Saline 20 μl i.c.

Administration of the vehicle saline (20 μl i.c.; n=5) had no significant effect on baseline R-R interval, mean arterial blood pressure, renal nerve activity or phrenic burst rate (figure 3.1 and table 5.1). The mean baseline variables measured before administration of saline were compared with those at each time point after saline using the Students paired t-test.

Buspirone 200 μg kg^{-1} i.c. (1.23 – 1.78 μmol)

Buspirone increased baseline R-R interval by 39 ± 11 ms 20 minutes after its administration (figure 3.1 and table 5.3). This was significant when compared to saline and remained increased for the duration of the experiment. Ongoing renal nerve activity was significantly increased after 5 minutes by 70 ± 38 %. This was associated with a significant increase in baseline mean arterial blood pressure of 6 ± 7 mmHg. After this time point, the ongoing level of renal nerve activity returned to control levels whereas baseline arterial blood pressure was reduced significantly below control levels. A maximal reduction in baseline arterial blood pressure of 17 ± 4 mmHg was observed after 20 minutes. At the same time, baseline phrenic burst rate was significantly increased by 26 ± 7 bursts min^{-1}.

WAY-100635 100 μg kg^{-1} i.v.

In protocol 1b, animals were pre-treated with an i.v. injection of WAY-100635 prior to an i.c. injection of buspirone. The effect of this pre-treatment on baseline values was therefore examined.

Intravenous administration of WAY-100635 had no significant effect on baseline R-R interval or mean arterial blood pressure when compared to saline (table 5.5). After 5 minutes, there was however a significant increase in ongoing renal nerve activity of 24 ± 6% and a decrease in baseline phrenic burst rate of 2 ± 1 bursts min^{-1}.

(1.14 – 1.33 μmol)

Buspirone 200 μg kg^{-1} i.c. (pre-treated with WAY-100635 100 μg kg^{-1}i.v.)

Pre-treatment with WAY-100635 attenuated the effects of buspirone on baseline R-R interval (figure 3.1 and table 5.7). In the presence of WAY-100635, buspirone still significantly increased baseline R-R interval by 10 ± 4 ms after 20 minutes. However, this was significantly less than the increase of 39 ± 11 ms produced at the same time by buspirone alone.

In figure 3.1 it can be seen that in the case of the other baseline variables, the effects caused by buspirone in the presence of the WAY-100635 did not

differ significantly from those caused by buspirone alone. However, at certain time points in between those shown in figure 3.1, pre-treatment with WAY-100635 did significantly attenuate some of the buspirone-mediated effects (see figure 3.13 and table 5.19). After 7.5 minutes, the initial increase in ongoing renal nerve activity of 76 ± 35 % caused by buspirone was significantly reduced to an increase of 23 ± 28 % by pre-treatment with WAY-100635. Also, after 37.5 minutes, the secondary fall in arterial blood pressure of 17 ± 2 mmHg was reduced to a fall of 7 ± 3 mmHg. At the same time, the buspirone-evoked increase in baseline phrenic burst rate of 33 ± 9 bursts min^{-1} was reduced to an increase of 13 ± 4 bursts min^{-1}.

Buspirone 200 μg kg^{-1} i.v.

Intravenous administration of buspirone had no significant effect on baseline R-R interval when compared to saline (figure 3.1 and table 5.9). Ongoing renal nerve activity was however reduced after i.v administration of buspirone reaching statistical significance after 20 minutes when the activity was reduced below control levels by 32 ± 7%. Intravenous administration of buspirone also reduced baseline mean arterial blood pressure by 15 ± 2 mmHg but this was significant only at 5 minutes. In contrast to i.c. administration, buspirone administered i.v. produced no initial increases in either ongoing renal nerve activity or baseline mean arterial blood pressure. Also, when administered i.v., buspirone did not significantly increase baseline phrenic burst rate. After 20 minutes, baseline phrenic burst rate was actually reduced below control levels by 4 ± 1 bursts min^{-1}.

WAY-100635 100 μg kg^{-1} i.c. (0.49 – 0.54 μmol)

Intracisternal administration of WAY-100635 had no significant effect on baseline R-R interval, phrenic burst rate or arterial blood pressure when compared to saline (figure 3.2 and table 5.23). However WAY-100635 significantly increased baseline renal nerve activity and this became significant

11.5 min after administration of the drug when an increase of 44 ± 17 % was observed..

A summary of the effects of the 5-HT_{1A} receptor ligands on baseline cardiorespiratory variables is given in table 3.1.

Table 3.1

Summary of the effects of 5-HT_{1A} receptor ligands on baseline mean arterial blood pressure (MABP), R-R interval, phrenic burst rate (PBR) and integrated renal nerve activity (IRNA).

0 = no significant change

↑ = significant increase within 20 minutes of drug administration

↓ = significant decrease within 20 minutes of drug administration

↑↓ = significant increase followed by significant decrease

Drug	MABP	R-R interval	PBR	IRNA
Buspirone i.c.	↑↓	↑	↑	↑
WAY-100635 i.v.	0	0	↓	↑
Buspirone i.c. / WAY-100635 i.v.	↑↓	↑	0	0
Buspirone i.v.	↓	0	↓	↓
WAY-100635 i.c.	0	0	0	↑

Figure 3.1

Histograms showing the effects of saline (20 μl i.c.; □; n=5), buspirone (200 μg kg^{-1} i.c.; ■; n=5), buspirone (200 μg kg^{-1} i.c.) 20 minutes after pre-treatment with WAY-100635 (100 μg kg^{-1} i.v.; ▨; n=5) and buspirone (200 μg kg^{-1} i.v.; ⊠; n=4) on baseline mean arterial blood pressure (MABP), R-R interval, phrenic burst rate (PBR) and integrated renal nerve activity (IRNA). Effects are shown at 5 min after administration of the drug or vehicle and thereafter at 15 minute intervals. Each column represents the mean change (Δ) from the pre-drug control values and the bars show s.e.mean.

Comparisons between groups have been made using two-way ANOVA and the least significant difference test.

*$p<0.05$, **$p<0.01$ compared to saline i.c.

†$p<0.05$, ††$p<0.01$ compared to buspirone i.c. alone.

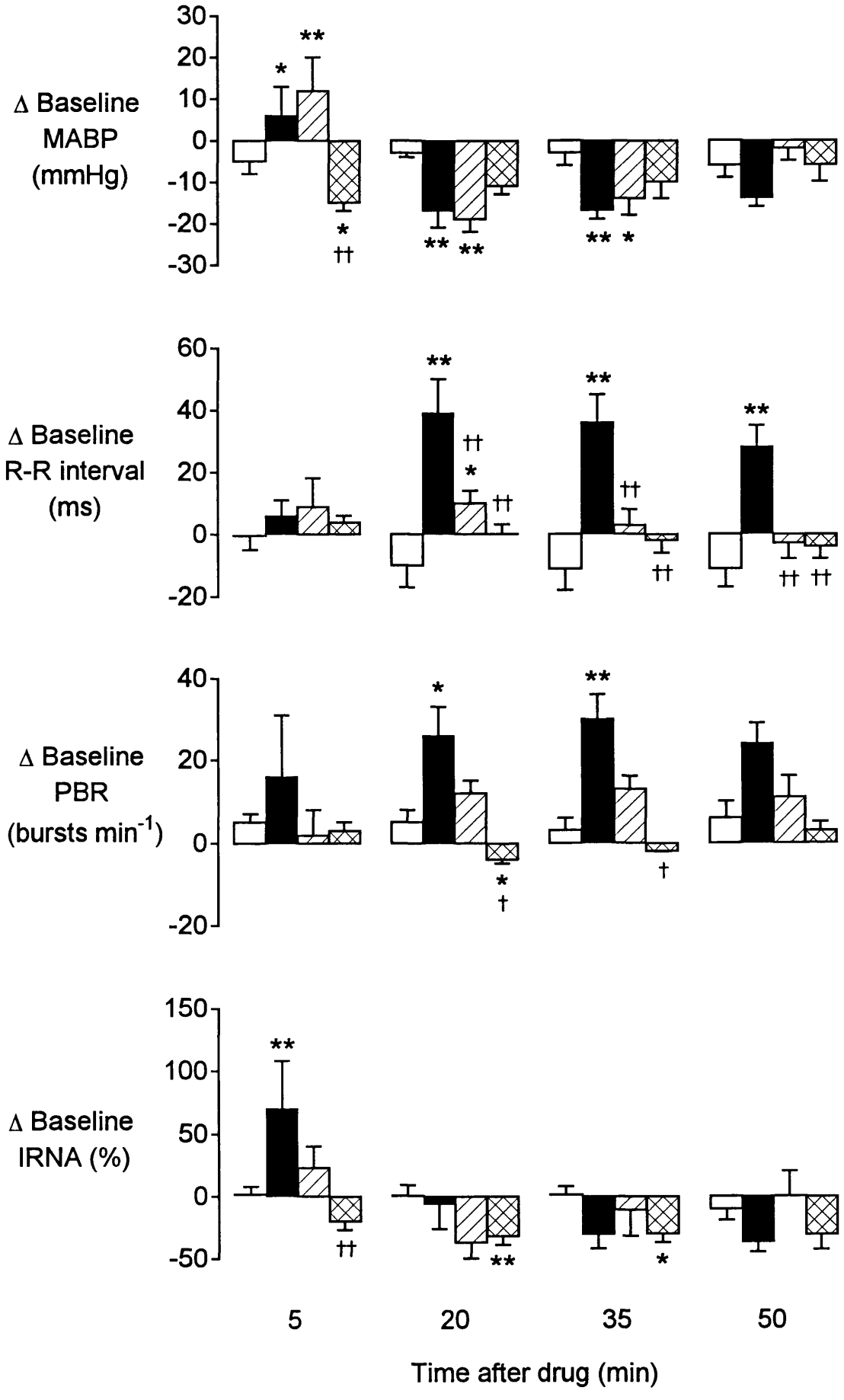
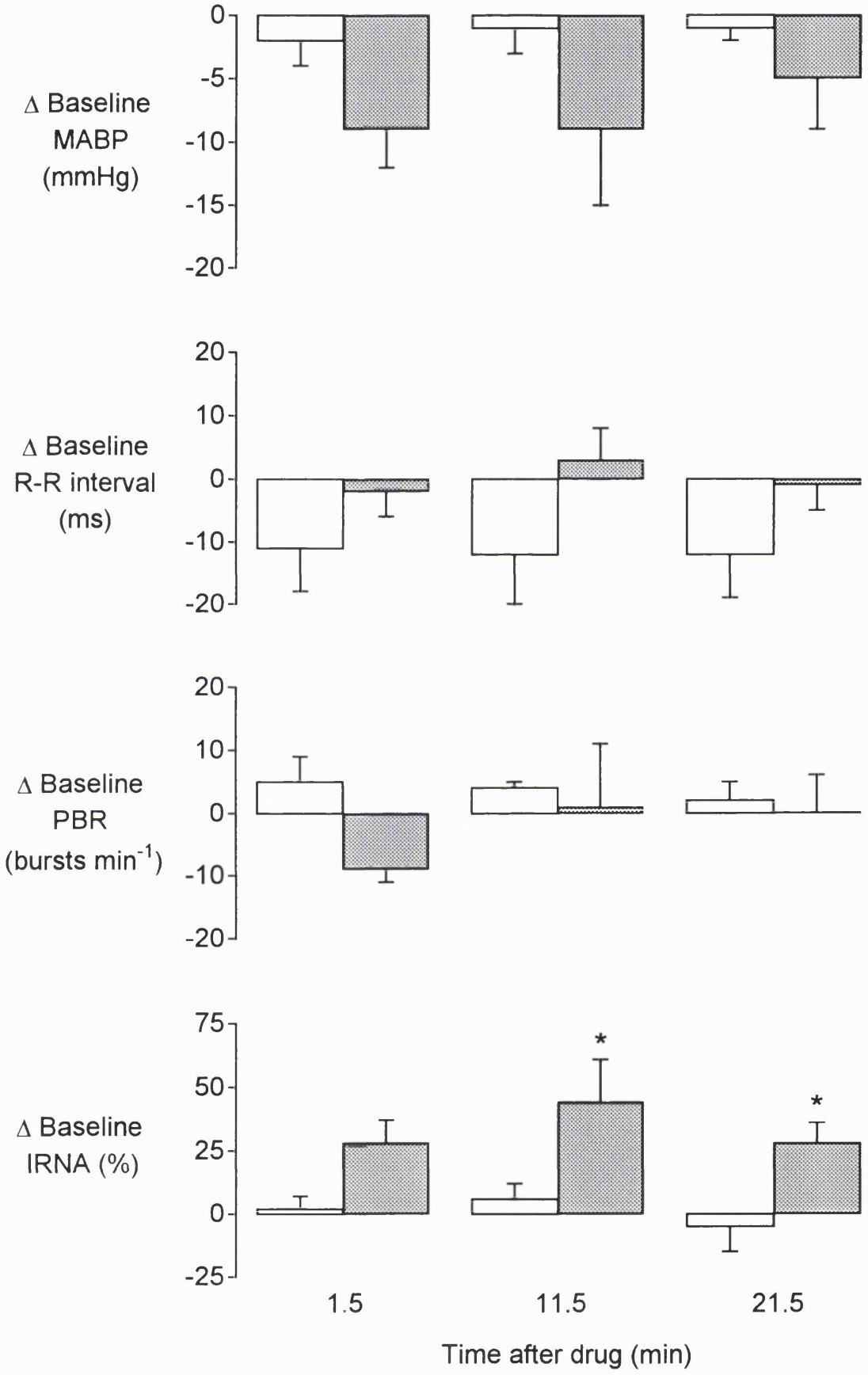

Δ Baseline MABP (mmHg)
30
20
10
0
-10
-20
-30
Δ Baseline R-R interval (ms)
60
40
20
0
-20
Δ Baseline PBR (bursts min-1)
40
20
0
-20
Δ Baseline IRNA (%)
150
100
50
0
-50
5
20
35
50
Time after drug (min)

Figure 3.2

Histograms showing the effects of saline (20 μl i.c.; □; n=5) and WAY-100635 (100 μg kg^{-1} i.c.; ▨; n=6) on baseline mean arterial blood pressure (MABP), R-R interval, phrenic burst rate (PBR) and integrated renal nerve activity (IRNA). Effects are shown at 1.5 min after administration of the drug and thereafter at 10 minute intervals. Each column represents the mean change (Δ) from the pre-drug control values and the bars show s.e.mean.

Comparisons between groups have been made using two-way ANOVA and the least significant difference test.

*$p<0.05$, **$p<0.01$, significant compared to saline i.c.


Δ Baseline MABP (mmHg)
0
-5
-10
-15
-20
Δ Baseline R-R interval (ms)
20
10
0
-10
-20
Δ Baseline PBR (bursts min-1)
20
10
0
-10
-20
Δ Baseline IRNA (%)
75
50
25
0
-25
*
*
1.5
11.5
21.5
Time after drug (min)

3.2 Effects of 5-HT$_{1A}$ receptor ligands on the cardiopulmonary reflex

Control stimulations

Stimulation of cardiopulmonary afferents by bolus injections of phenylbiguanide (7 - 40 μg kg^{-1}) into the right atrium of urethane anaesthetised rabbits evoked reflex changes in all variables measured. Taking the whole group of animals in which the cardiopulmonary reflex was elicited (n = 44; protocols 1, 2 and 4), control stimulations caused a reflex increase in R-R interval of 39 ± 3 ms from a baseline value of 240 ± 4 ms and a fall in the ongoing activity of the renal nerve of 46 ± 3 %. Mean arterial blood pressure was also reduced by 30 ± 1 mmHg from a baseline level of 81 ± 2 mmHg. Stimulation of cardiopulmonary afferents with PBG also caused an increase in phrenic nerve burst rate of 31 ± 2 bursts min^{-1} from a baseline rate of 71 ± 2 bursts min^{-1}.

The reflex changes in R-R interval, renal nerve activity and blood pressure usually occurred within 2 seconds of the injection of PBG and had returned to baseline levels within 30 seconds. The increase in phrenic nerve burst rate also usually occurred within 2 seconds however it often lasted longer than the other reflex effects and could take up to 2 minutes to return to normal baseline values. Control responses evoked by stimulating cardiopulmonary afferents are shown in figures 3.3, 3.4 and 3.9.

The effects of buspirone on the cardiopulmonary reflex were examined in protocols 1 and 2, however, the results obtained were virtually identical in both, therefore only those obtained from protocol 1 are described below. The results from protocol 4 in which the effects of i.c. WAY-100635 on the cardiopulmonary reflex were examined are also described.

Saline 20μl i.c.

After administration of the vehicle control saline, the reflex responses evoked by stimulating cardiopulmonary afferents with PBG were not significantly different from control stimulations (table 5.2). The reflex responses

obtained before administration of saline were compared with those at each time point after saline using the Students paired t-test.

Buspirone 200 μg kg^{-1} i.c. (1.23 – 1.78 μmol)

Intracisternal administration of the buspirone had significant modulatory effects on the reflex responses evoked by stimulating cardiopulmonary afferents when compared to saline (figure 3.6 and table 5.4). Buspirone potentiated the reflex increase in R-R interval evoked by bolus injection of PBG by 124 ± 56 ms 20 minutes after its administration. After 5 minutes, buspirone also significantly reduced the reflex renal sympathoinhibition and the hypotension by 69 ± 22 % and 12 ± 4 mmHg respectively. These inhibitory effects were also seen during the subsequent reflex stimulations. 5 minutes after i.c. administration of buspirone, the reflex increase in phrenic burst rate was significantly attenuated. At this time point, the reflex tachypnoea was 23 ± 7 bursts min^{-1} less than the control response.

Experimental traces showing the effects of buspirone after 20 minutes on the responses evoked by stimulating cardiopulmonary afferents with PBG are presented in figure 3.3.

WAY-100635 100 μg kg^{-1} i.v.

Pre-treatment with an intravenous injection of WAY-100635 had no significant effect on any of the reflex responses evoked by stimulating cardiopulmonary afferents when compared to saline (table 5.6).

(1.14 – 1.33 μmol)

Buspirone 200 μg kg^{-1} i.c. (pre-treated with WAY-100635 100 μg kg^{-1} i.v.)

Most of the effects of buspirone on the cardiopulmonary reflex were significantly reduced by pre-treatment with WAY-100635 (figure 3.6 and table 5.8). After pre-treatment with WAY-100635, buspirone still potentiated the reflex increase in R-R interval by 24 ± 9 ms after 20 minutes. This potentiation was

significant when compared to saline however, this was significantly less than the potentiation of 124 ± 56 ms produced by buspirone alone at the same time.

Pre-treatment with WAY-100635 significantly inhibited the effects of buspirone on the reflex renal sympathoinhibition. At 35 and 50 minutes after administration of buspirone alone, the reflex sympathoinhibitions were reduced by 45 ± 19 and 48 ± 18%. After pre-treatment with WAY-100635 the sympathoinhibitions evoked at the same times were actually increased by 21 ± 32 and 19 ± 27%. Pre-treatment with WAY-100635 also significantly attenuated the inhibition of the reflex hypotension caused by buspirone. After 5 minutes in the pre-treated group, the reflex hypotension was reduced by 1 ± 4 mmHg whereas buspirone alone caused a reduction of 12 ± 4 mmHg.

In addition, WAY-100635 also blocked the effect of buspirone on the tachypnoea evoked by stimulating cardiopulmonary afferents with PBG. In the buspirone alone group, the reflex tachypnoea was reduced by 23 ± 7 bursts min^{-1} after 5 minutes whereas in the pre-treated group, the respiratory response was only reduced by 3 ± 2 bursts min^{-1}.

Experimental traces showing the effects of i.c. buspirone after i.v. pre-treatment with WAY-100635 on the responses evoked by stimulating cardiopulmonary afferents are presented in figure 3.4.

Buspirone 200 $\mu g\ kg^{-1}$ i.v.

Intravenous administration of buspirone caused a small potentiation of the reflex increase in R-R interval after 5 minutes (figure 3.8 and table 5.10). This was significant when compared to saline, but was not statistically different from the effect caused by buspirone i.c. at the same time point. In contrast to buspirone administered i.c, buspirone i.v. had no significant effects on the reflex increases in R-R interval at any of the other time points.

Intravenous administration of buspirone reduced the reflex renal sympathoinhibitions. These effects were however later in onset than those caused by buspirone i.c. and only reached significance at 50 minutes when the

response was reduced by 36 ± 5%. 5 minutes after its administration, buspirone also significantly reduced the reflex hypotension evoked by cardiopulmonary afferent stimulation by 10 ± 1 mmHg. Similar effects were seen after 20 and 35 minutes. These effects did not differ significantly from those caused by buspirone administered i.c.

Similar to i.c. administration, buspirone i.v. significantly reduced the reflex increase in phrenic burst rate after 5 minutes. At this time point, the tachypnoea was reduced by 14 ± 8 bursts min^{-1}.

WAY-100635 100 $\mu g\ kg^{-1}$ i.c. (0.44 – 0.56 μmol)

Intracisternal administration of WAY-100635 attenuated all the effects of cardiopulmonary afferent stimulation on the variables recorded (figure 3.11 and table 5.12). 5 minutes after administration, the reflex increase in R-R interval was significantly reduced by 46 ± 6 ms. At this time point, WAY-100635 also reduced the reflex renal sympathoinhibitions, however this only reached statistical significance after 15 minutes when a decrease of 35 ± 12 % was observed. The reflex hypotensions evoked by cardiopulmonary afferent stimulation were significantly reduced 5 and 15 minutes after i.c. administration of WAY-100635 by 22 ± 4 and 9 ± 1 mmHg respectively. After 5 minutes, the reflex tachypnoea was also significantly reduced by 16 ± 8 bursts min^{-1}.

Experimental traces showing the effects of WAY-100635 i.c. after 5 minutes on the responses evoked by stimulating cardiopulmonary afferents are presented in figure 3.9.

The effects of the 5-HT_{1A} receptor ligands on the reflex responses evoked by stimulating cardiopulmonary afferents with PBG are summarised in table 3.2.

Table 3.2

Summary of the effects of 5-HT_{1A} receptor ligands on the reflex decrease in mean arterial blood pressure (MABP) and integrated renal nerve activity (IRNA) and the increase in R-R interval and phrenic burst rate (PBR) evoked by stimulating cardiopulmonary afferents with PBG.

0 = no significant change

↑ = significant increase within 20 minutes of drug administration

↓ = significant decrease within 20 minutes of drug administration

(↓) = significant decrease more than 20 minutes after drug administration

Drug	Decrease MABP	Increase R-R interval	Increase PBR	Decrease RNA
Buspirone i.c.	↓	↑	↓	↓
WAY-100635 i.v.	0	0	0	0
Buspirone i.c. / WAY-100635 i.v.	0	↑	0	0
Buspirone i.v.	↓	↑	↓	(↓)
WAY-100635 i.c.	↓	↓	↓	↓

Figure 3.3

The responses evoked by stimulating cardiopulmonary afferents with phenylbiguanide (PBG) during the control period and 20 minutes after administration of buspirone (200 μg kg^{-1} i.c.) in an atenolol (1 mg kg^{-1}) pre-treated rabbit.

From the top, the traces show arterial blood pressure (ABP), heart rate (HR), phrenic nerve activity (PNA) and integrated renal nerve activity (IRNA).

Constant doses of phenylbiguanide were injected into the right atrium at the points marked by the arrows.

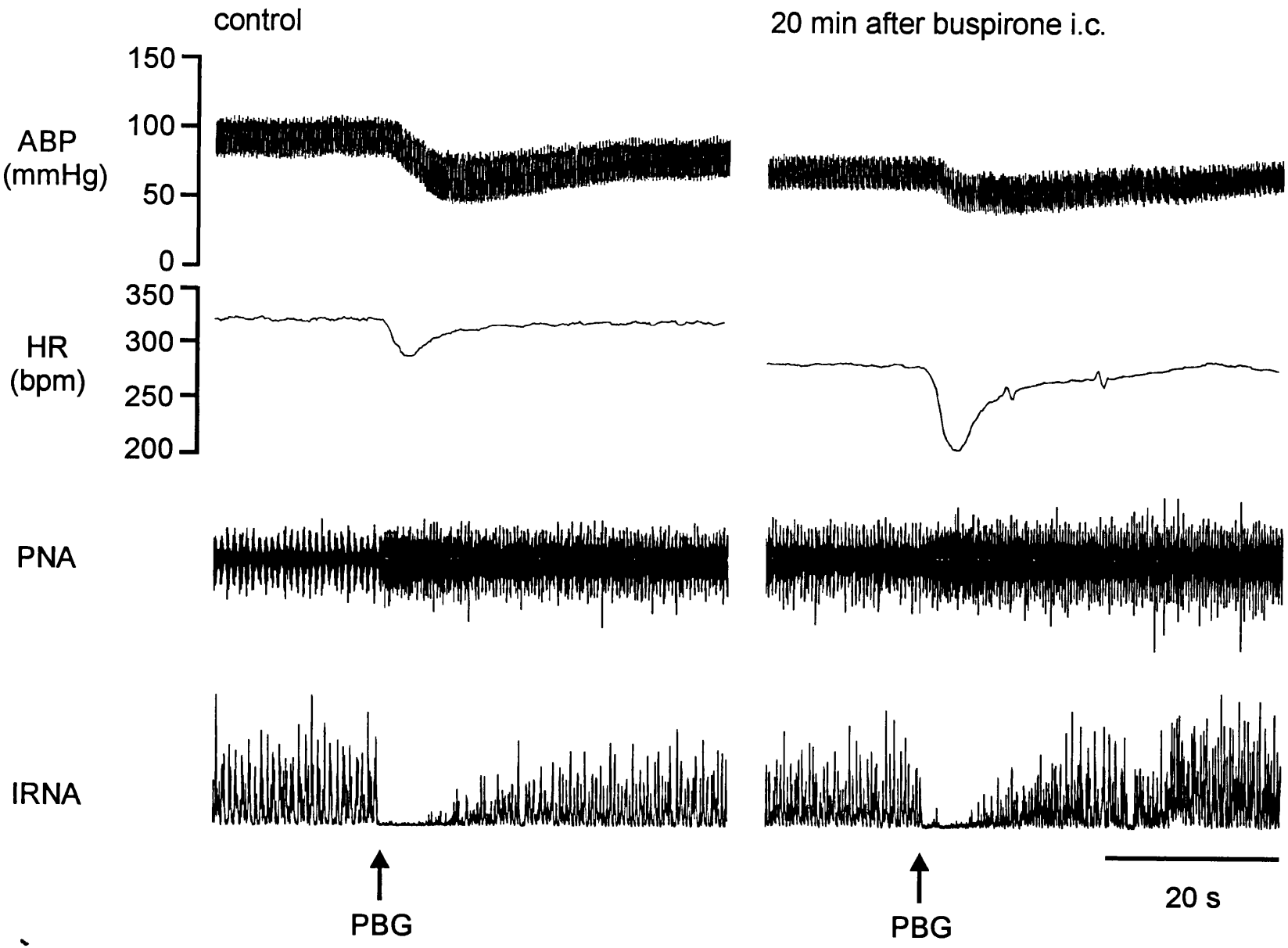

control
20 min after buspirone i.c.
150
100
50
0
ABP
(mmHg)
350
300
250
200
HR
(bpm)
PNA
IRNA
PBG
PBG
20 s

Figure 3.4

The responses evoked by stimulating cardiopulmonary afferents with phenylbiguanide (PBG) during the control period, 5 minutes after administration of WAY-100635 (100 μg kg^{-1} i.v.) and then 20 minutes after the subsequent administration of buspirone (200 μg kg^{-1} i.c.) in an atenolol (1 mg kg^{-1}) pre-treated rabbit.

From the top, the traces show arterial blood pressure (ABP), heart rate (HR), phrenic nerve activity (PNA) and integrated renal nerve activity (IRNA).

Constant doses of phenylbiguanide were injected into the right atrium at the points marked by the arrows.

Note that i.v. pre-treatment with WAY-100635 attenuates the potentiating effects of i.c. buspirone on the reflex bradycardia.

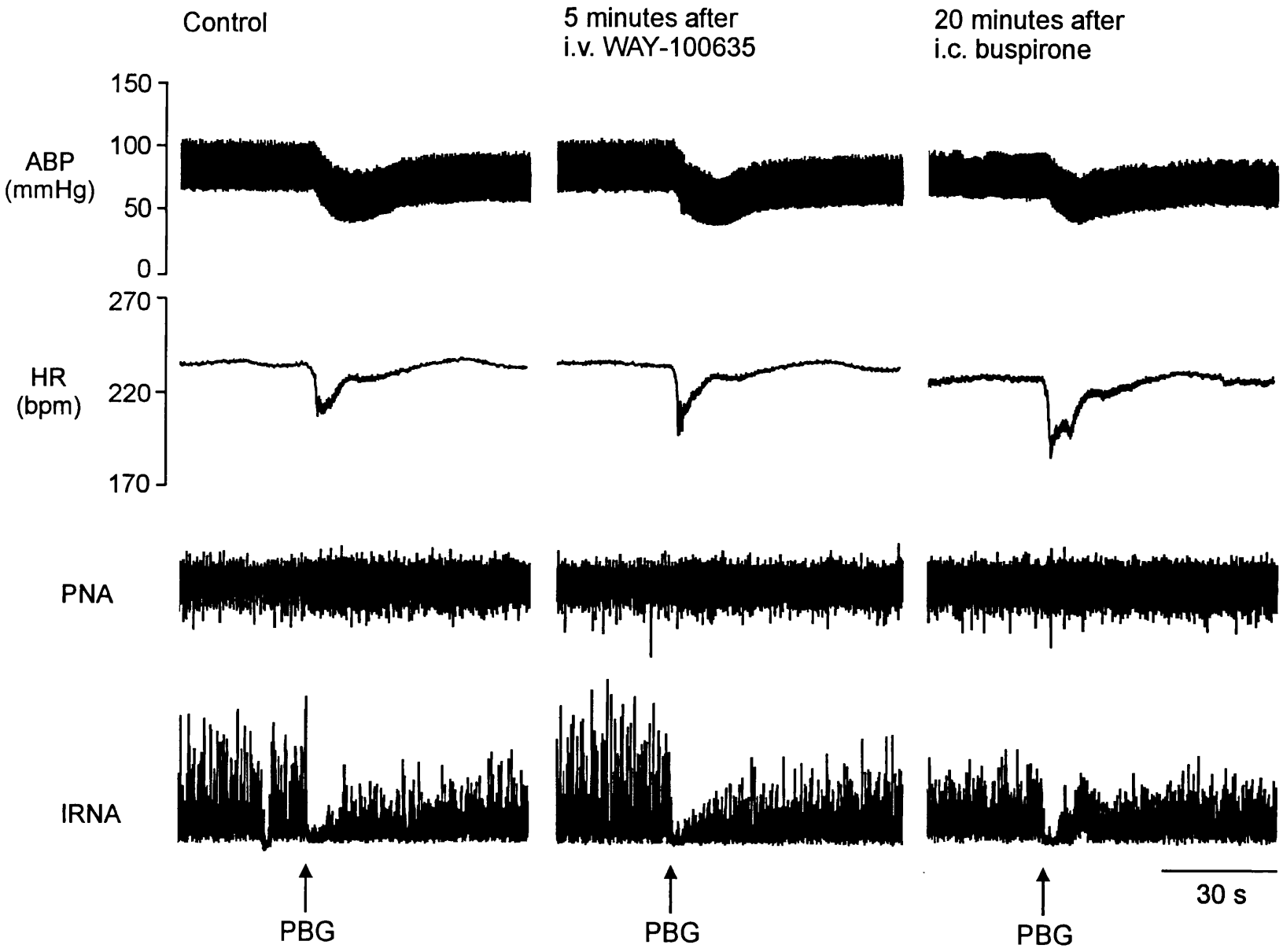

Control
5 minutes after
i.v. WAY-100635
20 minutes after
i.c. buspirone
150
100
50
0
ABP
(mmHg)
270
220
170
HR
(bpm)
PNA
IRNA
PBG
PBG
PBG
30 s

Figure 3.5

Histograms showing the effects of saline (20 μl i.c.; ☐ ; n=5), buspirone (200 μg kg^{-1} i.c.; ■ ; n=5) and buspirone (200 μg kg^{-1} i.c.) 20 minutes after pre-treatment with WAY-100635 (100 μg kg^{-1} i.v.; ▨ ; n=5) on baseline mean arterial blood pressure (MABP), R-R interval, phrenic burst rate (PBR) and integrated renal nerve activity (IRNA). Baseline variables were measured immediately prior to the stimulation of cardiopulmonary afferents (see figure 3.6). Effects are shown at 5 min after administration of the drug or vehicle and thereafter at 15 minute intervals. Each column represents the mean change (Δ) from the pre-drug control values and the bars show s.e.mean.

Comparisons between groups have been made using two-way ANOVA and the least significant difference test.

*$p<0.05$, **$p<0.01$ compared to saline i.c.

†$p<0.05$, ††$p<0.01$ compared to buspirone i.c. alone.

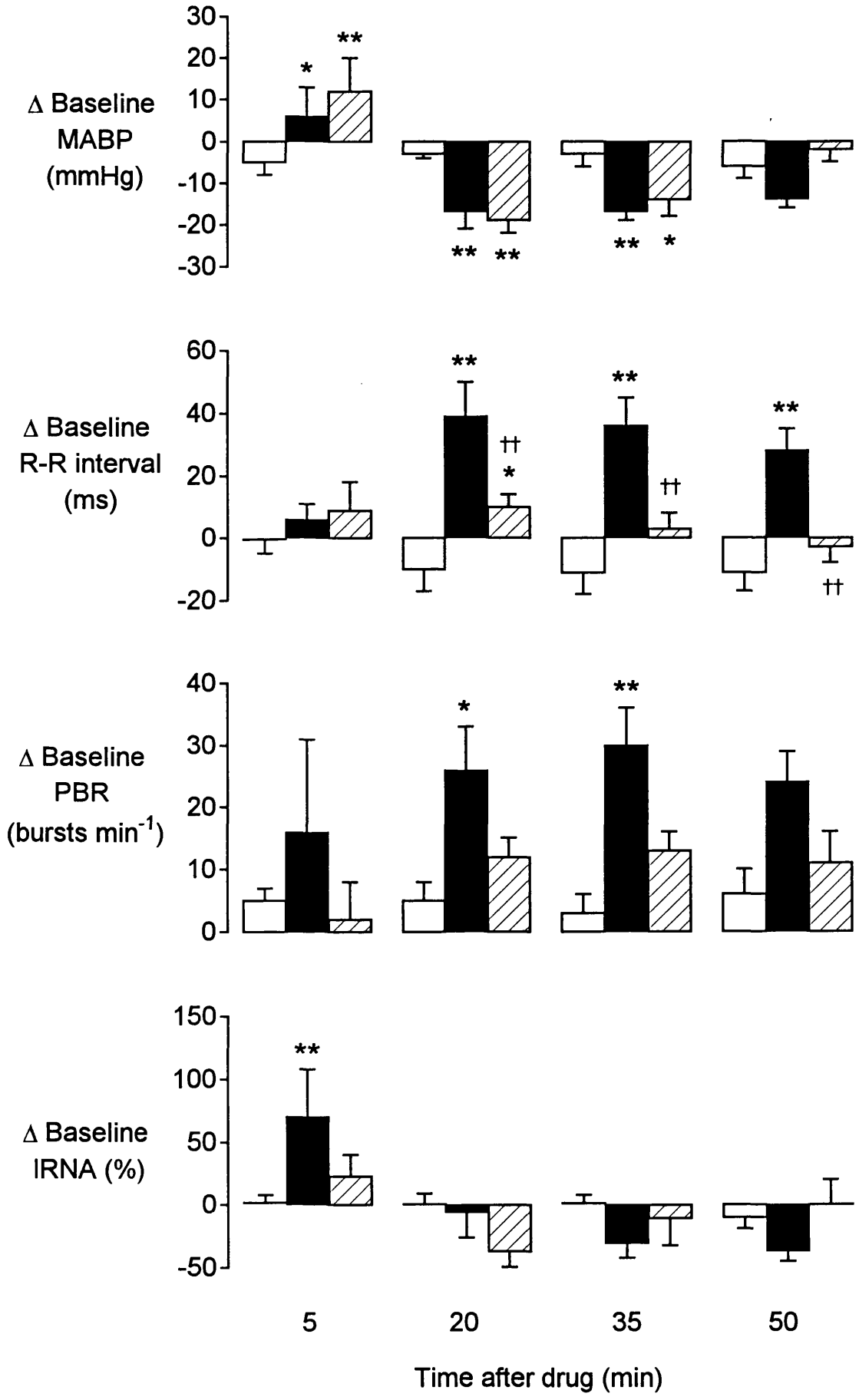

Δ Baseline MABP (mmHg)
30
20
10
0
-10
-20
-30
Δ Baseline R-R interval (ms)
60
40
20
0
-20
Δ Baseline PBR (bursts min-1)
40
30
20
10
0
Δ Baseline IRNA (%)
150
100
50
0
-50
5
20
35
50
Time after drug (min)

Figure 3.6

Histograms showing the effects of saline (20 μl i.c.; □ ; n=5), buspirone (200 μg kg^{-1} i.c.; ■ ; n=5) and buspirone (200 μg kg^{-1} i.c.) 20 minutes after pre-treatment with WAY-100635 (100 μg kg^{-1} i.v.; ▨ ; n=5) on the reflex increases in R-R interval and phrenic burst rate (PBR) and the decreases in mean arterial blood pressure (MABP) and integrated renal nerve activity (IRNA) evoked by stimulating cardiopulmonary afferents with PBG. Stimulations were performed 5 minutes after administration of the drug or vehicle and thereafter at 15 minute intervals. Each column represents the mean change (Δ) from the pre-drug control values and the bars show s.e.mean.

Comparisons between groups have been made using two-way ANOVA and the least significant difference test.

*$p<0.05$, **$p<0.01$ compared to saline i.c.

†$p<0.05$, ††$p<0.01$ compared to buspirone i.c. alone.

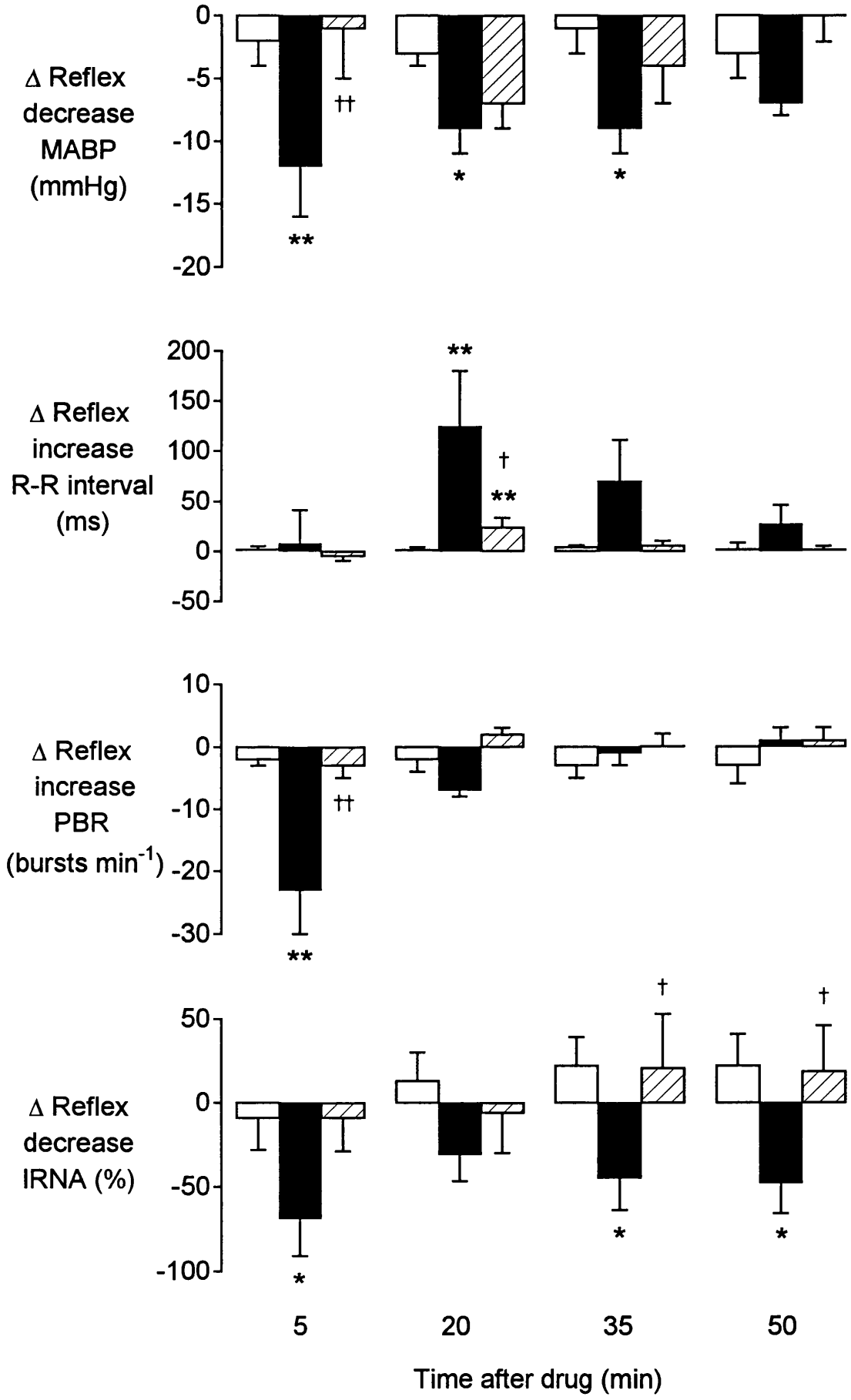

Δ Reflex decrease MABP (mmHg)
0
-5
-10
-15
-20
**
*
*
††
Δ Reflex increase R-R interval (ms)
200
150
100
50
0
-50
**
**
†
Δ Reflex increase PBR (bursts min-1)
10
0
-10
-20
-30
**
††
Δ Reflex decrease IRNA (%)
50
0
-50
-100
*
*
*
†
†
5
20
35
50
Time after drug (min)

Figure 3.7

Histograms showing the effects of saline (20 μl i.c.; □; n=5), buspirone (200 μg kg^{-1} i.c.; ■; n=5) and buspirone (200 μg kg^{-1} i.v.; ⊠; n=4) on baseline mean arterial blood pressure (MABP), R-R interval, phrenic burst rate (PBR) and integrated renal nerve activity (IRNA). Baseline variables were measured immediately prior to the stimulation of cardiopulmonary afferents (see figure 3.8). Effects are shown at 5 min after administration of the drug or vehicle and thereafter at 15 minute intervals. Each column represents the mean change (Δ) from the pre-drug control values and the bars show s.e.mean.

Comparisons between groups have been made using two-way ANOVA and the least significant difference test.

*$p<0.05$, **$p<0.01$, significant compared to saline i.c.

†$p<0.05$, ††$p<0.01$, significant compared to buspirone i.c.

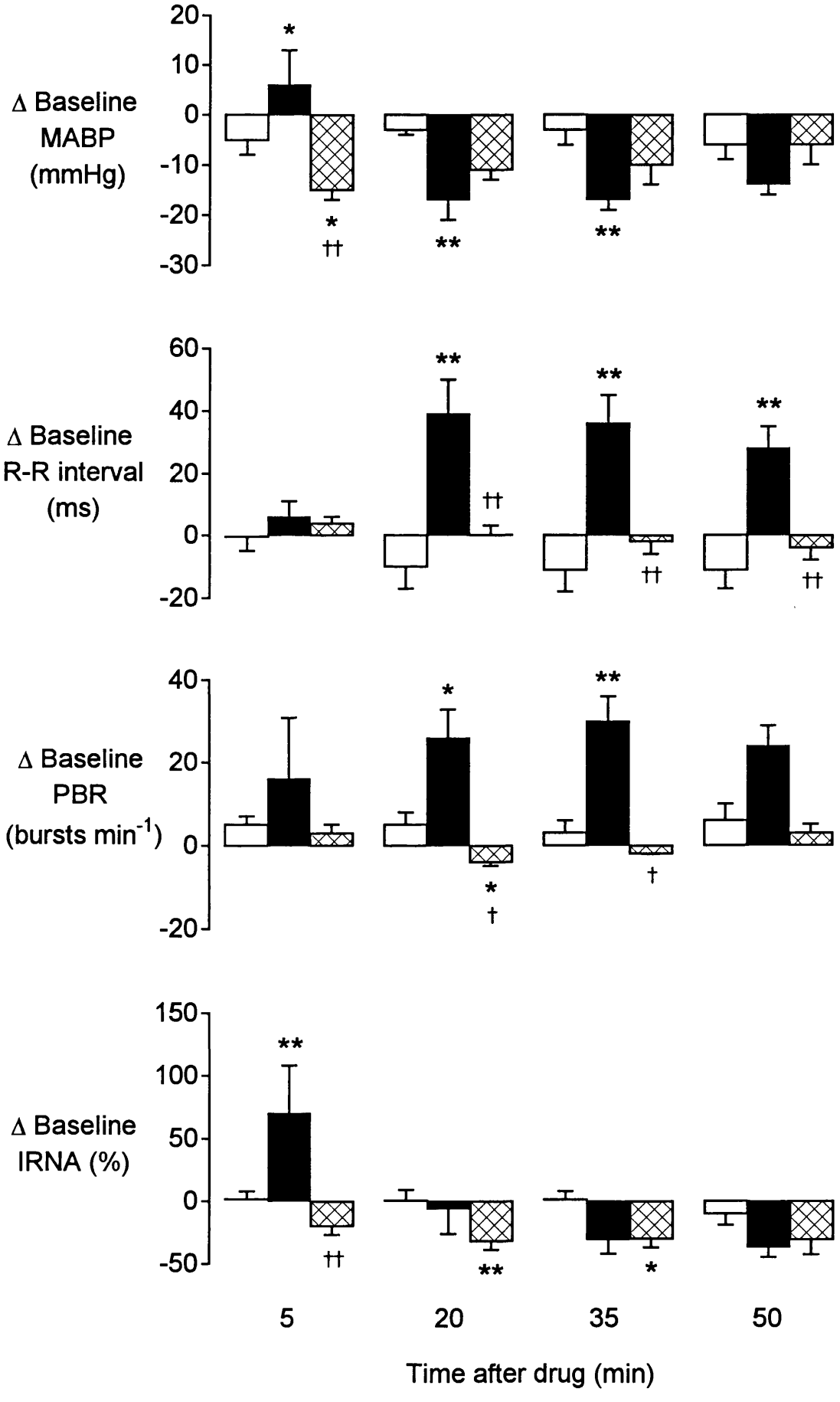

Δ Baseline MABP (mmHg)
20
10
0
-10
-20
-30
Δ Baseline R-R interval (ms)
60
40
20
0
-20
Δ Baseline PBR (bursts min-1)
40
20
0
-20
Δ Baseline IRNA (%)
150
100
50
0
-50
5
20
35
50
Time after drug (min)

Figure 3.8

Histograms showing the effects of saline (20 μl i.c.; □; n=5), buspirone (200 μg kg^{-1} i.c.; ■; n=5) and buspirone (200 μg kg^{-1} i.v.; ⊠; n=4) on the reflex increases in R-R interval and phrenic burst rate (PBR) and the decreases in mean arterial blood pressure (MABP) and integrated renal nerve activity (IRNA) evoked by stimulating cardiopulmonary afferents with PBG. Stimulations were performed 5 minutes after administration of the drug or vehicle and thereafter at 15 minute intervals. Each column represents the mean change (Δ) from the pre-drug control values and the bars show s.e.mean.

Comparisons between groups have been made using two-way ANOVA and the least significant difference test.

*$p<0.05$, **$p<0.01$, significant compared to saline i.c.

†$p<0.05$, ††$p<0.01$, significant compared to buspirone i.c.

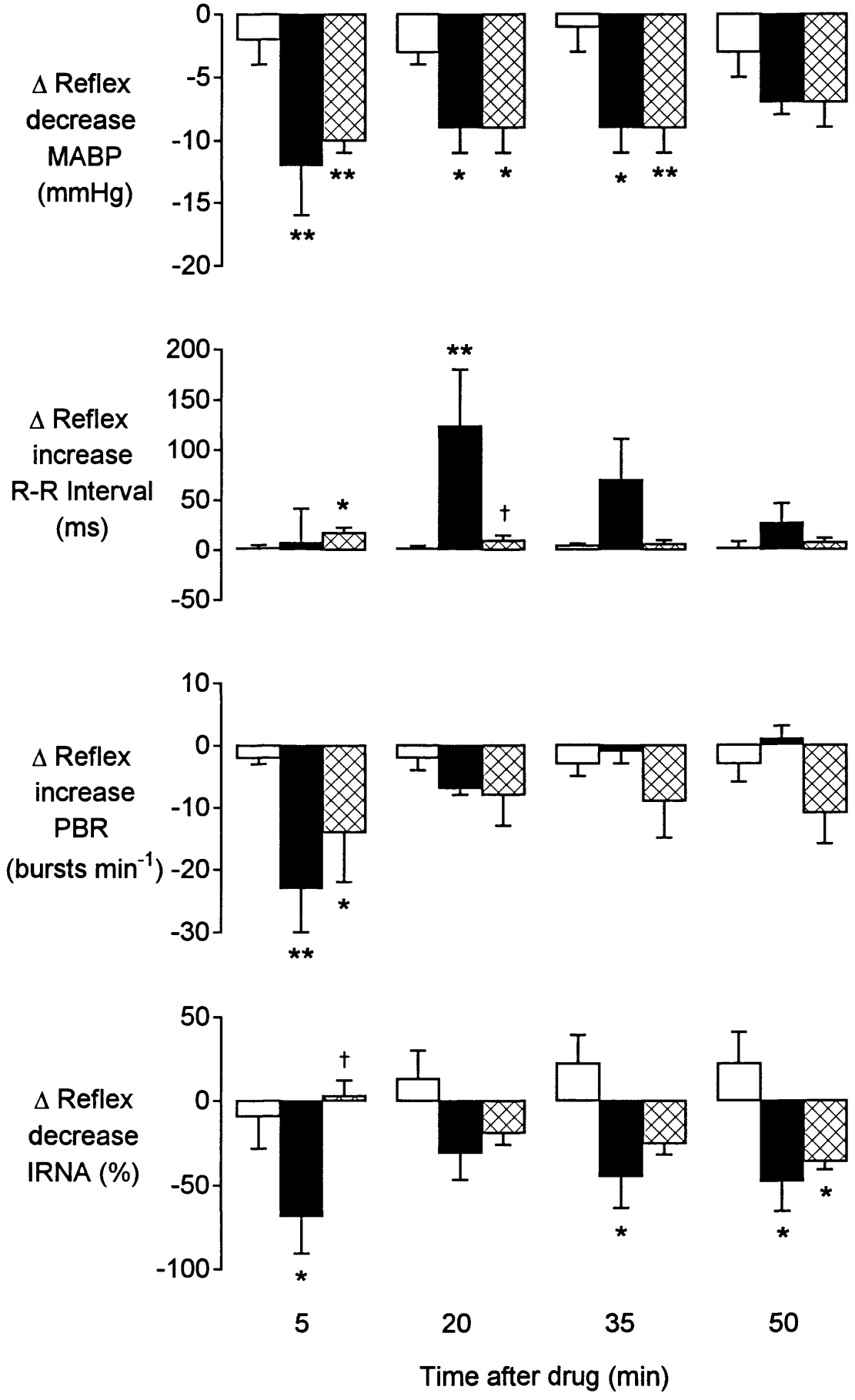

Δ Reflex decrease MABP (mmHg)
0
-5
-10
-15
-20
Δ Reflex increase R-R Interval (ms)
200
150
100
50
0
-50
Δ Reflex increase PBR (bursts min-1)
10
0
-10
-20
-30
Δ Reflex decrease IRNA (%)
50
0
-50
-100
5
20
35
50
Time after drug (min)

Figure 3.9

The responses evoked by stimulating cardiopulmonary afferents with phenylbiguanide (PBG) during the control period and 5 minutes after administration of WAY-100635 (100 μg kg^{-1} i.c.) in an atenolol (1 mg kg^{-1}) pre-treated rabbit.

From the top, the traces show arterial blood pressure (ABP), heart rate (HR), integrated phrenic nerve activity (IPNA) and integrated renal nerve activity (IRNA).

Constant doses of phenylbiguanide were injected into the right atrium at the points marked by the arrows.

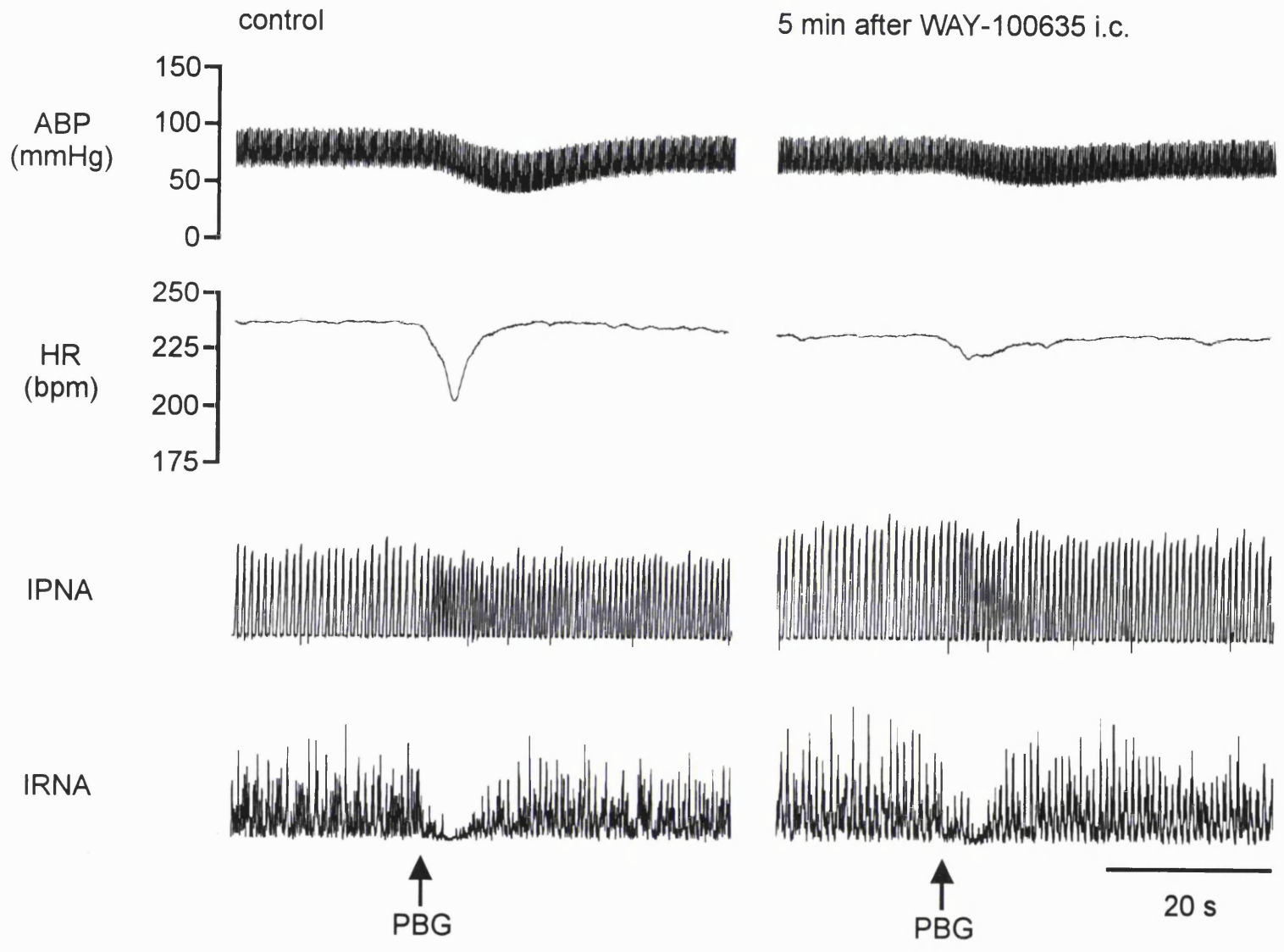

control
5 min after WAY-100635 i.c.
150
100
50
0
ABP
(mmHg)
250
225
200
175
HR
(bpm)
IPNA
IRNA
PBG
PBG
20 s

Figure 3.10

Histograms showing the effects of saline (20 μl i.c.; □; n=5) and WAY-100635 (100 μg kg^{-1} i.c.; ▨; n=6) on baseline mean arterial blood pressure (MABP), R-R interval, phrenic burst rate (PBR) and integrated renal nerve activity (IRNA). Baseline variables were measured immediately prior to the stimulation of cardiopulmonary afferents (see figure 3.11). Effects are shown at 5 and 15 minutes after administration of the drug or vehicle. Each column represents the mean change (Δ) from the pre-drug control values and the bars show s.e.mean.

Comparisons between groups have been made using two-way ANOVA and the least significant difference test.

*$p<0.05$, **$p<0.01$, significant compared to saline i.c.

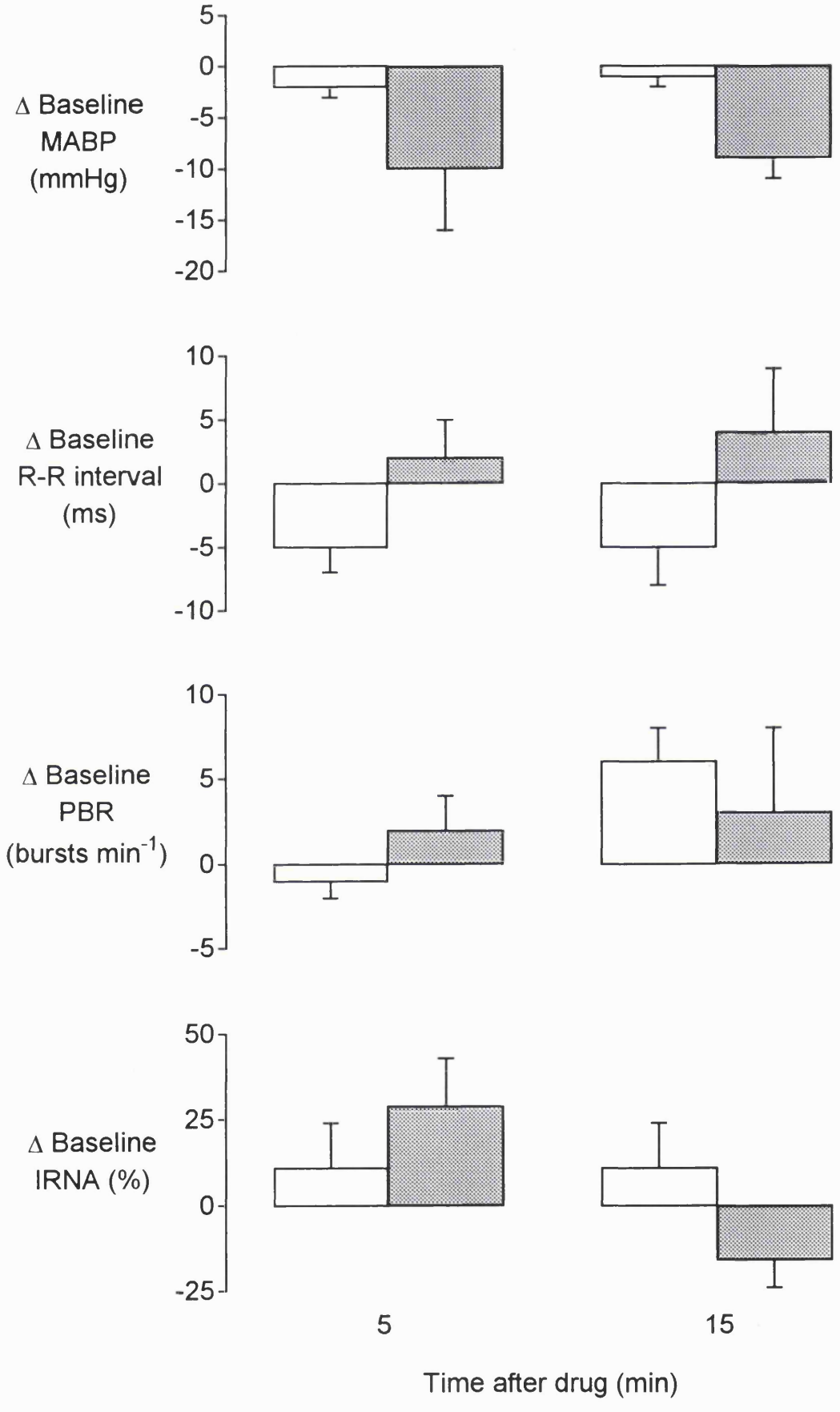
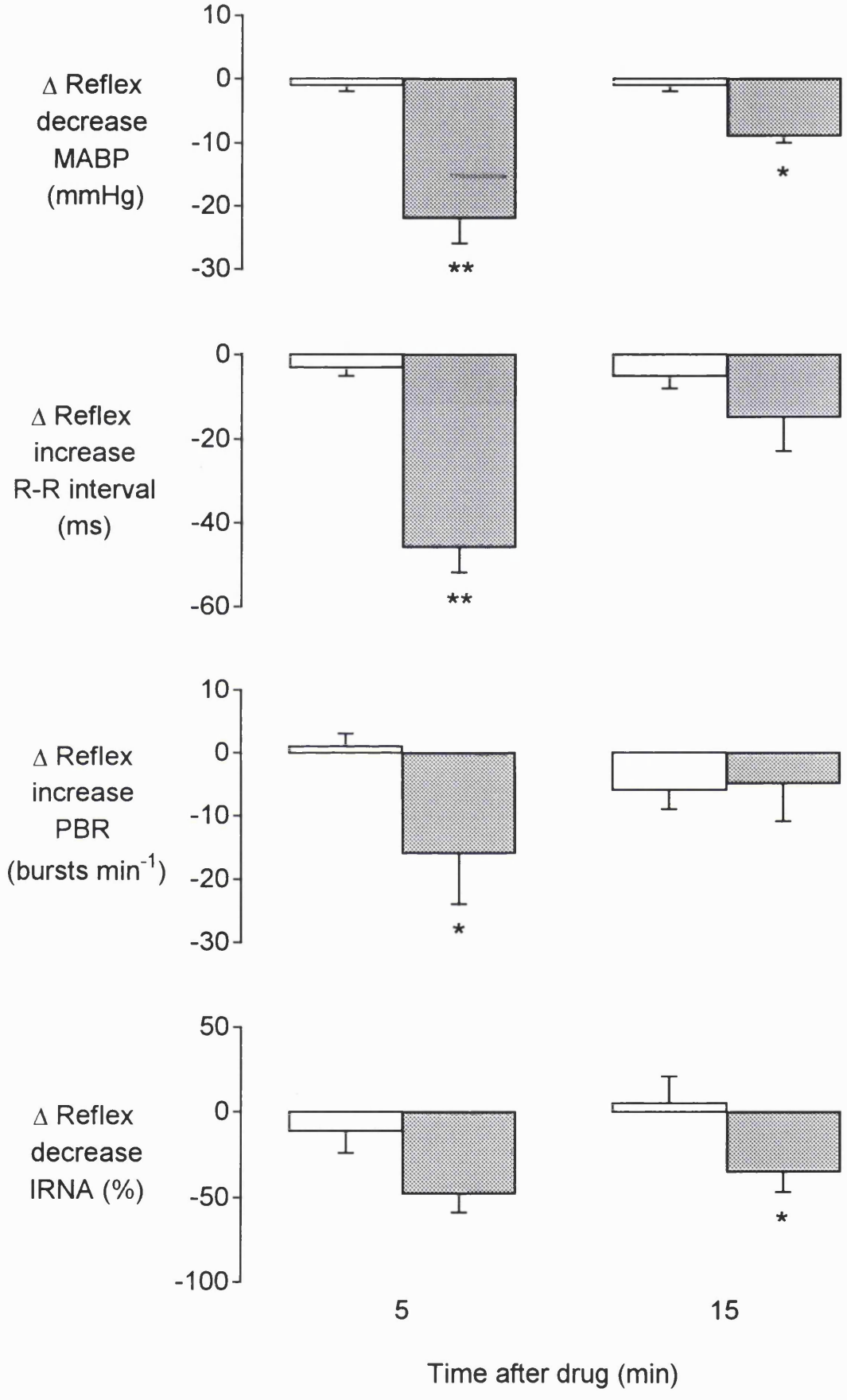

Δ Baseline MABP (mmHg)
5
0
-5
-10
-15
-20
Δ Baseline R-R interval (ms)
10
5
0
-5
-10
Δ Baseline PBR (bursts min-1)
10
5
0
-5
Δ Baseline IRNA (%)
50
25
0
-25
5
15
Time after drug (min)

Figure 3.11

Histograms showing the effects of saline (20 μl i.c.; □ ; n=5) and WAY-100635 (100 μg kg^{-1} i.c.; ▨; n=6) on the reflex increases in R-R interval and phrenic burst rate (PBR) and the decreases in mean arterial blood pressure (MABP) and integrated renal nerve activity (IRNA) evoked by stimulating cardiopulmonary afferents with PBG. Stimulations were performed 5 and 15 minutes after administration of the drug or vehicle. Each column represents the mean change (Δ) from the pre-drug control values and the bars show s.e.mean.

Comparisons between groups have been made using two way ANOVA and the least significant difference test.
*$p<0.05$, **$p<0.01$, significant compared to saline i.c.


Δ Reflex decrease MABP (mmHg)
10
0
-10
-20
-30
**
*
Δ Reflex increase R-R interval (ms)
0
-20
-40
-60
**
Δ Reflex increase PBR (bursts min-1)
10
0
-10
-20
-30
*
Δ Reflex decrease IRNA (%)
50
0
-50
-100
*
5
15
Time after drug (min)

3.3 Effects of 5-HT$_{1A}$ receptor ligands on the baroreceptor reflex

Control stimulations

In the groups of animals in which the baroreceptor reflex was elicited (n = 25; protocols 1 and 3), control stimulations of the left aortic nerve (5 - 7.5 V, 1 ms, 5 -160 Hz) evoked a reflex increase in R-R interval, an inhibition of integrated renal sympathetic nerve activity and a reflex hypotension. The magnitude of the reflex responses became greater with increasing stimulation frequency (see table 3.3 overleaf). Maximal responses appeared to be obtained at 80 Hz since the responses evoked by stimulation at 160 Hz were not significantly greater (paired t-test). Stimulation of the aortic nerve at 20 Hz and above caused a small but significant increase in the phrenic burst rate (PBR). This response did not increase significantly with increasing stimulation frequencies. The reflex responses evoked by stimulation of the left aortic nerve were transient and variables had usually returned to baseline values within 30 seconds. Control responses evoked by stimulating the left aortic nerve at 40, 80 and 160 Hz are shown in figure 3.12.

The effects of buspirone and WAY-100635 on the responses evoked by aortic nerve stimulation were examined in protocols 1 and 3 respectively. The results obtained are described below.

Table 3.3

The table shows the average size of the control reflex responses in the groups of animals in which the baroreceptor reflex was elicited by stimulation of the left aortic nerve. Stimulation of the left aortic nerve evoked increases in R-R interval and phrenic burst (PBR) and decreases in mean arterial blood pressure (MABP) and integrated renal nerve activity (IRNA).

Stimulation Freq (Hz)	5	10	20	40	80	160
Decrease MABP (mmHg)	14 ± 1	20 ± 2	26 ± 2	28 ± 2	29 ± 2	29 ± 2
Increase R-R interval (ms)	5 ± 1	7 ± 1	12 ± 1	16 ± 2	27 ± 3	32 ± 5
Increase PBR (bursts min^{-1})	0 ± 1	2 ± 1	3 ± 1	3 ± 1	4 ± 1	3 ± 1
Decrease IRNA (%)	29 ± 4	38 ± 5	49 ± 5	55 ± 4	53 ± 4	58 ± 5

Saline 20μl i.c.

After intracisternal administration of saline the reflex responses evoked by stimulating the aortic nerve at increasing frequencies were not significantly different from the control responses (table 5.14). The mean control reflex responses obtained before administration of the saline were compared with those at each time point after saline using the Students paired t-test.

Buspirone 200 μg kg^{-1} i.c. (1.23 – 1.78 μmol)

Following intracisternal administration of buspirone, most of the reflex responses evoked by stimulation of the aortic nerve were significantly different compared to the saline control data (figures 3.14-3.16 and table 5.16).

Buspirone significantly potentiated the reflex increases in R-R interval evoked by aortic nerve stimulation when compared to saline. This effect was most prominent during the first set of aortic nerve stimulations beginning 7.5 minutes after administration of buspirone. In this case, the reflex increases in R-R interval evoked by stimulation at 20, 40, 80 and 160 Hz were potentiated by 31 ± 24, 58 ± 29, 89 ± 29 and 100 ± 25 ms respectively. Similar results were obtained during the second set of baroreceptor stimulations 22.5 min after buspirone. However during the final set of baroreceptor stimulations which began 37.5 min after administration of buspirone, only the increase in R-R interval evoked at 80 Hz remained significantly potentiated.

After 22.5 minutes, the reflex renal sympathoinhibitions evoked by stimulating the aortic nerve tended to be reduced by buspirone. At some frequencies of stimulation, this attained statistical significance. The reflex hypotensions were however significantly reduced at most frequencies and time points. For example, during the first set of stimulations following administration of buspirone, the hypotensions evoked by stimulating the aortic nerve at 80 and 160 Hz were 9 ± 2 and 10 ± 2 mmHg smaller than the control responses.

Buspirone had no significant effects on the slight increases in phrenic burst rate that accompanied aortic nerve stimulation.

Experimental traces showing the effects of buspirone on the responses evoked by stimulating the aortic nerve are presented in figure 3.12.

WAY-100635 100 μg kg^{-1} i.v.

During the set of aortic nerve stimulations 7.5 minutes after intravenous administration of WAY-100635, the reflex hypotensions evoked at 5, 10 and 20 Hz were significantly reduced when compared to the saline group. However, at all frequencies of aortic nerve stimulation, i.v. WAY-100635 had no significant effects on the reflex increases in R-R interval, the reflex renal sympathoinhibitions or the phrenic nerve responses (table 5.18).

(1.14 – 1.33 μmol)

Buspirone 200 μg kg^{-1} i.c.^(pre-treated with WAY-100635 100 μg kg^{-1} i.v.)

Pre-treatment with WAY-100635 attenuated most of the modulatory effects of buspirone on the responses evoked by aortic nerve stimulation (figures 3.14-3.16 and table 5.20).

After i.c. administration of buspirone in the presence of WAY-100635 the reflex increases in R-R interval were still significantly potentiated at the higher frequencies of aortic nerve stimulation when compared to saline. In most cases however, the potentiations were significantly less than those caused by buspirone alone at the same time and frequency . For example, during the first set of stimulations following administration of buspirone alone, the reflex increases in R-R interval evoked at 40, 80 and 160 Hz stimulation were potentiated by 58 ± 29, 89 ± 29 and 100 ± 25 ms respectively, in the pre-treated group this was reduced to potentiations of 22 ± 9, 35 ± 7 and 42 ± 10 ms. Pre-treatment with WAY-100635 also reduced the effects of buspirone on the reflex increases in R-R interval during the second and third set of stimulations.

The reductions in the renal sympathoinhibitions that were occasionally significant in the buspirone alone group, were attenuated in the pre-treated group. For example, during the third set of stimulation after administration of buspirone alone, the reflex sympathoinhibitions evoked at 10 and 20 Hz were

reduced by 38 ± 19 and 41 ± 20 % respectively. After administration of buspirone in the presence of WAY-100635, the renal sympathoinhibitions evoked at the same time and frequencies were 58 ± 27 and 47 ± 21 % greater than control responses. The effects of buspirone on the reflex hypotensions were also significantly attenuated at most frequencies of stimulation when pre-treated with WAY-100635. For example, the hypotensions evoked at 5 and 20 Hz during the second set of stimulations were reduced by 7 ± 3 and 10 ± 2 mmHg after buspirone alone but by 0 ± 3 and 2 ± 3 mmHg in the pre-treated group. .

Buspirone 200 μg kg^{-1} i.v.

Intravenous administration of buspirone had no significant effect on the reflex increases in R-R interval evoked by aortic nerve stimulation apart from at 80 Hz during the first set of baroreceptor stimulations (figures 3.18-3.20 and table 5.22). At this stimulation frequency, the reflex increase in R-R interval was significantly potentiated by 21 ± 11 ms when compared to saline. This however, was significantly less than the potentiation of 89 ± 29 ms caused by buspirone i.c.

Buspirone i.v. tended to reduce the reflex renal sympathoinhibitions evoked by stimulating the aortic nerve, however, his effect rarely reached statistical significance when compared to saline. Buspirone i.v. also reduced the reflex hypotensions evoked by baroreceptor afferent stimulation. This reached significance at a number of frequencies during the first and second sets of aortic nerve stimulations when compared to saline. The effects of buspirone i.v. on the reflex sympathoinhibitions and hypotensions were not however significantly different to those obtained when buspirone was given i.c.

As with buspirone administered i.c, i.v. buspirone had no effect on the slight increases in phrenic burst rate evoked by baroreceptor afferent stimulation.

WAY-100635 100 μg kg^{-1} i.c. (0.49 – 0.54 μmol)

Intracisternal administration of WAY-100635 had significant inhibitory effects on most of the responses evoked by aortic nerve stimulation when compared to saline (figure 3.23 and table 5.24).

During the first set of stimulations beginning 1.5 minutes after administration of the drug, the reflex increases in R-R interval evoked by stimulating the aortic nerve at 80 and 160 Hz were significantly reduced by 27 ± 6 and 29 ± 6 ms respectively. The reflex renal sympathoinhibition evoked at 160 Hz stimulation was also significantly reduced by 73 ± 14 %. In addition, the reflex hypotensions evoked by stimulation at both 80 and 160 Hz were significantly reduced by 13 ± 3 and 19 ± 3 mmHg respectively. WAY-100635 had no effect however on the slight increases in phrenic burst rate evoked by stimulating the aortic nerve.

Experimental traces showing the effects of i.c. WAY-100635 on the responses evoked by stimulating baroreceptor afferents at maximal frequencies are presented in figure 3.21 and 3.29.

The effects of the 5-HT$_{1A}$ receptor ligands on the reflex responses evoked by stimulating the left aortic nerve are summarised in table 3.4.

Table 3.4

Summary of the main effects of 5-HT_{1A} receptor ligands on the reflex decrease in mean arterial blood pressure (MABP) and integrated renal nerve activity (IRNA) and the increase in R-R interval and phrenic burst rate (PBR) evoked by stimulating aortic baroreceptor afferents.

0 = no significant change

↑ = significant increase within 20 minutes of drug administration

↓ = significant decrease within 20 minutes of drug administration

(↓) = significant decrease more than 20 minutes after drug administration

Drug	Decrease MABP	Increase R-R interval	Increase PBR	Decrease RNA
Buspirone i.c.	↓	↑	0	(↓)
WAY-100635 i.v.	↓	0	0	0
Buspirone i.c. / WAY-100635 i.v.	0	↑	0	0
Buspirone i.v.	↓	0	0	(↓)
WAY-100635 i.c.	↓	↓	0	↓

Figure 3.12

The responses evoked by stimulating the left aortic nerve at 40, 80 and 160 Hz during the control period and during the first set of stimulations after administration of buspirone (200 μg kg^{-1} i.c.) in an atenolol (1 mg kg^{-1}) pre-treated rabbit.

From the top, the traces show arterial blood pressure (ABP), heart rate (HR), and integrated renal nerve activity (IRNA).

The aortic nerve was electrically stimulated at each frequency at points marked by the arrows.

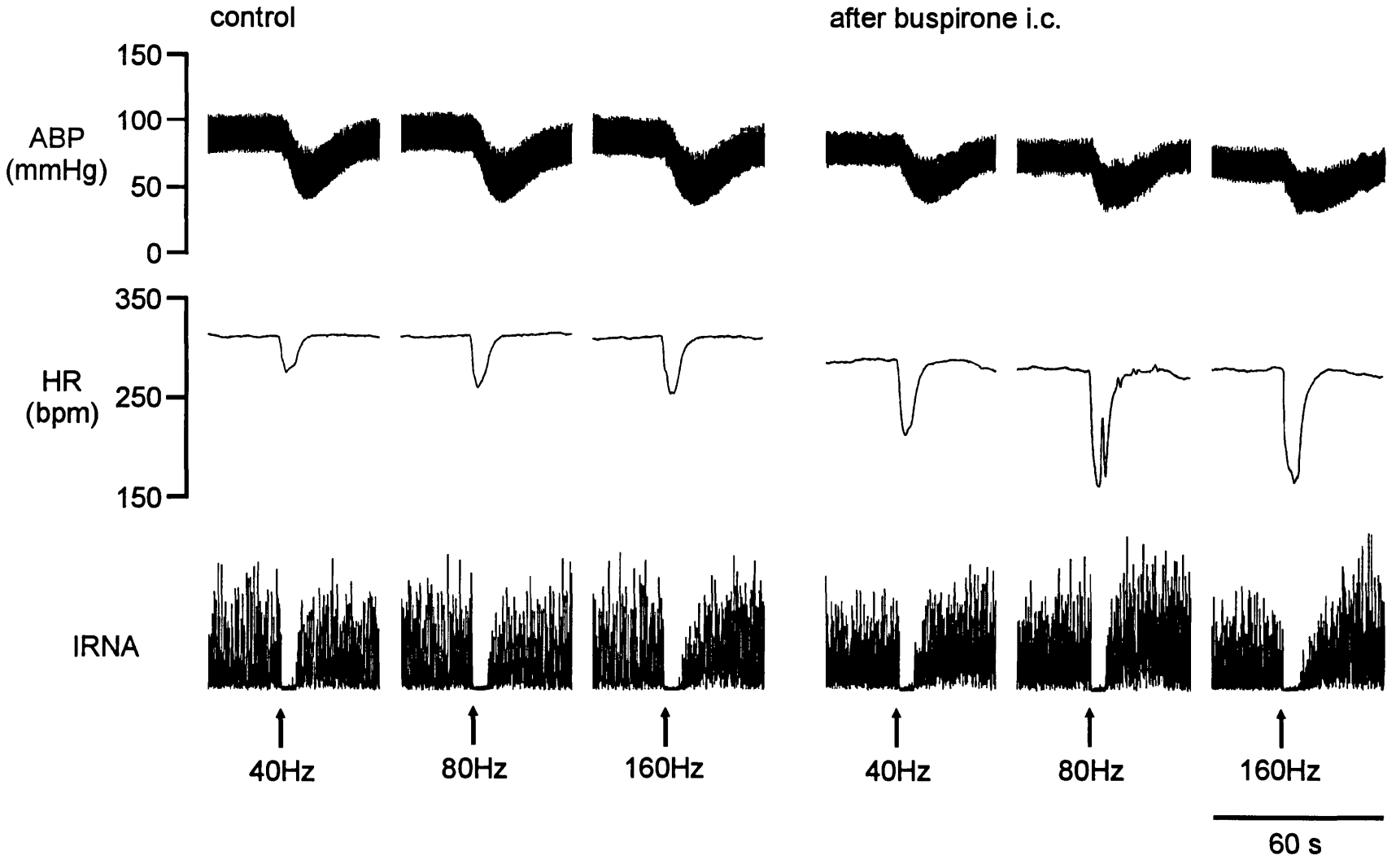

control
after buspirone i.c.
150
100
50
0
ABP
(mmHg)
350
250
150
HR
(bpm)
IRNA
40Hz
80Hz
160Hz
40Hz
80Hz
160Hz
60 s

Figure 3.13

Histograms showing the effects of saline (20 μl i.c.; □; n=5), buspirone (200 μg kg^{-1} i.c.; ■; n=5) and buspirone (200 μg kg^{-1} i.c.) 20 minutes after pre-treatment with WAY-100635 (100 μg kg^{-1} i.v.; ▨; n=5) on baseline mean arterial blood pressure (MABP), R-R interval, phrenic burst rate (PBR) and integrated renal nerve activity (IRNA). Baseline variables were measured immediately prior to the sets of aortic nerve stimulations (see figures 3.14 - 3.16). Effects are shown at 7.5 min after administration of the drug or vehicle and thereafter at 15 minute intervals. Each column represents the mean change (Δ) from the pre-drug control values and the bars show s.e.mean.

Comparisons between groups have been made using two-way ANOVA and the least significant difference test.

*$p<0.05$, **$p<0.01$ compared to saline i.c.

†$p<0.05$, ††$p<0.01$ compared to buspirone i.c. alone.

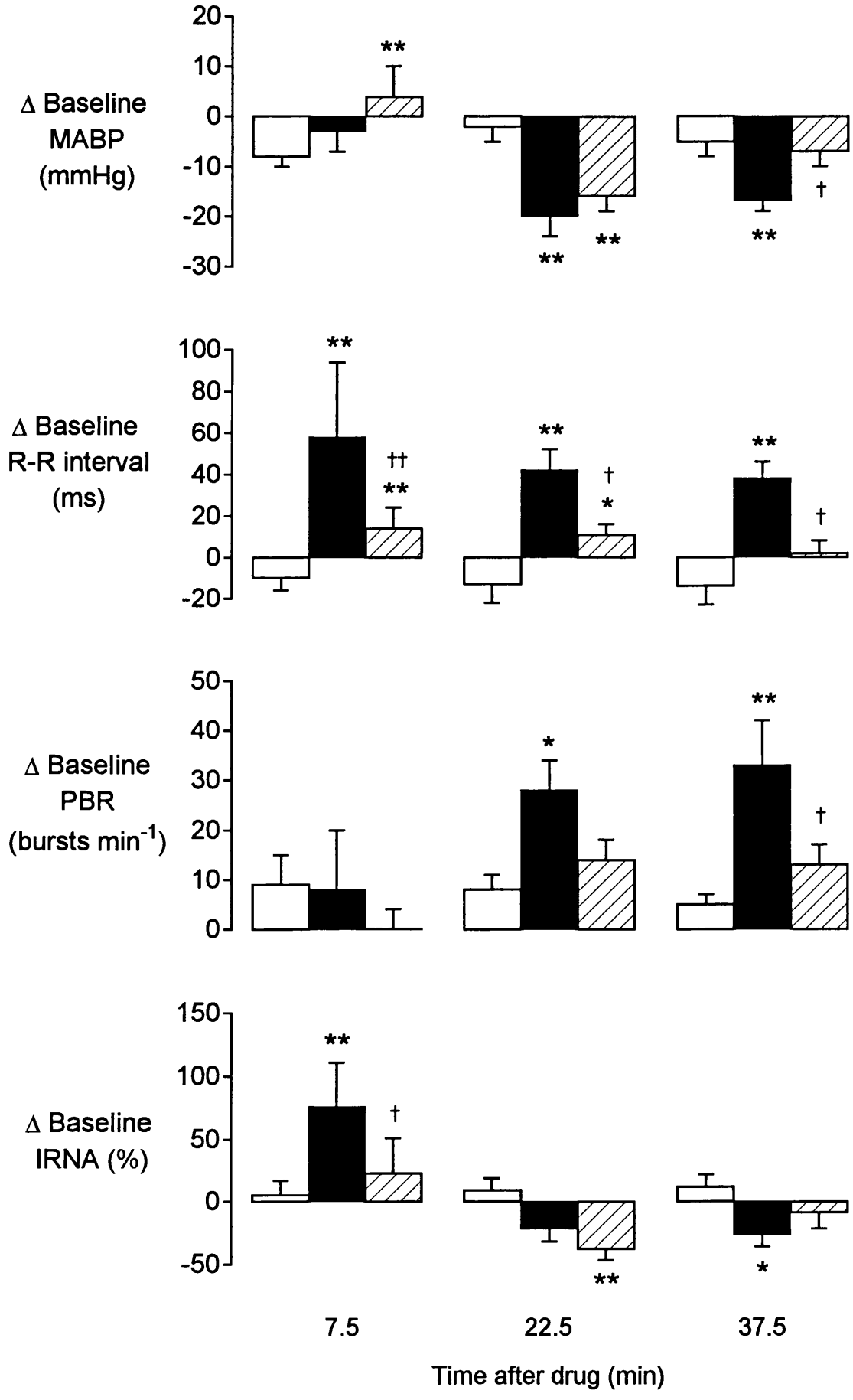

Δ Baseline MABP (mmHg)
20
10
0
-10
-20
-30
Δ Baseline R-R interval (ms)
100
80
60
40
20
0
-20
Δ Baseline PBR (bursts min-1)
50
40
30
20
10
0
Δ Baseline IRNA (%)
150
100
50
0
-50
7.5
22.5
37.5
Time after drug (min)

Figure 3.14

Histograms showing the effects of saline (20 μl i.c.; □; n=5), buspirone (200 μg kg^{-1} i.c.; ■; n=5) and buspirone (200 μg kg^{-1} i.c.) 20 minutes after pre-treatment with WAY-100635 (100 μg kg^{-1} i.v.; ▨; n=5) on the reflex increases in R-R interval and phrenic burst rate (PBR) and the decreases in mean arterial blood pressure (MABP) and integrated renal nerve activity (IRNA) evoked by stimulating the left aortic nerve at six frequencies. Data is shown from the **first set of aortic nerve stimulations** beginning 7.5 min after administration of the drug or vehicle. Each column represents the mean change (Δ) from the pre-drug control values and the bars show s.e.mean.

Comparisons between groups have been made using three-way ANOVA and the least significant difference test.

*$p<0.05$, **$p<0.01$ compared to saline i.c.

†$p<0.05$, ††$p<0.01$ compared to buspirone i.c. alone.

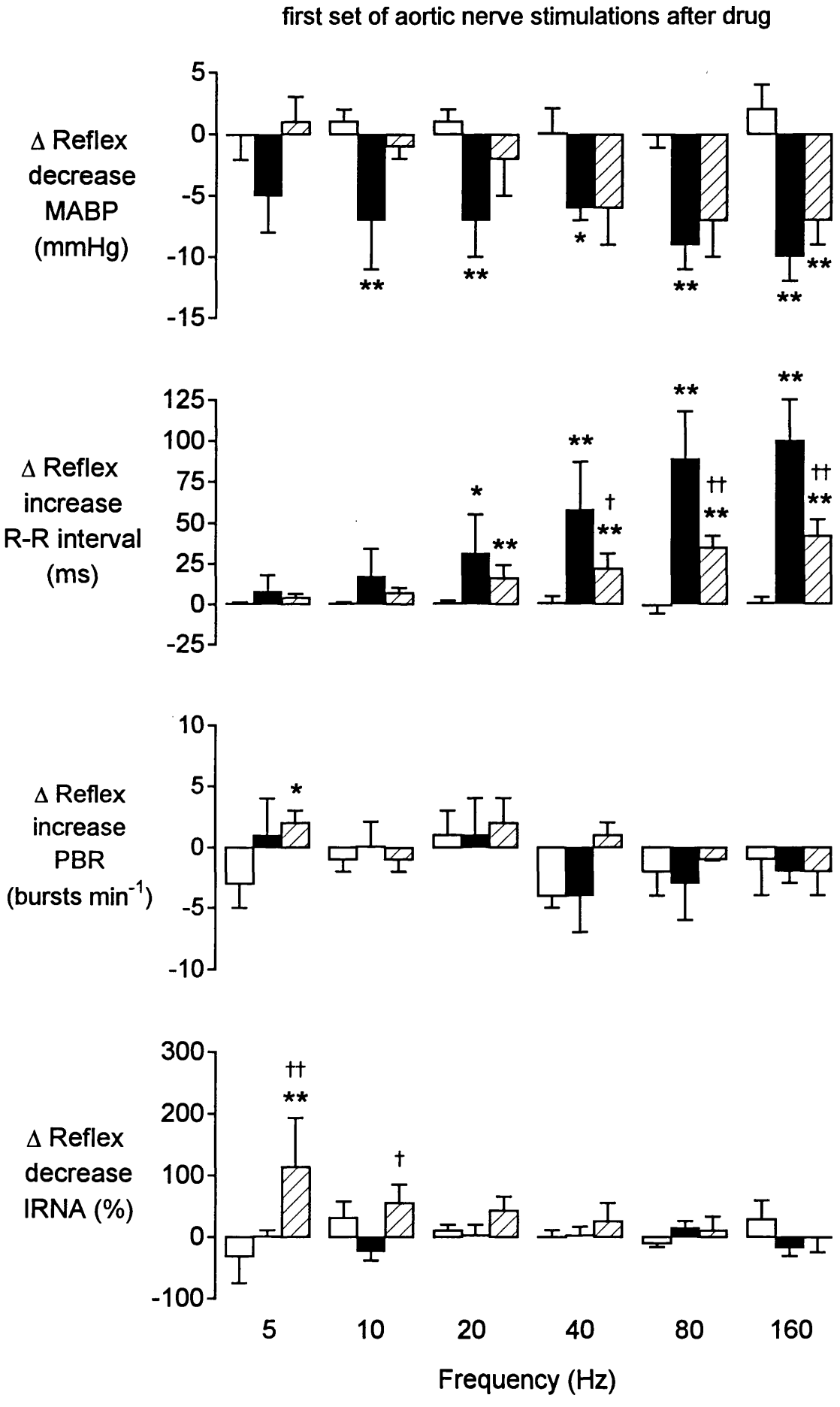

first set of aortic nerve stimulations after drug
Δ Reflex decrease MABP (mmHg)
5
0
-5
-10
-15
Δ Reflex increase R-R interval (ms)
125
100
75
50
25
0
-25
Δ Reflex increase PBR (bursts min-1)
10
5
0
-5
-10
Δ Reflex decrease IRNA (%)
300
200
100
0
-100
5
10
20
40
80
160
Frequency (Hz)

Figure 3.15

Histograms showing the effects of saline (20 μl i.c.; □; n=5), buspirone (200 μg kg^{-1} i.c.; ■; n=5) and buspirone (200 μg kg^{-1} i.c.) 20 minutes after pre-treatment with WAY-100635 (100 μg kg^{-1} i.v.; ▨; n=5) on the reflex increases in R-R interval and phrenic burst rate (PBR) and the decreases in mean arterial blood pressure (MABP) and integrated renal nerve activity (IRNA) evoked by stimulating the left aortic nerve at six frequencies. Data is shown from the **second set of aortic nerve stimulations** beginning 22.5 min after administration of the drug or vehicle. Each column represents the mean change (Δ) from the pre-drug control values and the bars show s.e.mean.

Comparisons between groups have been made using three-way ANOVA and the least significant difference test.

*$p<0.05$, **$p<0.01$ compared to saline i.c.

†$p<0.05$, ††$p<0.01$ compared to buspirone i.c. alone.

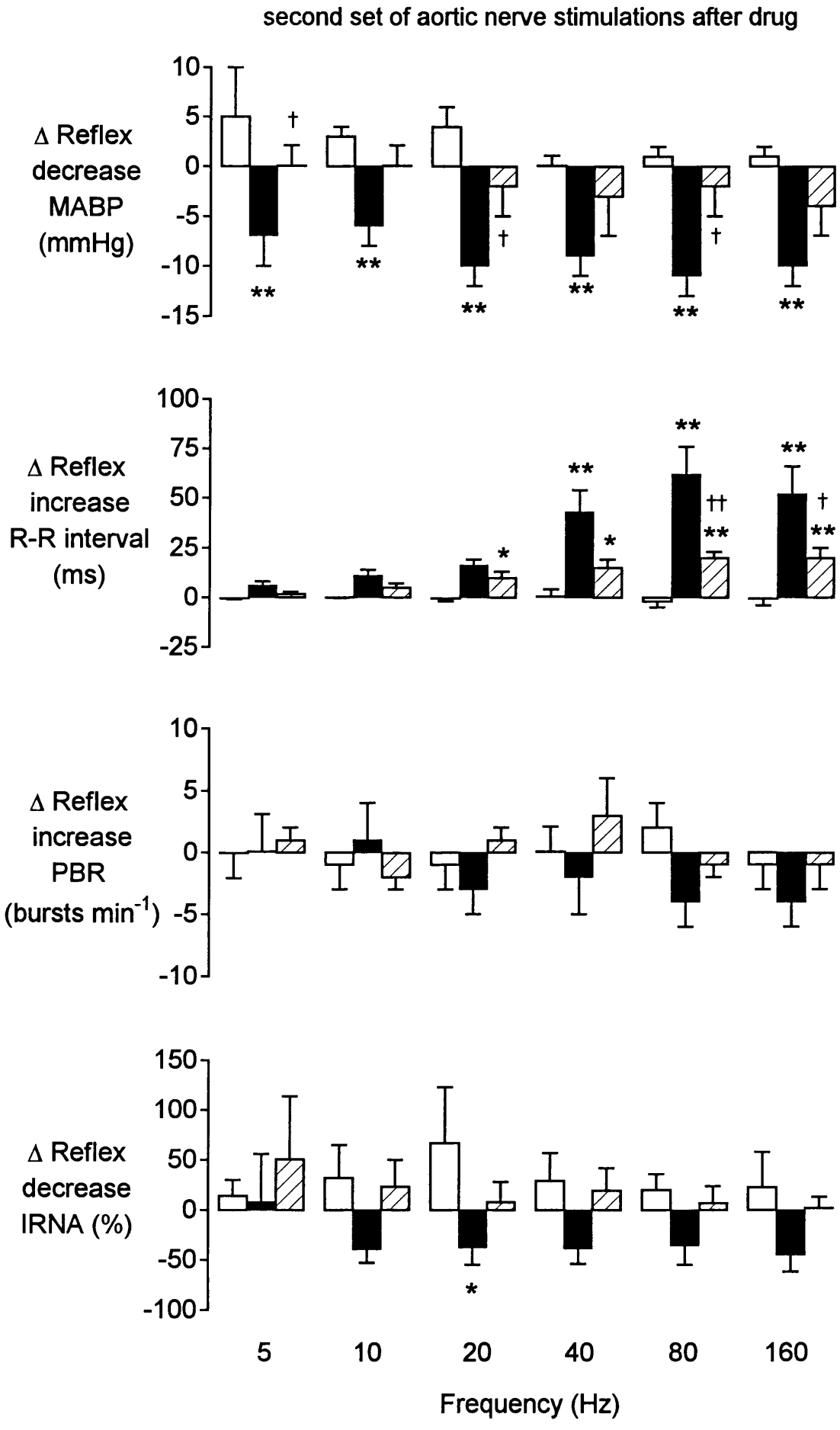

second set of aortic nerve stimulations after drug
Δ Reflex decrease MABP (mmHg)
10
5
0
-5
-10
-15
Δ Reflex increase R-R interval (ms)
100
75
50
25
0
-25
Δ Reflex increase PBR (bursts min-1)
10
5
0
-5
-10
Δ Reflex decrease IRNA (%)
150
100
50
0
-50
-100
5
10
20
40
80
160
Frequency (Hz)

Figure 3.16

Histograms showing the effects of saline (20 μl i.c.; □; n=5), buspirone (200 μg kg^{-1} i.c.; ■; n=5) and buspirone (200 μg kg^{-1} i.c.) 20 minutes after pre-treatment with WAY-100635 (100 μg kg^{-1} i.v.; ▨; n=5) on the reflex increases in R-R interval and phrenic burst rate (PBR) and the decreases in mean arterial blood pressure (MABP) and integrated renal nerve activity (IRNA) evoked by stimulating the left aortic nerve at six frequencies. Data is shown from the **third set of aortic nerve stimulations** beginning 37.5 min after administration of the drug or vehicle. Each column represents the mean change (Δ) from the pre-drug control values and the bars show s.e.mean.

Comparisons between groups have been made using three-way ANOVA and the least significant difference test.

*$p<0.05$, **$p<0.01$ compared to saline i.c.

†$p<0.05$, ††$p<0.01$ compared to buspirone i.c. alone.

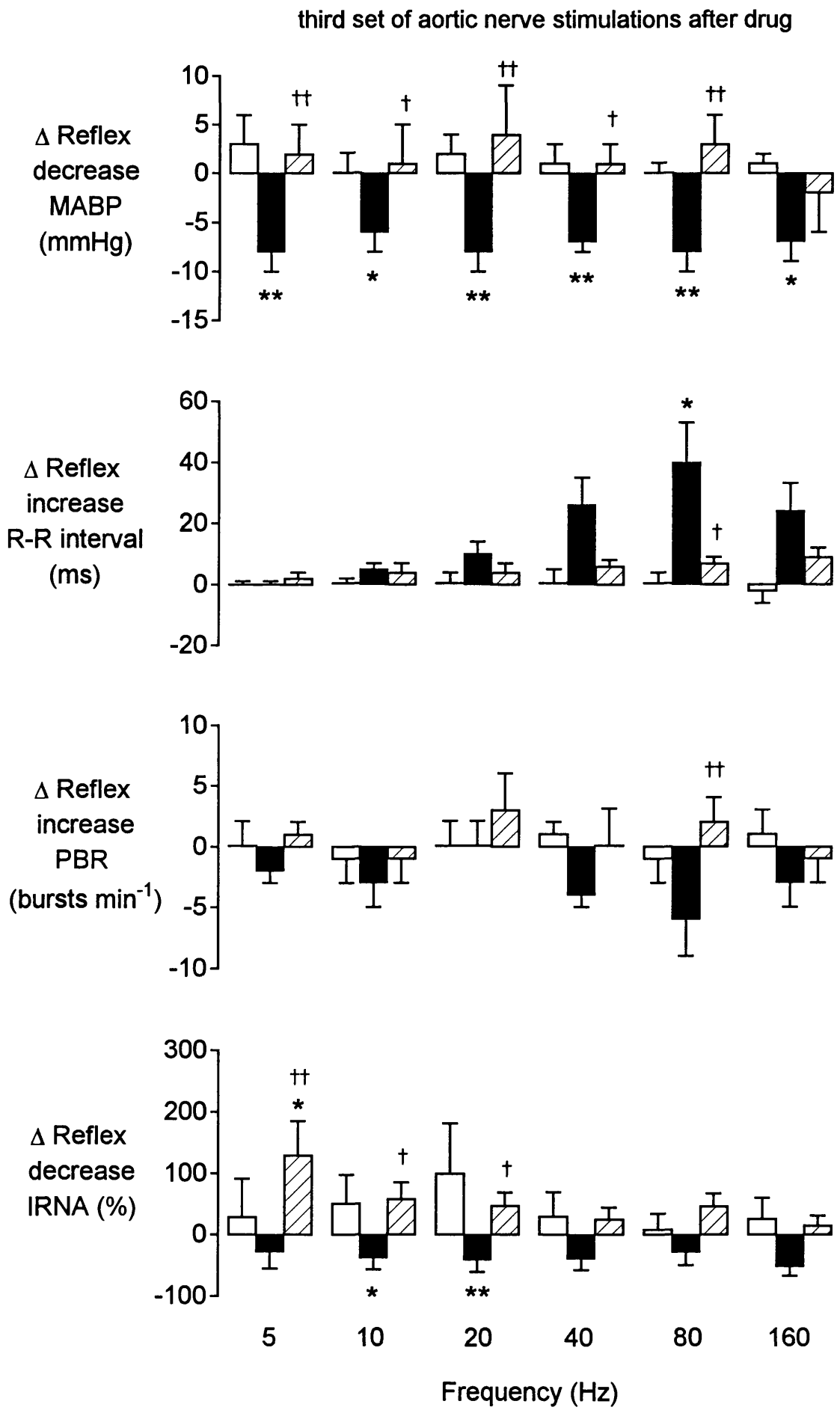

third set of aortic nerve stimulations after drug
Δ Reflex decrease MABP (mmHg)
10
5
0
-5
-10
-15
Δ Reflex increase R-R interval (ms)
60
40
20
0
-20
Δ Reflex increase PBR (bursts min-1)
10
5
0
-5
-10
Δ Reflex decrease IRNA (%)
300
200
100
0
-100
5
10
20
40
80
160
Frequency (Hz)

Figure 3.17

Histograms showing the effects of saline (20 μl i.c.; □; n=5), buspirone (200 μg kg^{-1} i.c.; ■; n=5) and buspirone (200 μg kg^{-1} i.v.; ⊠; n=4) on baseline mean arterial blood pressure (MABP), R-R interval, phrenic burst rate (PBR) and integrated renal nerve activity (IRNA). Baseline variables were measured immediately prior to the sets of aortic nerve stimulations (see figures 3.18 - 3.20). Effects are shown at 7.5 min after administration of the drug or vehicle and thereafter at 15 minute intervals. Each column represents the mean change (Δ) from the pre-drug control values and the bars show s.e.mean.

Comparisons between groups have been made using two-way ANOVA and the least significant difference test.

*$p<0.05$, **$p<0.01$ compared to saline i.c.

†$p<0.05$, ††$p<0.01$ compared to buspirone i.c. alone.

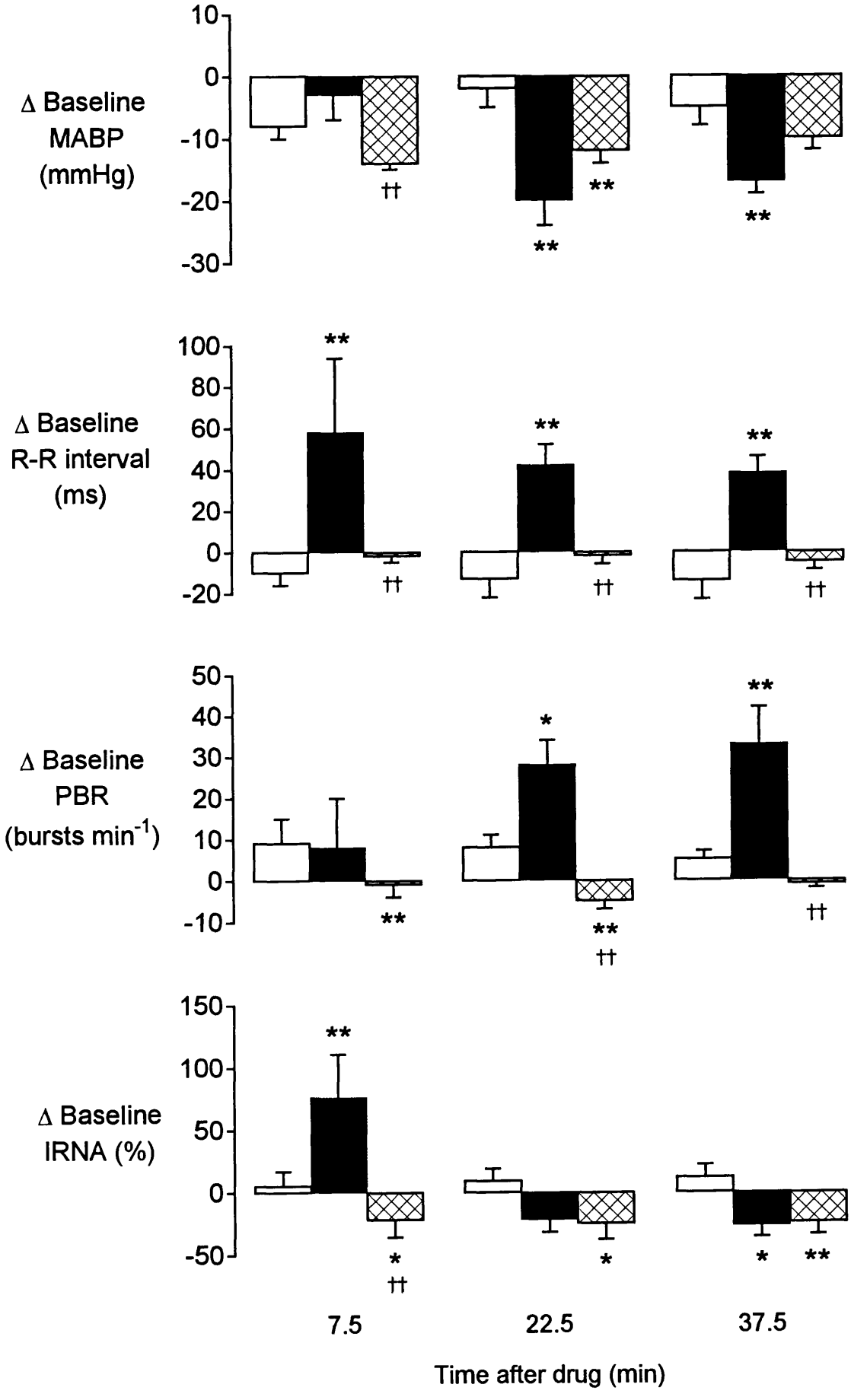

Δ Baseline MABP (mmHg)
10
0
-10
-20
-30
††
**
**
**
Δ Baseline R-R interval (ms)
100
80
60
40
20
0
-20
**
**
**
††
††
††
Δ Baseline PBR (bursts min-1)
50
40
30
20
10
0
-10
**
*
**
**
††
††
Δ Baseline IRNA (%)
150
100
50
0
-50
**
*
*
*
**
††
7.5
22.5
37.5
Time after drug (min)

Figure 3.18

Histograms showing the effects of saline (20 μl i.c.; □; n=5), buspirone (200 μg kg^{-1} i.c.; ■; n=5) and buspirone (200 μg kg^{-1} i.v.; ⊠; n=4) on the reflex increases in R-R interval and phrenic burst rate (PBR) and the decreases in mean arterial blood pressure (MABP) and integrated renal nerve activity (IRNA) evoked by stimulating the left aortic nerve at six frequencies. Data is shown from the **first set of aortic nerve stimulations** beginning 7.5 min after administration of the drug or vehicle. Each column represents the mean change (Δ) from the pre-drug control values and the bars show s.e.mean.

Comparisons between groups have been made using three-way ANOVA and the least significant difference test.

*$p<0.05$, **$p<0.01$ compared to saline i.c.

†$p<0.05$, ††$p<0.01$ compared to buspirone i.c. alone.

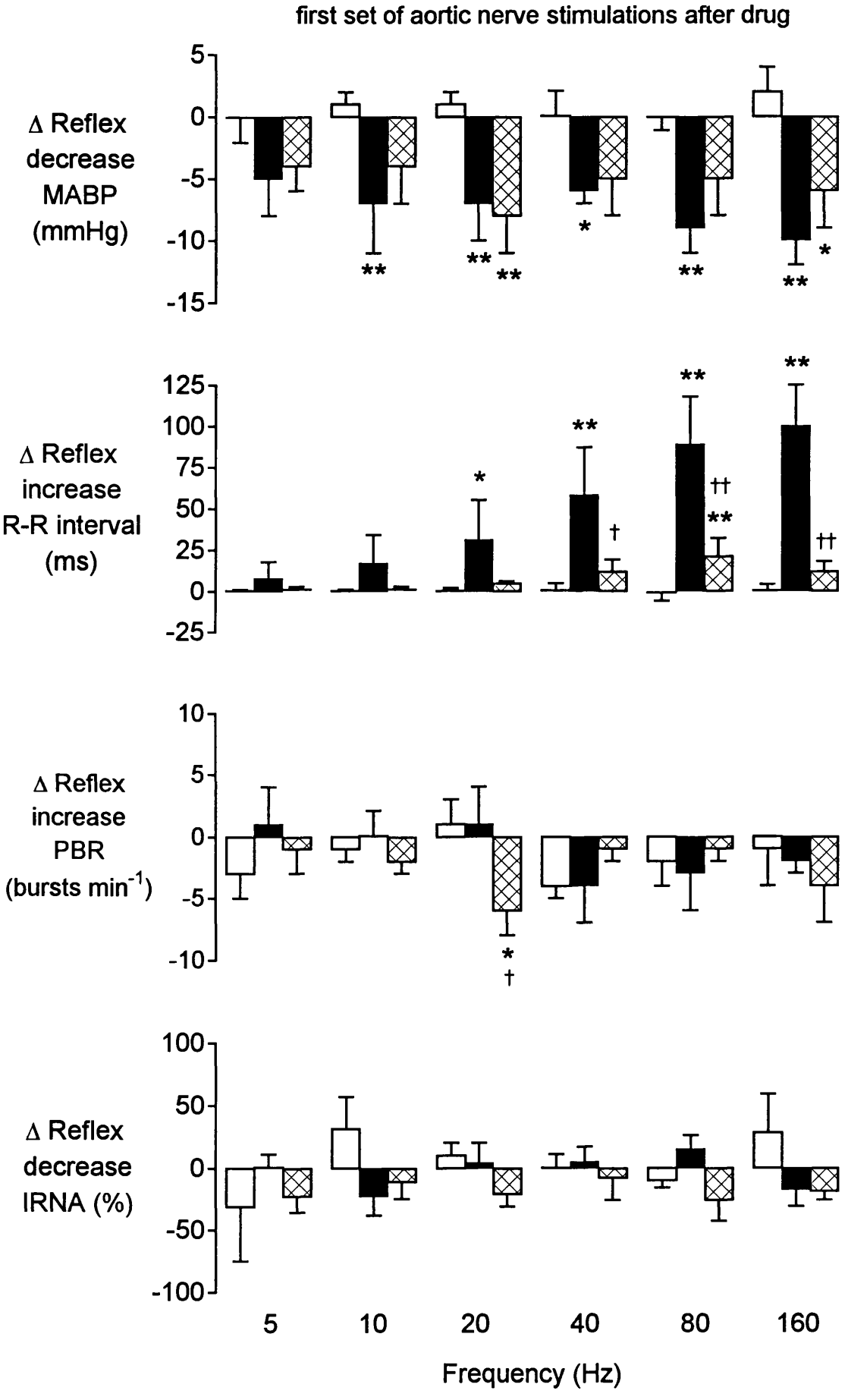

first set of aortic nerve stimulations after drug
Δ Reflex decrease MABP (mmHg)
5
0
-5
-10
-15
Δ Reflex increase R-R interval (ms)
125
100
75
50
25
0
-25
Δ Reflex increase PBR (bursts min-1)
10
5
0
-5
-10
Δ Reflex decrease IRNA (%)
100
50
0
-50
-100
5
10
20
40
80
160
Frequency (Hz)

Figure 3.19

Histograms showing the effects of saline (20 μl i.c.; □; n=5), buspirone (200 μg kg^{-1} i.c.; ■; n=5) and buspirone (200 μg kg^{-1} i.v.; ⊠; n=4) on the reflex increases in R-R interval and phrenic burst rate (PBR) and the decreases in mean arterial blood pressure (MABP) and integrated renal nerve activity (IRNA) evoked by stimulating the left aortic nerve at six frequencies. Data is shown from the **second set of aortic nerve stimulations** beginning 22.5 min after administration of the drug or vehicle. Each column represents the mean change (Δ) from the pre-drug control values and the bars show s.e.mean.

Comparisons between groups have been made using three-way ANOVA and the least significant difference test.

*$p<0.05$, **$p<0.01$ compared to saline i.c.

†$p<0.05$, ††$p<0.01$ compared to buspirone i.c. alone.

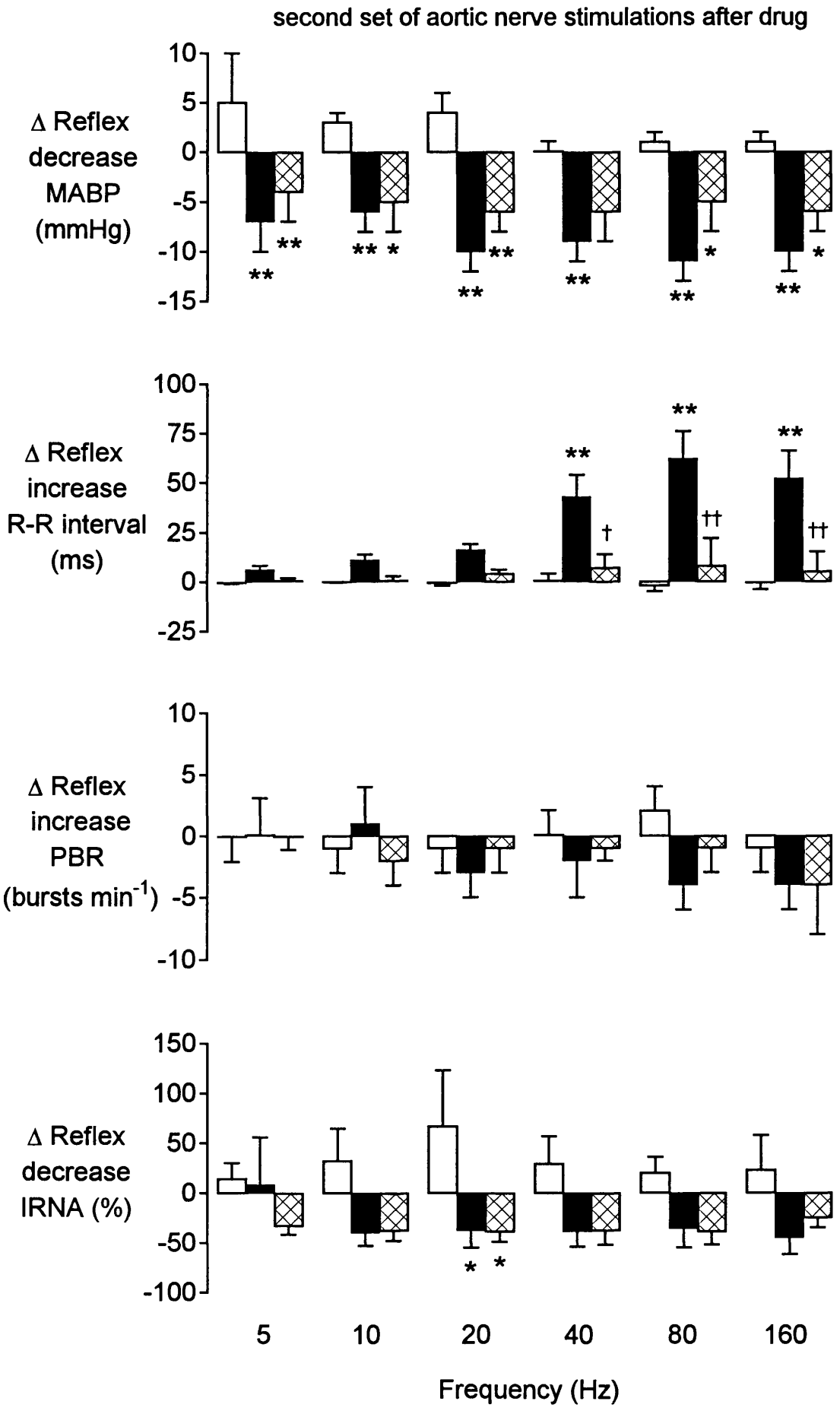

second set of aortic nerve stimulations after drug
Δ Reflex decrease MABP (mmHg)
10
5
0
-5
-10
-15
Δ Reflex increase R-R interval (ms)
100
75
50
25
0
-25
Δ Reflex increase PBR (bursts min-1)
10
5
0
-5
-10
Δ Reflex decrease IRNA (%)
150
100
50
0
-50
-100
5
10
20
40
80
160
Frequency (Hz)

Figure 3.20

Histograms showing the effects of saline (20 μl i.c.; □; n=5), buspirone (200 μg kg^{-1} i.c.; ■; n=5) and buspirone (200 μg kg^{-1} i.v.; ⊠; n=4) on the reflex increases in R-R interval and phrenic burst rate (PBR) and the decreases in mean arterial blood pressure (MABP) and integrated renal nerve activity (IRNA) evoked by stimulating the left aortic nerve at six frequencies. Data is shown from the **third set of aortic nerve stimulations** beginning 37.5 min after administration of the drug or vehicle. Each column represents the mean change (Δ) from the pre-drug control values and the bars show s.e.mean.

Comparisons between groups have been made using three-way ANOVA and the least significant difference test.

*$p<0.05$, **$p<0.01$ compared to saline i.c.

†$p<0.05$, ††$p<0.01$ compared to buspirone i.c. alone.

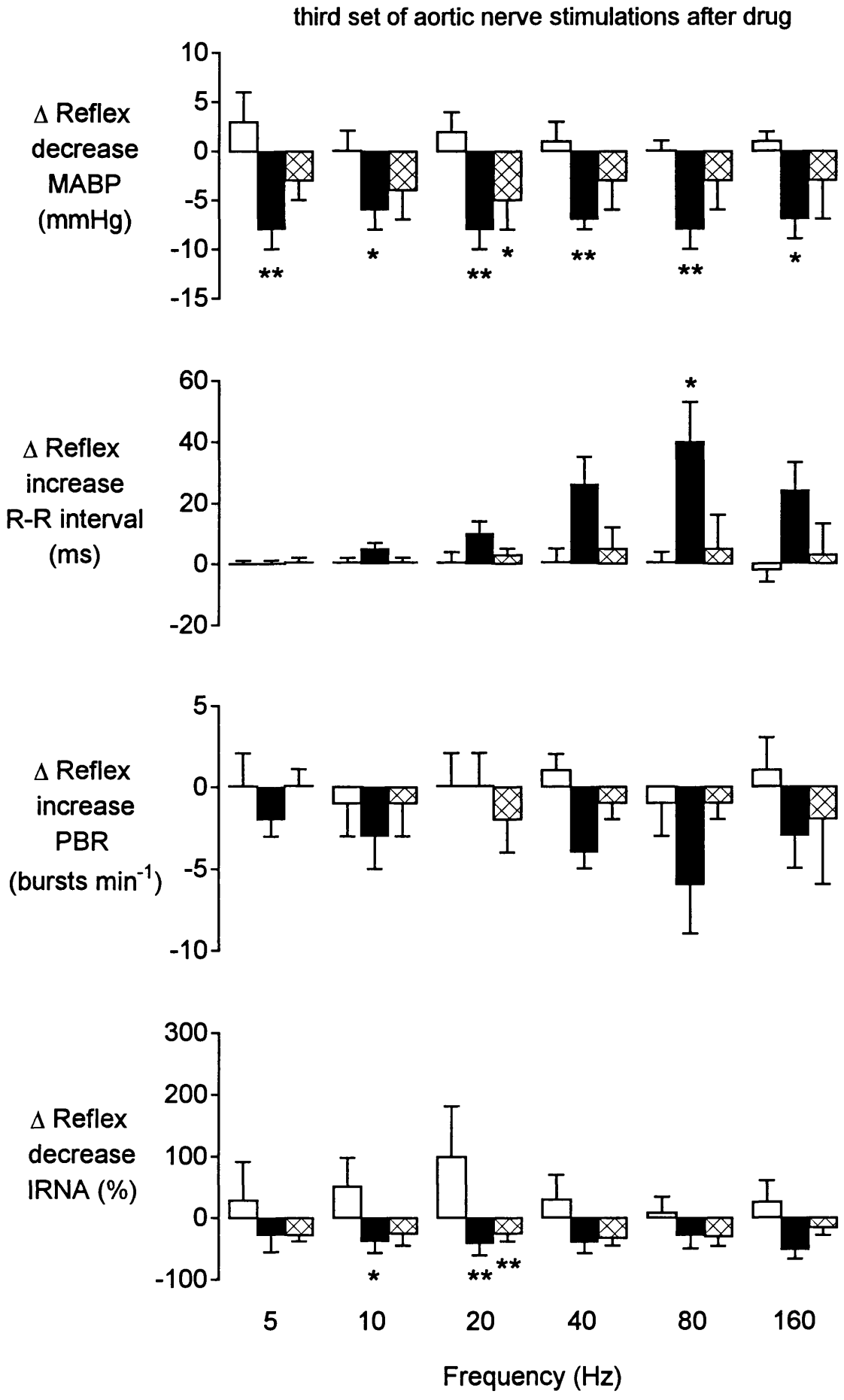

third set of aortic nerve stimulations after drug
Δ Reflex decrease MABP (mmHg)
10
5
0
-5
-10
-15
**
*
**
*
**
**
*
Δ Reflex increase R-R interval (ms)
60
40
20
0
-20
*
Δ Reflex increase PBR (bursts min-1)
5
0
-5
-10
Δ Reflex decrease IRNA (%)
300
200
100
0
-100
*
**
**
5
10
20
40
80
160
Frequency (Hz)

Figure 3.21

The responses evoked by stimulating the left aortic nerve at 80 and 160 Hz during the control period and during the first set of stimulations after administration of WAY-100635 (100 μg kg^{-1} i.c.) in an atenolol (1 mg kg^{-1}) pre-treated rabbit.

From the top, the traces show arterial blood pressure (ABP), heart rate (HR), and integrated renal nerve activity (IRNA).

The aortic nerve was electrically stimulated at each frequency at points marked by the arrows.

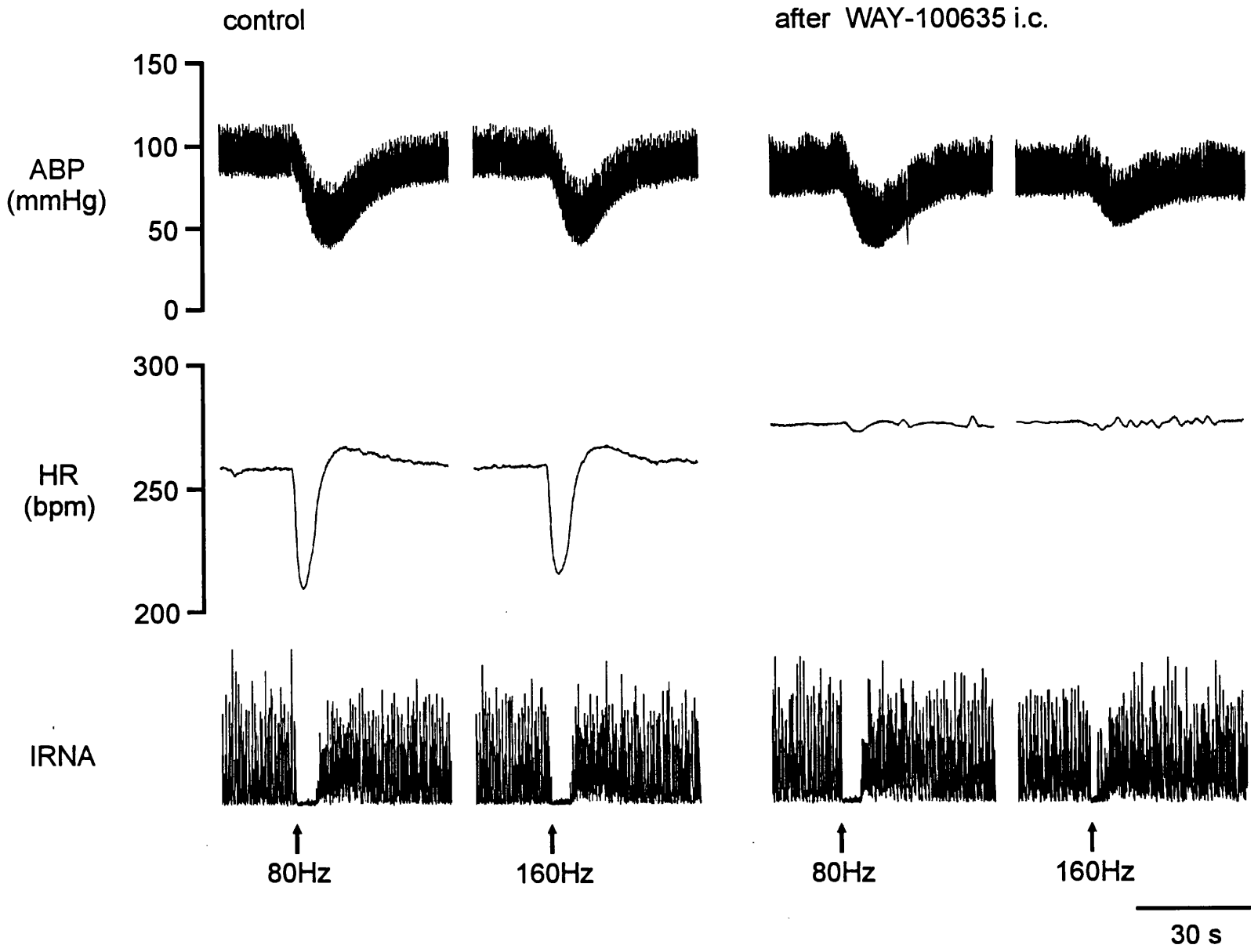

control
after WAY-100635 i.c.
150
100
50
0
ABP
(mmHg)
300
250
200
HR
(bpm)
IRNA
80Hz
160Hz
80Hz
160Hz
30 s

Figure 3.22

Histograms showing the effects of saline (20 μl i.c.; □; n=5) and WAY-100635 (100 μg kg^{-1} i.c.; ▨; n=6) on baseline mean arterial blood pressure (MABP), R-R interval, phrenic burst rate (PBR) and integrated renal nerve activity (IRNA). Baseline variables were measured immediately prior to the sets of aortic nerve stimulations (see figure 3.23). Effects are shown at 1.5 minutes after administration of the drug or vehicle and thereafter at 10 minute intervals. Each column represents the mean change (Δ) from the pre-drug control values and the bars show s.e.mean.

Comparisons between groups have been made using two way ANOVA and the least significant difference test.

*$p<0.05$, **$p<0.01$, significant compared to saline i.c.

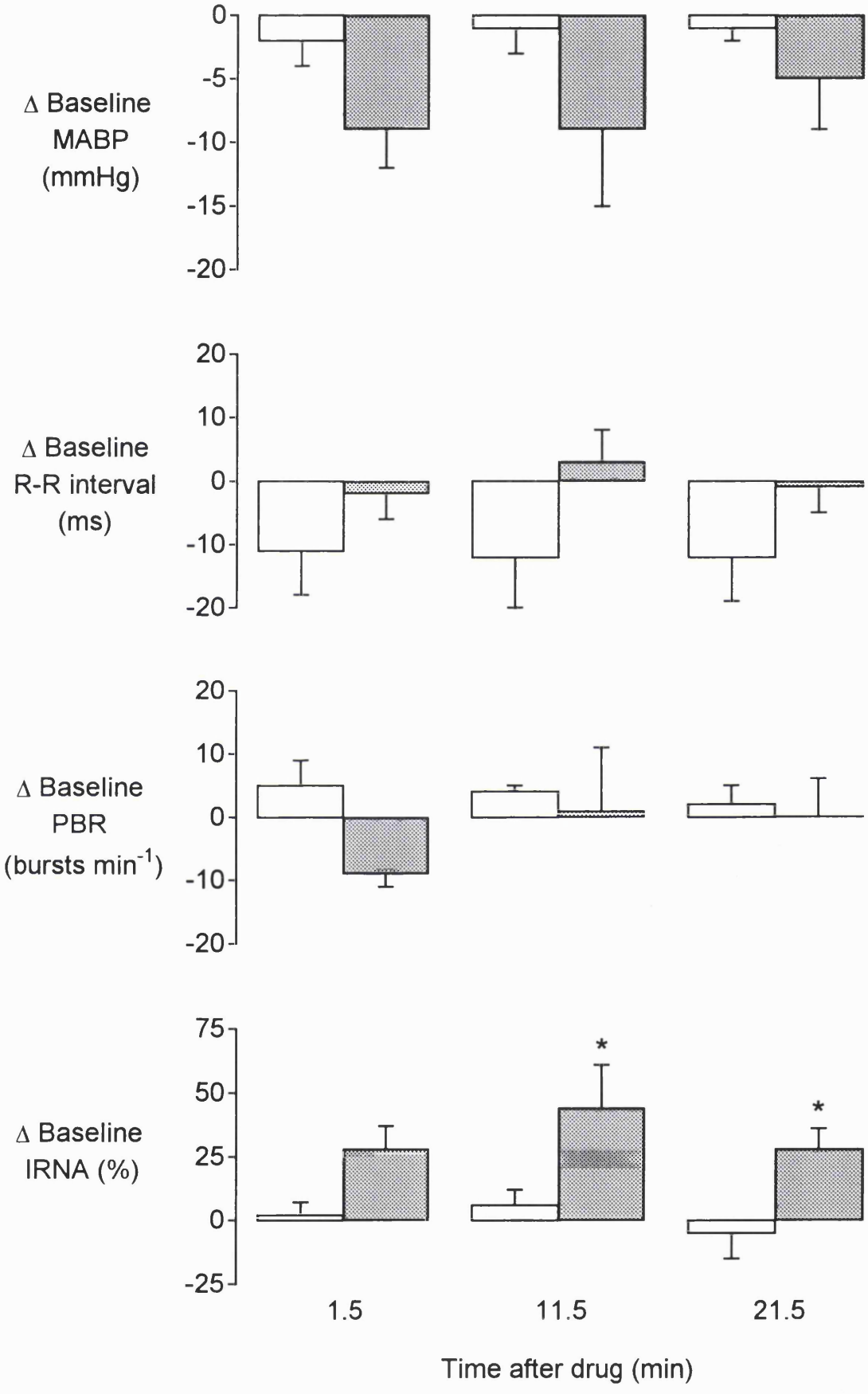
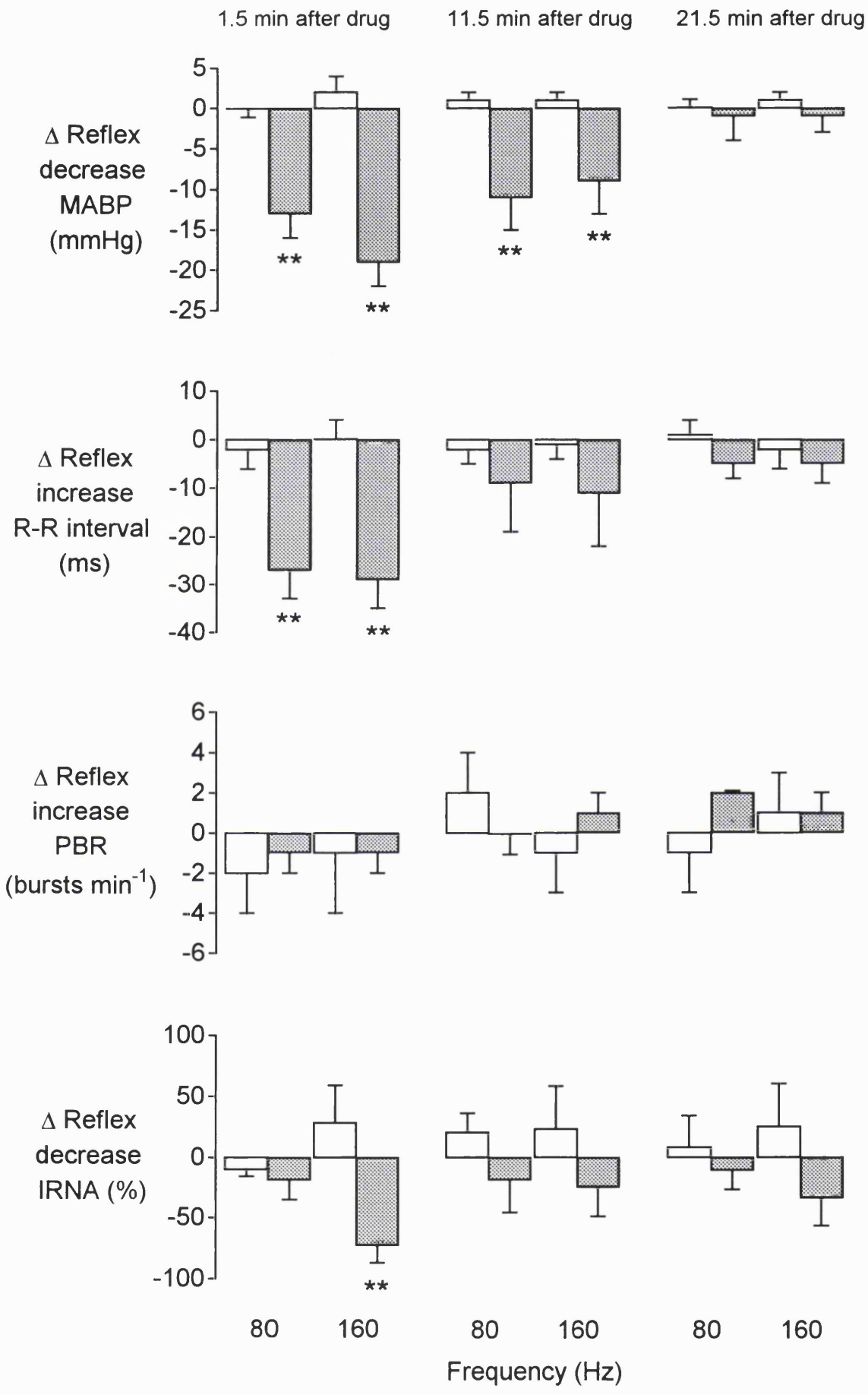

Δ Baseline MABP (mmHg)
0
-5
-10
-15
-20
Δ Baseline R-R interval (ms)
20
10
0
-10
-20
Δ Baseline PBR (bursts min-1)
20
10
0
-10
-20
Δ Baseline IRNA (%)
75
50
25
0
-25
*
*
1.5
11.5
21.5
Time after drug (min)

Figure 3.23

Histograms showing the effects of saline (20 μl i.c.; □ ; n=5) and WAY-100635 (100 μg kg^{-1} i.c.; ▨; n=6) on the reflex increases in R-R interval and phrenic burst rate (PBR) and the decreases in mean arterial blood pressure (MABP) and integrated renal nerve activity (IRNA) evoked by the stimulation of the left aortic nerve at 80 and 160 Hz. Sets of stimulations were performed 1.5 minutes after administration of the drug or vehicle and thereafter at 10 minute intervals. Each column represents the mean change (Δ) from the pre-drug control values and the bars show s.e.mean.

Comparisons between groups have been made using three-way ANOVA and the least significant difference test.

*$p<0.05$, **$p<0.01$, significant compared to saline i.c.


1.5 min after drug
11.5 min after drug
21.5 min after drug
Δ Reflex decrease MABP (mmHg)
5
0
-5
-10
-15
-20
-25
**
**
**
**
Δ Reflex increase R-R interval (ms)
10
0
-10
-20
-30
-40
**
**
Δ Reflex increase PBR (bursts min-1)
6
4
2
0
-2
-4
-6
Δ Reflex decrease IRNA (%)
100
50
0
-50
-100
**
80
160
80
160
80
160
Frequency (Hz)

3.4 Effects of 5-HT$_{1A}$ receptor ligands on the chemoreceptor reflex

Control stimulations

In the groups of animals in which the chemoreceptor reflex was elicited by bolus injections of NaCN (8 - 189 μg kg^{-1}) into the carotid artery (n = 25; protocols 2 and 3), control stimulations evoked a reflex increase in R-R interval of 29 ± 3 ms, an increase in ongoing renal sympathetic nerve activity of 74 ± 14 % and a small reflex hypertension of 8 ± 1 mmHg. Stimulation of chemoreceptor afferents also caused an increase in phrenic burst rate of 14 ± 1 bursts min^{-1}. The reflex changes described above usually occurred within 2 s following injection of NaCN. The responses were short lasting and returned to normal values within 30 seconds. Control responses evoked by stimulating chemoreceptor afferents are shown in figures 3.24 and 3.29.

The effects of buspirone and WAY-100635 on the responses evoked by chemoreceptor afferent stimulation were examined in protocols 2 and 3 respectively. The results from these experiments are described below.

Saline 20 μl i.c.

Intracisternal injection of the vehicle saline had no significant effect on the reflex responses evoked by stimulating chemoreceptor afferent with NaCN throughout the protocol (table 5.26). The mean control responses obtained before administration of the saline were compared with those at each time point after saline using the Students paired t-test.

Buspirone 200 μg kg^{-1} i.c. (1.14 – 1.68 μmol)

Intracisternal administration of buspirone had significant effects on the reflex responses evoked by stimulating chemoreceptor afferent with NaCN when compared to saline (figure 3.26 and table 5.28). The reflex increases in R-R interval evoked were significantly potentiated by 55 ± 23 and 73 ± 23 ms 10 and 20 minutes after administration of buspirone respectively. After 20 minutes, the reflex renal sympathoexcitation was significantly increased by 826

± 304 % and in addition, a significant potentiation of 465 ± 128 % was seen during the reflex that was elicited 30 minutes after buspirone. The reflex increases in mean arterial blood pressure were also significantly potentiated by 5 ± 4 and 7 ± 2 mmHg 10 and 20 minutes after administration of the drug respectively. However, buspirone had no significant effects on the reflex increases in phrenic burst rate.

Experimental traces showing the effects of buspirone on the responses evoked by stimulating chemoreceptor afferents are presented in figure 3.24.

WAY-100635 100 μg kg^{-1} i.v.

Pre-treatment with an intravenous injection of WAY-100635 had no significant effect on the reflex responses evoked by stimulating chemoreceptor afferents with NaCN when compared to saline (table 5.30).

(1.09 – 1.21 μmol)

Buspirone 200 μg kg^{-1} i.c. (pre-treated with WAY-100635 100 μg kg^{-1} i.v.)

Pre-treatment with WAY-100635 attenuated most of the effects of i.c. buspirone on the chemoreceptor reflex (figure 3.26 and table 5.32).

Pre-treatment with WAY-100635 significantly reduced the potentiation of the reflex increase in R-R interval evoked by buspirone. After 10 and 20 minutes, the potentiations of 55 ± 23 and 73 ± 23 ms evoked by buspirone alone were reduced to 7 ± 13 and 16 ± 5 ms.

After 20 and 30 minutes, the potentiations of the renal sympathoexcitation were also significantly blocked by pre-treatment with WAY-100635. They were reduced from potentiations of 826 ± 304 and 465 ± 128 % after buspirone alone to small increases of 10 ± 24 and 48 ± 10 % in the pre-treated group. The potentiation of the reflex hypertension after 20 minutes was significantly attenuated by pre-treatment with the WAY-100635. After buspirone alone, the reflex hypertension was potentiated by 7 ± 2 mmHg, however after pre-treatment with WAY-100635, this was reduced to an increase of 1 ± 2 mmHg.

As with buspirone alone, buspirone in the presence of the WAY-100635 had no significant effects on the reflex increases in phrenic burst rate.

Buspirone 200 μg kg^{-1} i.v.

The reflex increases in R-R interval evoked by stimulating chemoreceptor afferents were potentiated 10 and 20 minutes after intravenous administration of buspirone (figure 3.28 and table 5.34). These were significant when compared to saline but did not differ significantly from the potentiations caused by buspirone i.c. although the potentiation after 20 minutes did tend to be smaller. Buspirone i.v. also potentiated the reflex increase in renal nerve activity after 10 minutes by 448 ± 367 %. This was significant when compared to saline, however similar potentiations did not occur at any other times during the protocol. Intravenous administration of buspirone significantly potentiated the reflex hypertension evoked by chemoreceptor stimulation at all time points. These effects did not differ significantly from those produced by i.c. buspirone. In contrast to buspirone administered i.c., buspirone i.v. significantly reduced the reflex increase in phrenic burst rate evoked by chemoreceptor stimulation by 11 ± 1 bursts min^{-1} after 10 minutes.

WAY-100635 100 μg kg^{-1} i.c. (0.49 – 0.54 μmol)

Intracisternal administration of WAY-100635 had no significant effect on any of the reflex responses evoked by stimulating chemoreceptor afferents when compared to saline (figure 3.31 and table 5.36).

Experimental traces showing the reflex responses evoked by carotid chemoreceptor stimulation before and after i.c. administration of WAY-100635 are presented in figure 3.29.

The effects of the 5-HT$_{1A}$ receptor ligands on the reflex responses evoked by stimulating carotid chemoreceptor afferents with NaCN are summarised in table 3.5.

Table 3.5

Summary of the main effects of 5-HT_{1A} receptor ligands on the reflex increase in mean arterial blood pressure (MABP), R-R interval, phrenic burst rate (PBR) and integrated renal nerve activity (IRNA) evoked by stimulating carotid chemoreceptor afferents with NaCN.

0 = no significant change

↑ = significant increase within 20 minutes of drug administration

↓ = significant decrease within 20 minutes of drug administration

Drug	Increase MABP	Increase R-R interval	Increase PBR	Increase IRNA
Buspirone i.c.	↑	↑	0	↑
WAY-100635 i.v.	0	0	0	0
Buspirone i.c. / WAY-100635 i.v.	↑	0	0	0
Buspirone i.v.	↑	↑	↓	↑
WAY-100635 i.c.	0	0	0	0

Figure 3.24

The responses evoked by stimulating carotid chemoreceptor afferents with sodium cyanide (NaCN) during the control period and 20 and 30 minutes after administration of buspirone (200 $\mu g\ kg^{-1}$ i.c.) in an atenolol (1 $mg\ kg^{-1}$) pre-treated rabbit.

From the top, the traces show arterial blood pressure (ABP), heart rate (HR), integrated phrenic nerve activity (IPNA) and integrated renal nerve activity (IRNA).

Constant doses of NaCN were injected into the left lingual artery at the points marked by the arrows.

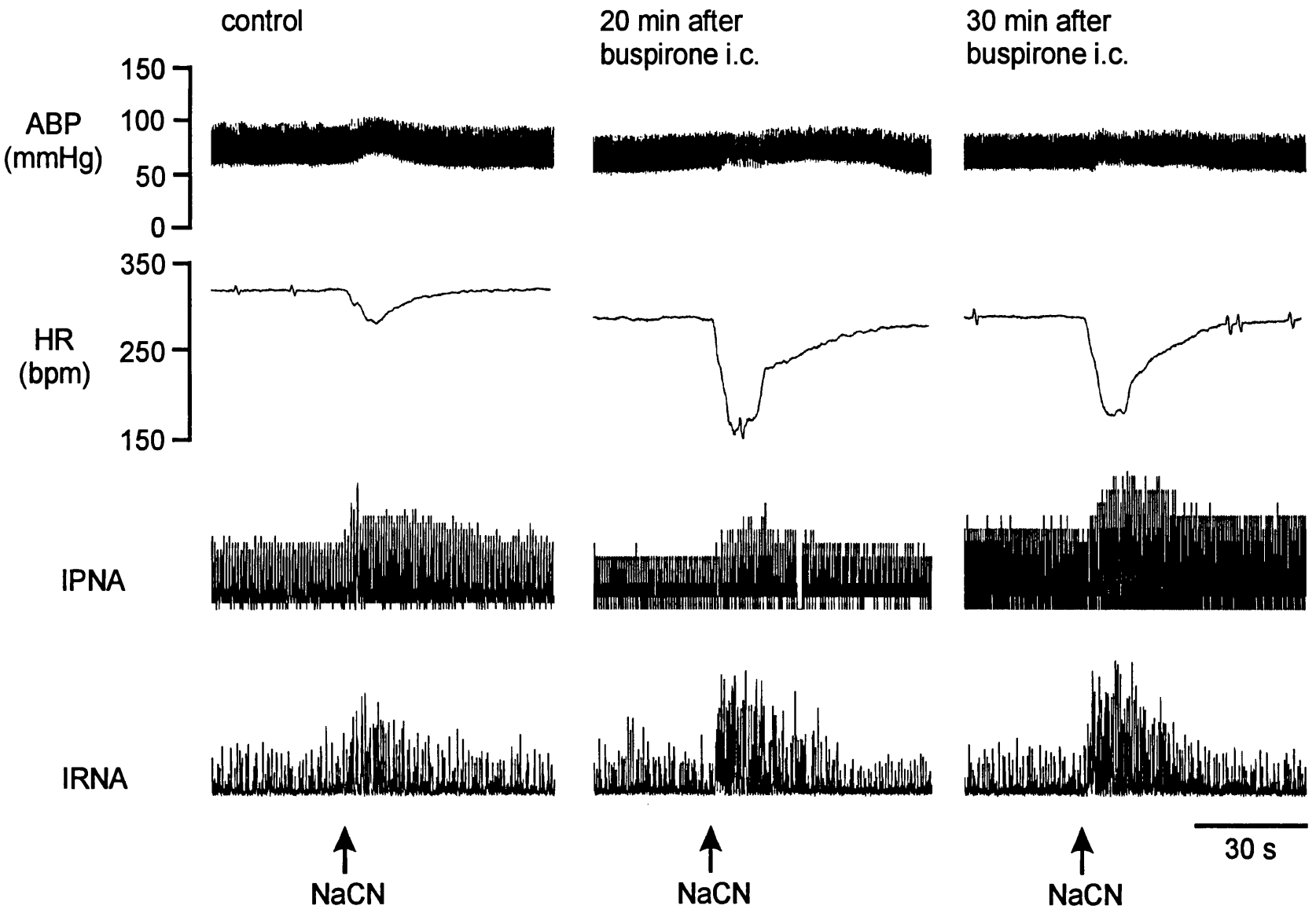

control
20 min after
buspirone i.c.
30 min after
buspirone i.c.
150
100
50
0
ABP
(mmHg)
350
250
150
HR
(bpm)
IPNA
IRNA
NaCN
NaCN
NaCN
30 s

Figure 3.25

Histograms showing the effects of saline (20 μl i.c.; □; n=5), buspirone (200 μg kg^{-1} i.c.; ■; n=5) and buspirone (200 μg kg^{-1} i.c.) 20 minutes after pre-treatment with WAY-100635 (100 μg kg^{-1} i.v.; ▨; n=5) on baseline mean arterial blood pressure (MABP), R-R interval, phrenic burst rate (PBR) and integrated renal nerve activity (IRNA). Baseline variables were measured immediately prior to the stimulation of chemoreceptor afferents (see figure 3.26). Effects are shown at 10 min after administration of the drug or vehicle and thereafter at 10 minute intervals. Each column represents the mean change (Δ) from the pre-drug control values and the bars show s.e.mean.

Comparisons between groups have been made using two-way ANOVA and the least significant difference test.

*$p<0.05$, **$p<0.01$ compared to saline i.c.

†$p<0.05$, ††$p<0.01$ compared to buspirone i.c. alone.

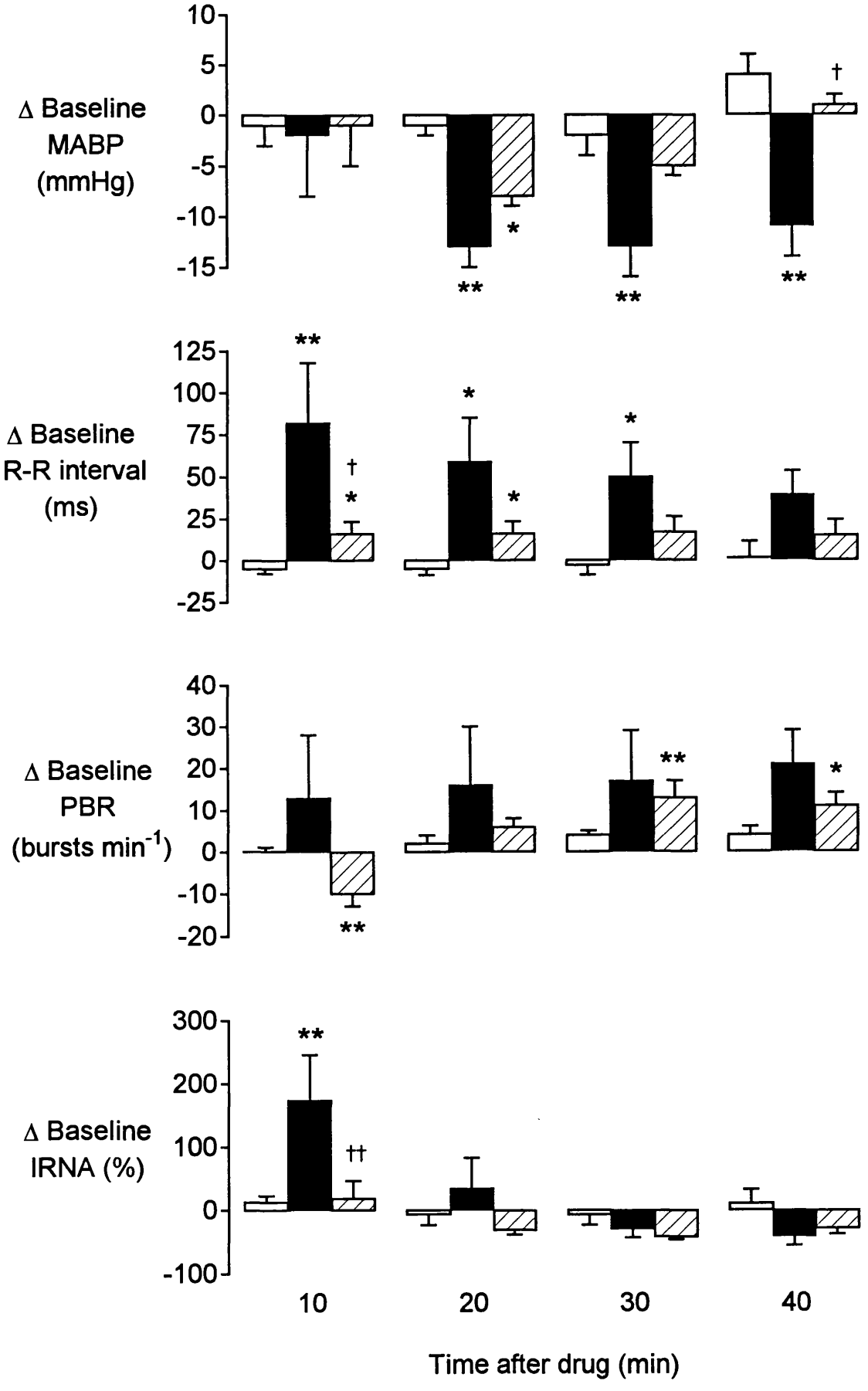

Δ Baseline MABP (mmHg)
10
5
0
-5
-10
-15
†
*
**
**
**
Δ Baseline R-R interval (ms)
125
100
75
50
25
0
-25
**
†
*
*
*
*
Δ Baseline PBR (bursts min-1)
40
30
20
10
0
-10
-20
**
**
*
Δ Baseline IRNA (%)
300
200
100
0
-100
**
††
10
20
30
40
Time after drug (min)

Figure 3.26

Histograms showing the effects of saline (20 μl i.c.; □; n=5), buspirone (200 μg kg^{-1} i.c.; ■; n=5) and buspirone (200 μg kg^{-1} i.c.) 20 minutes after pre-treatment with WAY-100635 (100 μg kg^{-1} i.v.; ▨; n=5) on the reflex increases in mean arterial blood pressure (MABP), R-R interval, phrenic burst rate (PBR) and integrated renal nerve activity (IRNA) evoked by stimulating carotid chemoreceptor afferents with NaCN. Stimulations were performed 10 minutes after administration of the drug or vehicle and thereafter at 10 minute intervals. Each column represents the mean change (Δ) from the pre-drug control values and the bars show s.e.mean.

Comparisons between groups have been made using two-way ANOVA and the least significant difference test.

*$p<0.05$, **$p<0.01$ compared to saline i.c.

†$p<0.05$, ††$p<0.01$ compared to buspirone i.c. alone.

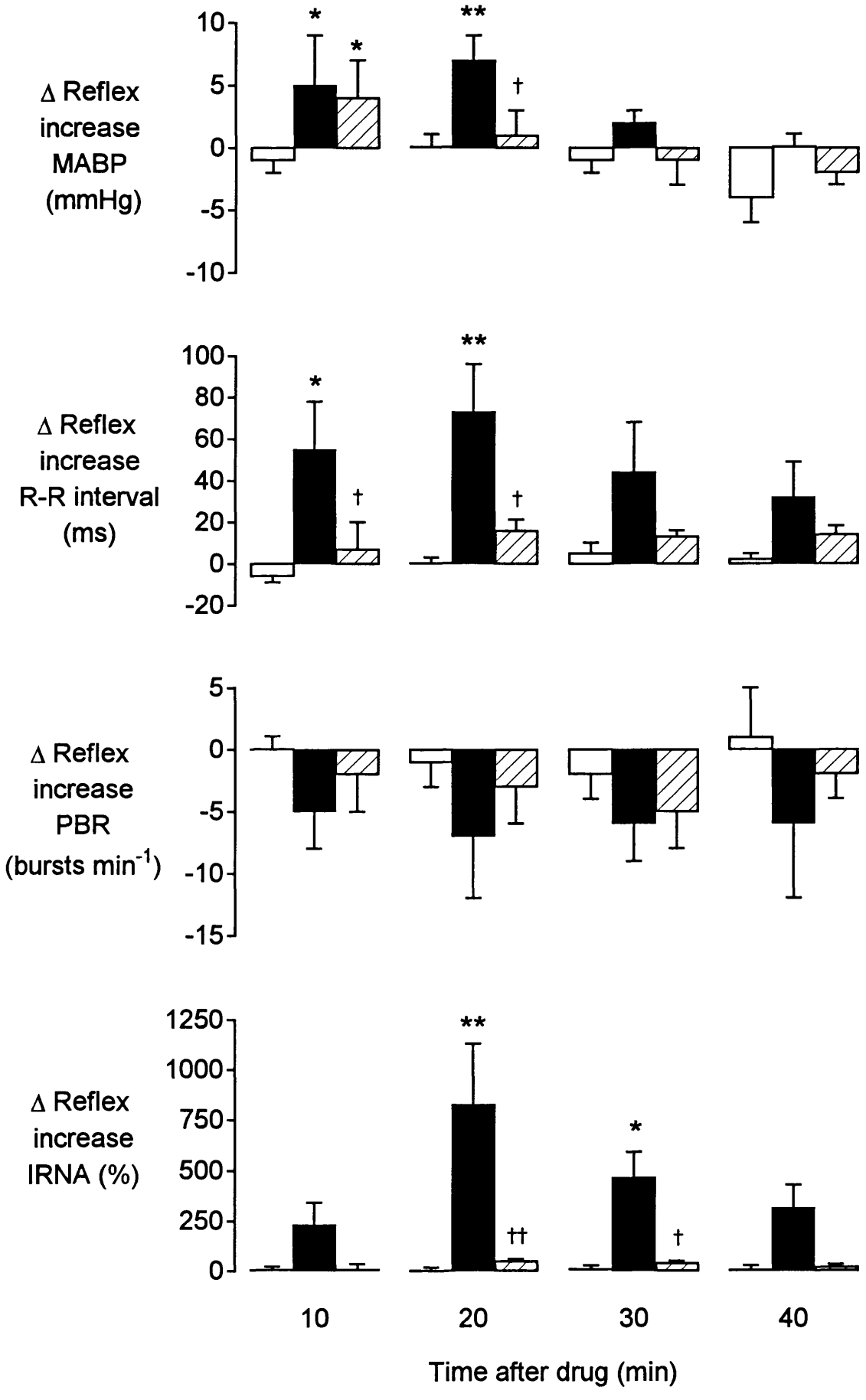

Δ Reflex increase MABP (mmHg)
10
5
0
-5
-10
*
*
**
†
Δ Reflex increase R-R interval (ms)
100
80
60
40
20
0
-20
*
†
**
†
Δ Reflex increase PBR (bursts min-1)
5
0
-5
-10
-15
Δ Reflex increase IRNA (%)
1250
1000
750
500
250
0
**
*
††
†
10
20
30
40
Time after drug (min)

Figure 3.27

Histograms showing the effects of saline (20 μl i.c.; □; n=5), buspirone (200 μg kg^{-1} i.c.; ■; n=5) and buspirone (200 μg kg^{-1} i.v.; ⊠; n=4) on baseline mean arterial blood pressure (MABP), R-R interval, phrenic burst rate (PBR) and integrated renal nerve activity (IRNA). Baseline variables were measured immediately prior to the stimulation of chemoreceptor afferents (see figure 3.28). Effects are shown at 10 min after administration of the drug or vehicle and thereafter at 10 minute intervals. Each column represents the mean change (Δ) from the pre-drug control values and the bars show s.e.mean.

Comparisons between groups have been made using two-way ANOVA and the least significant difference test.

*$p<0.05$, **$p<0.01$, significant compared to saline i.c.

†$p<0.05$, ††$p<0.01$, significant compared to buspirone i.c.

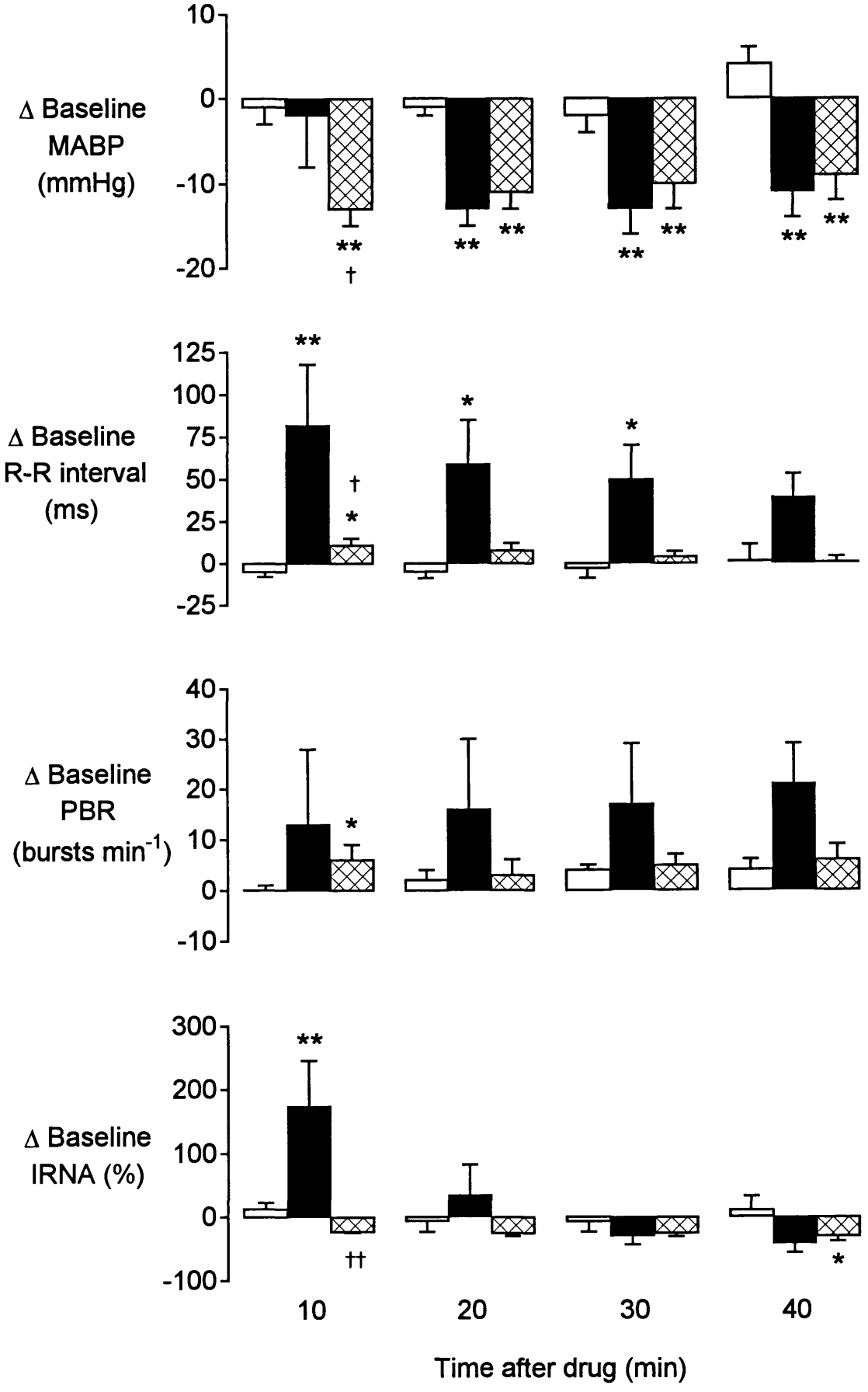

Δ Baseline MABP (mmHg)
10
0
-10
-20
**
†
**
**
**
**
**
**
Δ Baseline R-R interval (ms)
125
100
75
50
25
0
-25
**
†
*
*
*
Δ Baseline PBR (bursts min-1)
40
30
20
10
0
-10
*
Δ Baseline IRNA (%)
300
200
100
0
-100
**
††
*
10
20
30
40
Time after drug (min)

Figure 3.28

Histograms showing the effects of saline (20 μl i.c.; □; n=5), buspirone (200 μg kg^{-1} i.c.; ■; n=5) and buspirone (200 μg kg^{-1} i.v.; ⊠; n=4) on the reflex increases in mean arterial blood pressure (MABP), R-R interval, phrenic burst rate (PBR) and integrated renal nerve activity (IRNA) evoked by stimulating carotid chemoreceptor afferents with NaCN. Stimulations were performed 10 minutes after administration of the drug or vehicle and thereafter at 10 minute intervals. Each column represents the mean change (Δ) from the pre-drug control values and the bars show s.e.mean.

Comparisons between groups have been made using two-way ANOVA and the least significant difference test.

*$p<0.05$, **$p<0.01$, significant compared to saline i.c.

†$p<0.05$, ††$p<0.01$, significant compared to buspirone i.c.

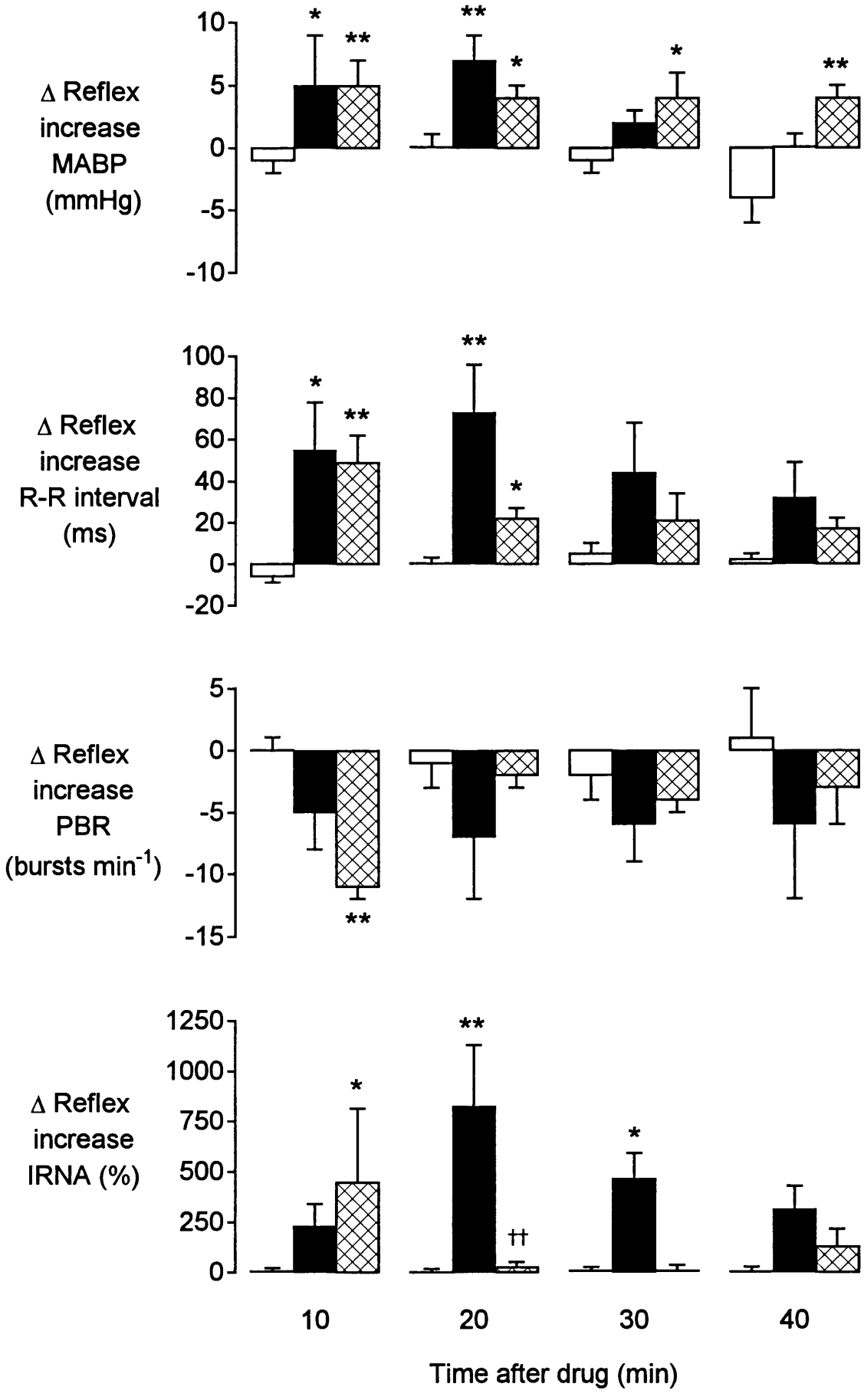

Δ Reflex increase MABP (mmHg)
10
5
0
-5
-10
Δ Reflex increase R-R interval (ms)
100
80
60
40
20
0
-20
Δ Reflex increase PBR (bursts min-1)
5
0
-5
-10
-15
Δ Reflex increase IRNA (%)
1250
1000
750
500
250
0
*
**
††
10
20
30
40
Time after drug (min)

Figure 3.29

The responses evoked by stimulating the left aortic nerve at 160 Hz during the control period and 3.5 minutes after administration of WAY-100635 (100 μg kg^{-1} i.c.) in an atenolol (1 mg kg^{-1}) pre-treated rabbit (Top traces). The aortic nerve was electrically stimulated at points shown by the arrows.

The responses evoked by stimulating carotid chemoreceptor afferents with sodium cyanide (NaCN) during the control period and 5 minutes after administration of WAY-100635 (100 μg kg^{-1} i.c.) in the same rabbit as above (Bottom traces). Constant doses of NaCN were injected into the left lingual artery at the points marked by the arrows.

The traces show arterial blood pressure (ABP), heart rate (HR), integrated phrenic nerve activity (IPNA) and integrated renal nerve activity (IRNA).

Note the inhibitory effects of WAY-100635 on the responses evoked by aortic nerve stimulation and the lack of effects on the responses evoked by chemoreceptor stimulation.

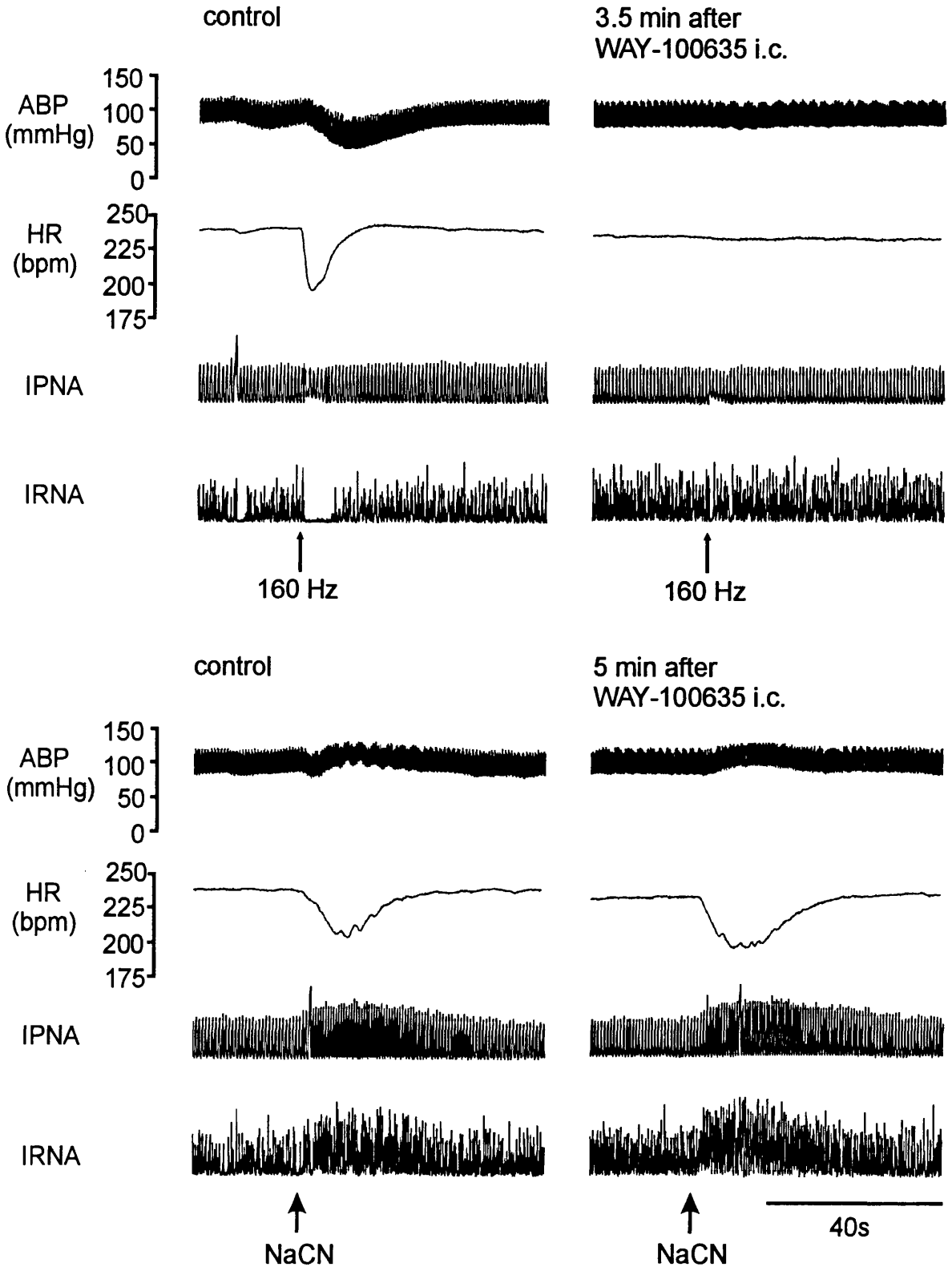

control
3.5 min after
WAY-100635 i.c.
ABP
(mmHg)
150
100
50
0
HR
(bpm)
250
225
200
175
IPNA
IRNA
160 Hz
160 Hz
control
5 min after
WAY-100635 i.c.
ABP
(mmHg)
150
100
50
0
HR
(bpm)
250
225
200
175
IPNA
IRNA
NaCN
NaCN
40s

Figure 3.30

Histograms showing the effects of saline (20 μl i.c.; □; n=5) and WAY-100635 (100 μg kg^{-1} i.c.; ▨; n=6) on baseline mean arterial blood pressure (MABP), R-R interval, phrenic burst rate (PBR) and integrated renal nerve activity (IRNA). Baseline variables were measured immediately prior to the stimulation of chemoreceptor afferents (see figure 3.31). Effects are shown at 5 min after administration of the drug or vehicle and thereafter at 10 minute intervals. Each column represents the mean change (Δ) from the pre-drug control values and the bars show s.e.mean.

Comparisons between groups have been made using two-way ANOVA and the least significant difference test.
*$p<0.05$, **$p<0.01$, significant compared to saline i.c.

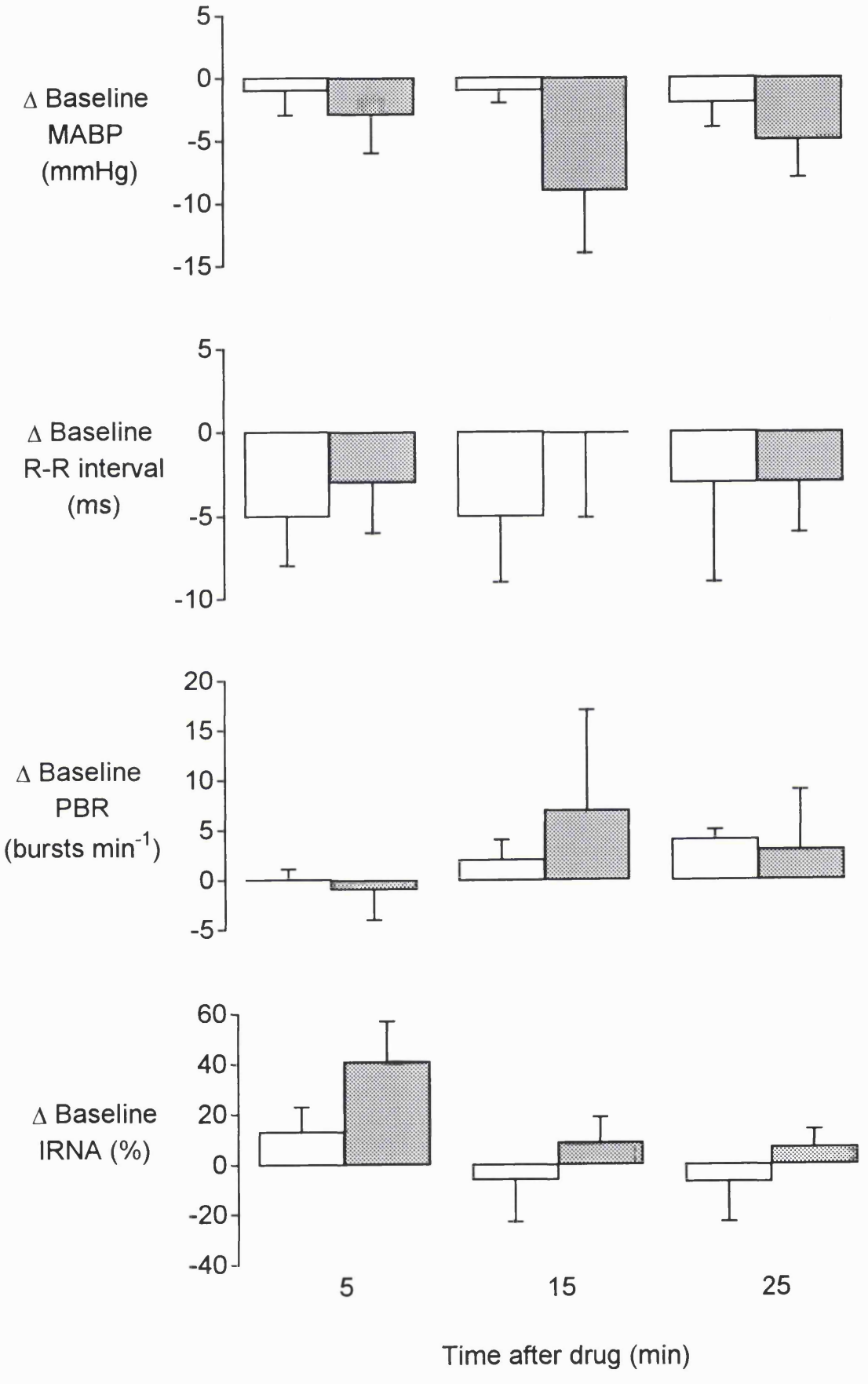
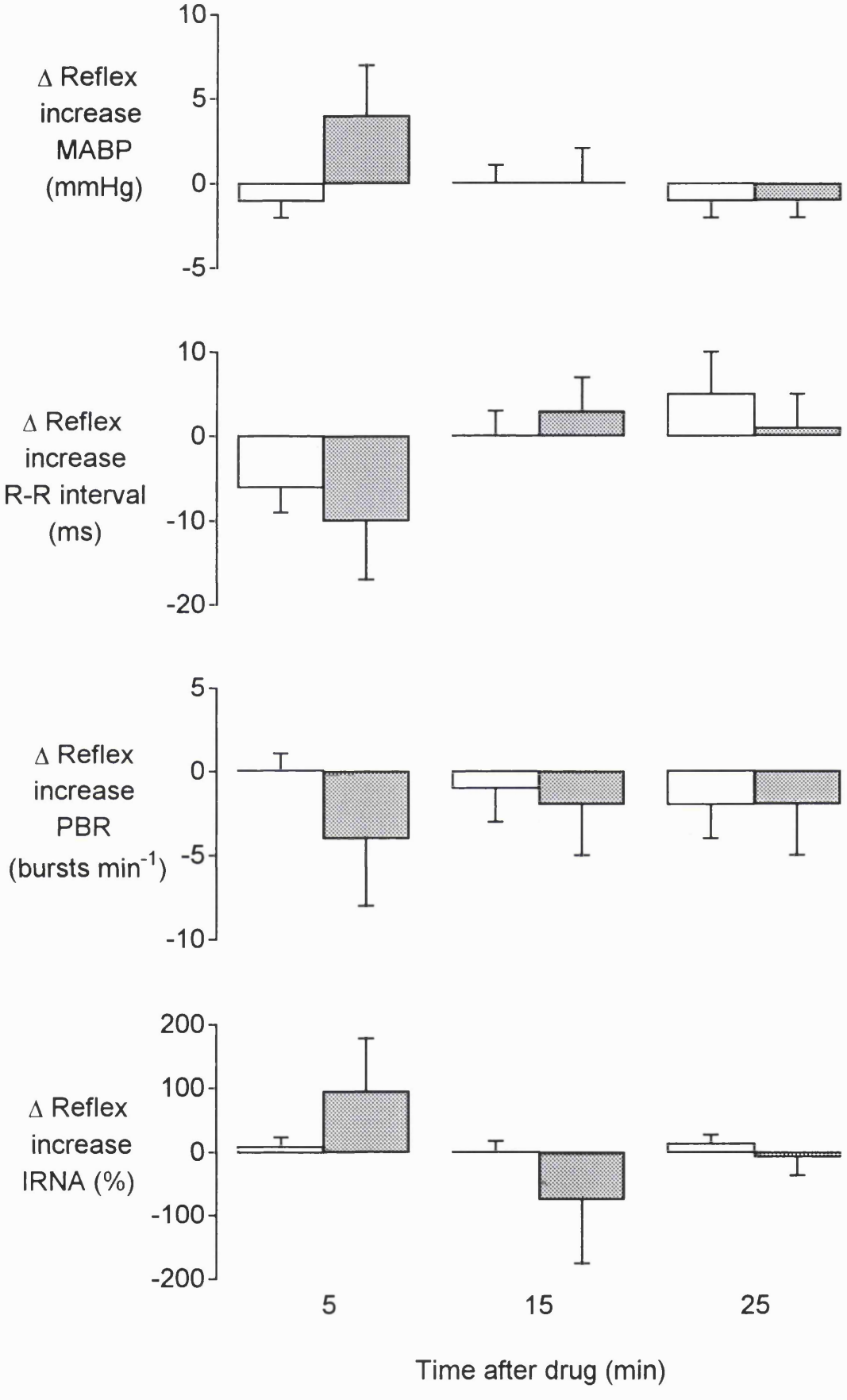

Δ Baseline MABP (mmHg)
5
0
-5
-10
-15
Δ Baseline R-R interval (ms)
5
0
-5
-10
Δ Baseline PBR (bursts min-1)
20
15
10
5
0
-5
Δ Baseline IRNA (%)
60
40
20
0
-20
-40
5
15
25
Time after drug (min)

Figure 3.31

Histograms showing the effects of saline (20 μl i.c.; □ ; n=5) and WAY-100635 (100 μg kg^{-1} i.c.; ▨; n=6) on the reflex increases in mean arterial blood pressure (MABP), R-R interval, phrenic burst rate (PBR) and integrated renal nerve activity (IRNA) evoked by stimulating carotid chemoreceptor afferents with NaCN. Stimulations were performed 5 minutes after administration of the drug or vehicle and thereafter at 10 minute intervals. Each column represents the mean change (Δ) from the pre-drug control values and the bars show s.e.mean.

Comparisons between groups have been made using two-way ANOVA and the least significant difference test.

*$p<0.05$, **$p<0.01$, significant compared to saline i.c.


Δ Reflex increase MABP (mmHg)
10
5
0
-5
Δ Reflex increase R-R interval (ms)
10
0
-10
-20
Δ Reflex increase PBR (bursts min-1)
5
0
-5
-10
Δ Reflex increase IRNA (%)
200
100
0
-100
-200
5
15
25
Time after drug (min)

3.5 Intracisternal injection of dye

To examine the potential site of action of the drugs applied i.c. 20 μl of Pontamine sky blue dye was injected intracisternally in a number of experiments. After 50 minutes (the duration of the longest experiment), heaviest staining was found on the dorsal surface of the brainstem in the region of the intracisternal cannula tip (figure 3.32a) There was also considerable staining on the ventral surface of the brainstem extending rostrally over central pontine areas and into the interpeduncular fossa of the midbrain (figure 3.32b). No staining was evident over the cerebellum or in forebrain regions. A saggital section of the entire brain revealed penetration of the dye beneath the pial surface into the brain parenchyma on both dorsal and ventral brainstem surfaces (figure 3.32c). The dye did not appear to have entered the ventricular system which is to be expected since in the rabbit there is no foramen of Magendie (Feldberg, 1976).

Figure 3.32

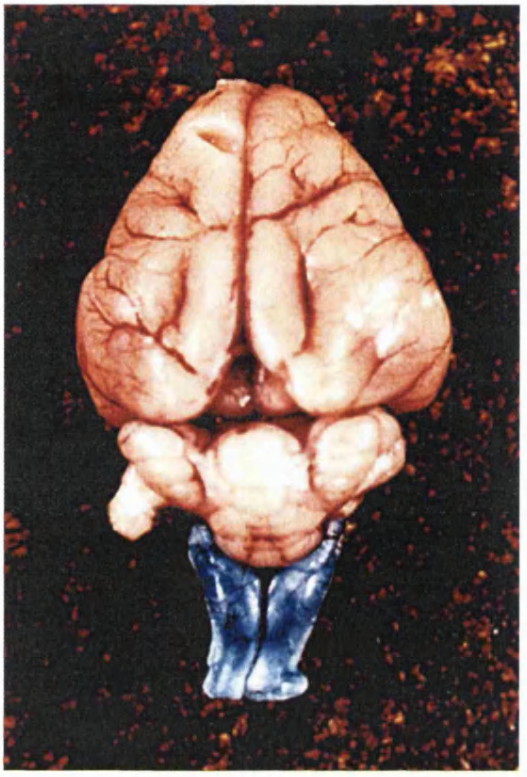
Staining of a dissected rabbit brain 50 minutes after intracisternal injection of 20 μl Pontamine sky blue.

a) dorsal surface

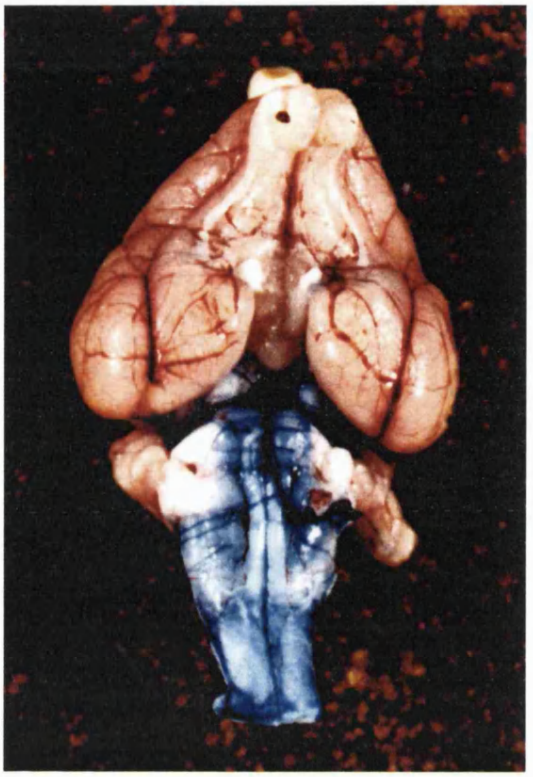
b) ventral surface

c) saggital section

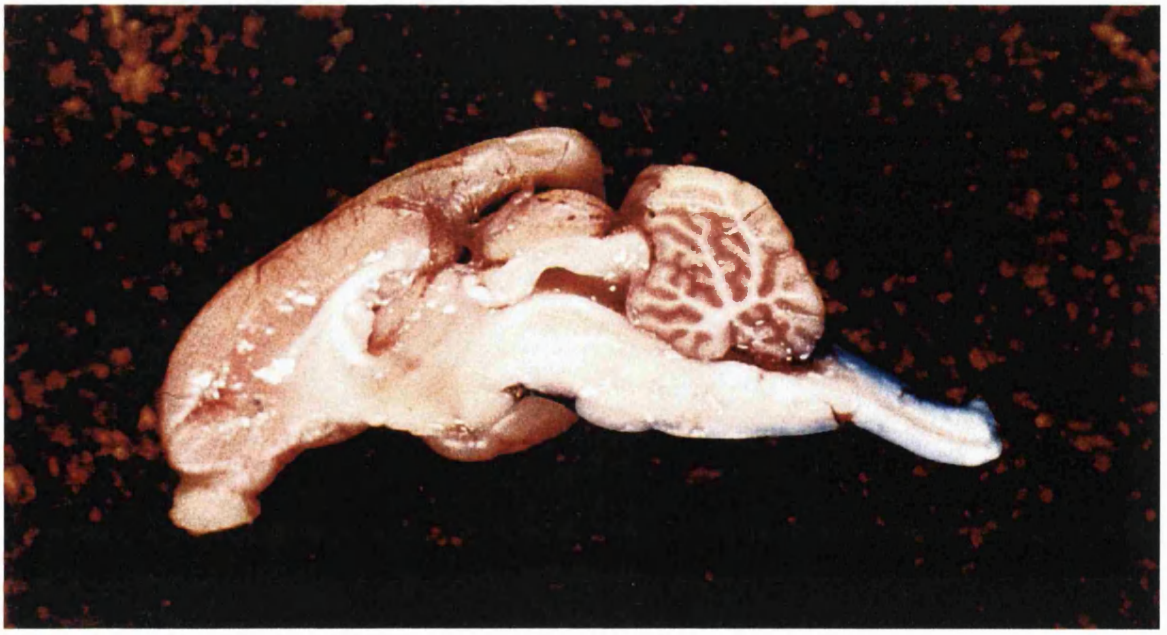
a



b



c



4 Discussion

4.1 Main findings

The present studies have examined the effects of buspirone on baseline cardiorespiratory variables and on the reflex responses evoked by stimulating cardiopulmonary, baroreceptor and chemoreceptor afferents in urethane-anaesthetised rabbits. Intracisternal administration of buspirone was found to have significant modulatory effects on baseline variables and on the evoked reflex responses. Most of these effects were attenuated by i.v. pre-treatment with the selective 5-HT_{1A} receptor antagonist WAY-100635 (Forster et al., 1995) indicating that the effects of buspirone were due to activation of 5-HT_{1A} receptors. The effects of central 5-HT_{1A} receptor blockade were also examined by administering WAY-100635 intracisternally. Whilst this only had significant effects on baseline renal nerve activity, the reflex responses evoked by stimulating cardiopulmonary and baroreceptor afferents were all significantly inhibited. These effects are discussed in more detail in sections 4.3 - 4.6.

4.2 Selectivity of 5-HT_{1A} receptor ligands used

Buspirone

In many previous studies, the effects of 5-HT_{1A} receptor activation on baseline cardiorespiratory variables and reflexly-evoked cardiovascular changes have been examined using the archetypal 5-HT_{1A} receptor agonist 8-OH-DPAT (Fozard et al., 1987; Futuro-Neto et al, 1993). Whilst in the majority of studies 8-OH-DPAT has been assumed to act selectively at the 5-HT_{1A} receptor subtype, recent studies in the anaesthetised rabbit have shown that in addition to its actions on 5-HT_{1A} receptors, 8-OH-DPAT can also exert significant effects via the activation of 5-$HT_{1B/1D}$ receptors (Dando et al., 1998). Therefore, in the present studies, the effects of 5-HT_{1A} receptor activation on certain cardiovascular reflexes were examined using the 5-HT_{1A} receptor partial agonist buspirone (Schoeffter and Hoyer, 1988). This compound has

high affinity for 5-HT_{1A} receptors (K_i = 9.3 nM) and is relatively inactive or only displays moderate affinity at 5-HT_{1D} (K_i > 12000 nM), 5-HT_2 (K_i = 178 nM), α_1-adrenoceptors (K_i > 2427 nM), α_2-adrenoceptors (K_i > 1042 nM) and D_1-dopamine receptors (K_i >1000 nM) (McCall et al., 1994a).

In the present studies buspirone was administered at a dose of 200 μg kg^{-1}. This dose has been previously shown to modulate the reflex responses evoked by stimulating cardiopulmonary and upper airway afferents in rats and rabbits when administered intracisternally. (Bogle et al., 1990; Futuro-Neto et al., 1993; Dando et al., 1994; Dando et al., 1998).

WAY-100635

WAY-100635 is an archiral analogue of the previously described 5-HT_{1A} receptor antagonist WAY-100135 (Fletcher et al., 1993a). In functional assays, WAY-100635 has been shown to act as a potent 5-HT_{1A} receptor antagonist at both presynaptic (somatodendritic) and postsynaptic 5-HT_{1A} receptors. In the isolated guinea pig ileum, WAY-100635 antagonises the 5-HT_{1A} receptor agonist actions of 5-CT with a pA_2 of 9.71 (Forster et al., 1995). WAY-100635 also antagonised the well characterised 8-OH-DPAT-induced behavioural syndrome in rats and guinea pigs and the 8-OH-DPAT-induced hypothermia in the mouse and the rat (Forster et al, 1995). The inhibitory effects of 8-OH-DPAT on the firing of serotonergic dorsal raphe neurones have also been shown to be antagonised by WAY-100635 (Wang et al., 1995; Forster et al., 1995; Martin et al., 1999). In anaesthetised rats, WAY-100635 at i.v. doses up to 100 μg kg^{-1} has been shown to have no significant effect on the firing rate of the serotonergic dorsal raphe neurones suggesting that this ligand does not possess any intrinsic agonist activity at pre-synaptic 5-HT_{1A} receptors (Forster et al., 1995). At higher doses, WAY-100635 has been shown to inhibit the firing of dorsal raphe neurones, however this effect has been attributed to α_1-adrenoceptor blockade (Martin et al., 1999). In conscious cats, the firing rate of serotonergic neurones of the dorsal raphe was increased by WAY-100635

indicating that these cells were under a tonic inhibitory control by endogenous 5-HT (Fornal et al., 1994).

Receptor binding assays have shown that WAY-100635 has a greater than 100-fold selectivity for the 5-HT_{1A} receptor relative to other 5-HT receptor subtypes and major neurotransmitter receptor sites (Forster et al., 1995). Whilst one study in the rat has shown that WAY-100635 can act as an antagonist at α_1-adrenoceptors (Martin et al., 1999), its affinity for 5-HT_{1A} receptors (K_i = 0.2 nM) exceeds that at α_1-adrenoceptors (K_i = 45 nM) by almost 200-fold (Johansson et al., 1997).

In the present studies WAY-100635 was administered at a dose of 100 μg kg^{-1}. This dose of WAY-100635 has been used in previous studies and shown to modulate the reflex responses evoked by stimulating upper airway afferents when administered intracisternally. (Dando et al., 1998).

4.3 Effects of 5-HT_{1A} receptor ligands on baseline variables

Effects of the 5-HT_{1A} receptor partial agonist buspirone

Heart rate

In the present experiments, intracisternal administration of buspirone significantly decreased baseline heart rate equating to an increase in R-R interval. This effect of i.c. buspirone was due to activation of 5-HT_{1A} receptors as it was attenuated by i.v. pre-treatment with the selective 5-HT_{1A} receptor antagonist WAY-100635, which itself had no effect on baseline R-R interval. The present findings also suggest that the receptors involved are located centrally, as the same dose of buspirone when administered intravenously had no effect on baseline R-R interval. As these experiments were performed in the presence of atenolol, the bradycardia produced by i.c. buspirone must have been due to an increase in cardiac vagal activity. This is in accord with other studies in rats (Gradin et al., 1995a), cats (Ramage and Fozard; 1987; Ramage et al., 1988; McCall et al., 1994b) and rabbits (Dando, 1995) in which buspirone

and other 5-HT_{1A} receptor agonists have been shown to elicit a vagal bradycardia.

It is unlikely that the effects of buspirone on baseline heart rate are secondary to its effects on central respiratory drive. Intracisternal administration of buspirone caused an increase in respiratory frequency (see below) which might be expected to raise baseline heart rate via inhibitory effects on cardiac vagal outflow (see Daly, 1986).

Respiratory rate

Intracisternal administration of buspirone had significant effects on central respiratory activity increasing the frequency of phrenic nerve discharges. This is consistent with previous findings in conscious rats in which buspirone also evoked an increase in respiratory rate (Mendelson et al., 1990). In the present study, the increase in phrenic burst rate was always reduced by i.v. pre-treatment with WAY-100635 and at certain time points, this reduction attained significance suggesting that the effects of buspirone were due to activation of 5-HT_{1A} receptors. In addition, buspirone administered i.v. had no effect on phrenic discharge frequency indicating that the 5-HT_{1A} receptors activated were central in origin. Similar increases in respiratory frequency have also been observed in cats following i.v. or fourth ventricular administration of the 5-HT_{1A} receptor agonist 8-OH-DPAT (Lalley et al., 1994; Rose et al., 1995).

In previous studies, the compound action potentials recorded in the phrenic nerve have been integrated and the amplitude of each burst measured to obtain an indication of tidal volume (Eldridge, 1971). This method was not routinely used in the present experiments since spontaneous muscular activity of the unparalysed animal sometimes caused slight movements of the recording electrode/phrenic nerve arrangement causing changes in amplitude of the phrenic nerve signal. However, in experiments in which movement did not occur, the 5-HT_{1A} receptor ligands used did not have any obvious effect on the amplitude of integrated phrenic bursts. A previous study in urethane-

anaesthetised rabbits has also shown that i.v. administration of 8-OH-DPAT dose not significantly affect the number of action potentials in each phrenic burst (Shepheard et al., 1990).

Arterial blood pressure and renal sympathetic nerve activity

Intracisternal administration of buspirone had a biphasic effect on mean arterial blood pressure causing a small but significant pressor response followed by a more prolonged hypotension. The initial transient pressor-response, which occurred during the first five minutes following i.c. buspirone, was accompanied by a renal sympathoexcitation. Both effects were centrally-mediated since they were not evident when the same dose of buspirone was administered i.v.

Although the renal sympathoexcitation was significantly attenuated by pre-treatment with the WAY-100635, the buspirone-evoked pressor response was not significantly reduced and in fact, tended to be greater in the presence of the 5-HT_{1A} receptor antagonist. These findings therefore suggest that the initial pressor response evoked by buspirone is not due to actions at 5-HT_{1A} receptors but may involve other mechanisms. Receptor binding profiles reveal that in addition to its high affinity for 5-HT_{1A} receptors, buspirone also binds with high affinity to dopamine-D_2 receptors (K_i = 13 nM) (McCall et al., 1994a). In the rat, buspirone antagonises the inhibition of dopaminergic neurones in the substantia nigra pars by amphetamine suggesting that it acts as an antagonist at dopamine-D_2 autoreceptors (Piercey et al, 1994). Antagonism at D_2 receptors may provide an explanation for the initial pressor effects of buspirone since in the urethane-anaesthetised rabbit, i.c. administration of the D_2 receptor antagonist sulpiride has also been shown to mediate increases in baseline arterial blood pressure (Dando, 1995).

Approximately 20 minutes after its i.c. administration, buspirone evoked a significant and prolonged fall in arterial blood pressure. A similar hypotensive effect has been described in the rat, rabbit and cat following i.c. or i.v.

administration buspirone and other 5-HT_{1A} receptor agonists (Fozard et al., 1987; Ramage and Fozard, 1987; Ramage et al., 1988, Wouters et al., 1988; Hof and Fozard; 1989; Romero et al., 1993, Futuro-Neto et al., 1993). This has been found to be due primarily to an inhibition of ongoing sympathetic nerve activity resulting in peripheral vasodilatation (Ramage et al., 1988; Wouters et al., 1988; Hof and Fozard, 1989). In the present experiments, administration of buspirone did cause a simultaneous reduction in ongoing renal sympathetic nerve activity however, this effect was weak and rarely reached significance. Studies in the rabbit suggest that in contrast to other species, the renal nerve is much less sensitive to the sympathoinhibitory effects of 8-OH-DPAT than other sympathetic outflows (Shepheard et al., 1990). This could explain the present findings and suggests that the fall in arterial blood pressure was due to vasodilatation in areas other than the renal vascular bed.

In a number of studies, the hypotensive potency of 5-HT_{1A} receptor agonists has been shown to be increased after central administration of the drug when compared to i.v. administration, thus confirming that the effect is mediated at a central site (Doods et al., 1988; Wouters et al., 1988). Surprisingly, in the present experiments, the fall in mean arterial blood pressure produced by i.v. buspirone was of similar magnitude to that produced by buspirone i.c. and was in fact more rapid in onset, occurring within 5 minutes of administration. Although these results might argue against a central site of action, it is unlikely that the hypotensive effects of buspirone are due to peripheral vasodilator mechanisms. In previous studies, buspirone has been shown to mediate vasoconstriction in the external carotid bed of intact or sympathectomised dogs (Terron et al., 1996) and also causes contractions of isolated rabbit aorta (Castillo et al., 1993). In addition, i.v. administration of buspirone caused an increase in arterial blood pressure in ganglion-blocked or pithed rats (Hanson et al., 1986; Castillo et al., 1995). These effects are all thought to be due to the activation of α_1-adrenoceptors in the vascular smooth

muscle since they were inhibited by pre-treatment with the α_1-adrenoceptor antagonist prazosin.

It is therefore possible that the dose of buspirone used in this study is large enough to reach maximally effective concentrations at central structures involved in the control of vasomotor tone whether administered i.v. or i.c. In this respect, the ED_{50} for the sympathoinhibitory effects of i.v. buspirone in rats was found to be 80 $\mu g\ kg^{-1}$ (Romero et al., 1993) suggesting that our dose of 200 $\mu g\ kg^{-1}$ may indeed have been supra-maximal. This however, does not explain why the onset latency of the hypotension was longer when buspirone was administered i.c. than when the drug was given i.v. It is possible that this could be due to the slow diffusion of the drug from the cisterna magna to the target sites. Alternatively, the hypotensive effect of i.c. buspirone could have been masked by the initial pressor effect that was evoked after central administration of the drug. Similar differences in onset latency of the evoked hypotension have been observed in cats following i.v. and i.c. administration of flesinoxan (Wouters et al., 1988).

At most time intervals, the fall in arterial blood pressure evoked by i.c. buspirone was less following pre-treatment with WAY-100635 however, this did not reach statistical significance until after 37.5 minutes. This may suggest that the hypotensive effects of buspirone before this time were due to actions at other receptor subtypes. It is more likely however, that the effects of i.c. buspirone were due to actions at 5-HT_{1A} receptors, but that when given i.v., the dose of WAY-100635 was not great enough to exert a significant blockade at the central 5-HT_{1A} receptors responsible. The reason why pre-treatment with WAY-100635 was able to significantly attenuate the buspirone-evoked bradycardia at all time points but not the hypotension is not known however, another study in cats has shown that the bradycardia induced by 8-OH-DPAT is more readily attenuated by the 5-HT_{1A} receptor antagonist pindolol than the evoked hypotension (Wouters et al., 1988).

Effects of the 5-HT_{1A} receptor antagonist WAY-100635

Heart rate

Intracisternal or intravenous administration of the selective 5-HT_{1A} receptor antagonist WAY-100635 had no significant effect on baseline R-R interval. It is possible that the dose of antagonist used was not sufficient to exert significant blockade at central 5-HT_{1A} receptors. This seems unlikely as when administered i.v., the dose was sufficient to significantly attenuate the effects of centrally applied buspirone on baseline heart rate. In a study in anaesthetised spinal cats, i.v. injection of WAY-100135, a structurally analogous 5-HT_{1A} receptor antagonist, also had no effect on baseline heart rate, even at doses ten times higher than those used in the present studies (McCall et al., 1994b). It therefore seems likely that in these anaesthetised preparations, 5-HT_{1A} receptors are not involved in the control of baseline cardiac vagal tone.

Respiratory rate

Intravenous administration of WAY-100635 caused a small, but significant decrease in baseline phrenic discharge frequency after 5 minutes. These results would agree with the previous findings that activation of 5-HT_{1A} receptors increases phrenic burst rate and would also suggest that endogenous 5-HT acts tonically at 5-HT_{1A} receptors to increase central respiratory frequency. A previous study in the cat has however produced conflicting results since in these experiments, i.v. administration of the 5-HT_{1A} receptor antagonist NAN-190 increased the frequency of phrenic bursts (Lalley et al., 1997). These differences may be due to the fact that unlike WAY-100635, NAN-190 is not a 'silent' 5-HT_{1A} receptor antagonist since it is known to exert partial agonist effects at presynaptic 5-HT_{1A} receptors (see Fletcher et al., 1993b).

Arterial blood pressure and renal sympathetic nerve activity

Ongoing renal sympathetic nerve activity was significantly increased following i.v. or i.c. WAY-100635 indicating that 5-HT exerts tonic inhibition of renal sympathetic nerve activity via activation of 5-HT_{1A} receptors. However, WAY-100635 had no significant effects on baseline arterial blood pressure suggesting that any changes in renal vascular conductance that occurred had a relatively minor effect on total peripheral resistance. The lack of effects of WAY-100635 on arterial blood pressure also indicates that the changes in ongoing renal sympathetic nerve activity were not due to any changes in baroreceptor activity.

4.4 Effects of 5-HT_{1A} receptor ligands on the cardiopulmonary reflex

Stimulation of cardiopulmonary afferents

The short latency between right atrial-injection and reflex responses (within 2s) suggests that the effects of PBG were due to stimulation of vagal afferent C-fibres originating in the cardiopulmonary region. It is unlikely that stimulation of arterial baroreceptors contributes to the PBG-evoked responses since in these studies, PBG evoked a simultaneous decrease in ongoing aortic nerve activity. Other studies in rabbits have also shown that the effects of right atrial PBG are not attenuated by sino-aortic denervation or bilateral vagotomy below the diaphragm thus confirming that PBG does not activate baroreceptor afferents and also eliminating the possibility of actions at abdominal sensory afferents (Evans et al., 1990). PBG has however been shown to excite carotid chemoreceptor afferents in the cat (McQueen and Mir, 1989). It is likely that this action opposes the reflex effects of PBG at cardiopulmonary afferents and may, in part, account for the exaggerated reflex responses evoked by PBG following sino-aortic denervation (Evans et al., 1990).

Effects of the 5-HT_{1A} receptor partial agonist buspirone

The results of the present study confirmed previous finding in urethane-anaesthetised rabbits that i.c. administration of buspirone potentiates the reflex vagal bradycardia evoked by stimulating cardiopulmonary afferents (Dando et al, 1994). Since the present study has shown that this effect was significantly attenuated by pre-treatment with WAY-100635 and was either absent or significantly less following i.v. buspirone, it can now be concluded that the potentiation results from activation of central 5-HT_{1A} receptors.

Previous studies in cats have shown that the reflex bradycardia evoked by right atrial injection of PBG is relatively unaffected by changes in central respiratory drive or pulmonary stretch receptor activity (Daly and Kirkman, 1988, 1989; Daly, 1991). Therefore, it is unlikely that the modulatory effects of buspirone are secondary to its effects on respiration. Even if the bradycardia evoked by cardiopulmonary afferent stimulation was modulated by respiratory activity in the same way as the chemoreceptor and baroreceptor bradycardias, the observed changes in respiration could not account for the potentiating effects of buspirone. Firstly, the increase in baseline central respiratory drive would tend to inhibit the activity of CVPNs. Secondly, although the reflex tachypnoea evoked by cardiopulmonary afferent stimulation was significantly inhibited 5 minutes after administration of buspirone i.c., it was unaffected at time points after buspirone when the reflex bradycardia was potentiated.

Both i.c. and i.v. buspirone significantly reduced the magnitude of the reflex hypotensions evoked by cardiopulmonary afferent stimulation. This appeared to be due at least in part to a depression of the central sympathowithdrawal since in many cases the associated renal sympathoinhibition was also significantly reduced. The reduction of the reflex hypotension that occurred 5 minutes after i.c. administration of buspirone was significantly attenuated by pre-treatment with WAY-100635. The inhibitory effects of buspirone on the reflex sympathoinhibitions were also reduced by

pre-treatment with WAY-100635 reaching significance after 35 minutes. Therefore these effects appear to be due to activation of 5-HT_{1A} receptors.

It could be argued that the effects of buspirone on the reflex hypotensions were secondary to its effects on baseline arterial blood pressure. Since buspirone significantly reduced the baseline level of mean arterial blood pressure, it might be expected that the magnitude of the hypotensions would also be reduced. Whilst this may be the case for the reflex hypotensions that were evoked 20 minutes after buspirone, it cannot explain the reduction of the hypotension that occurred after 5 minutes since at this time point, baseline mean arterial blood pressure was slightly greater than control levels. The effects of buspirone on the reflex renal sympathoinhibitions also seem unrelated to the concomitant changes in ongoing renal nerve activity. Inhibitory effects were observed after 5 minutes when the ongoing activity was significantly increased and also after 20 minutes when ongoing renal nerve had returned to control levels.

Effects of the 5-HT_{1A} receptor antagonist WAY-100635

Intracisternal administration of the selective 5-HT_{1A} receptor antagonist WAY-100635 significantly attenuated the vagal bradycardia evoked by stimulation of the cardiopulmonary afferents. These effects were presumably centrally-mediated, since in the pre-treatment studies, i.v. administration of WAY-100635 had no significant effect on the reflex responses evoked. Similar studies in α-chlorolose-anaesthetised rats have shown that i.c administration of the 5-HT_{1A} receptor antagonists spiperone, methiothepin and pindolol also attenuate the reflex bradycardia evoked by stimulating cardiopulmonary afferents (Bogle et al., 1990).

If the bradycardia evoked by right atrial injection of PBG was subject to respiratory modulation, the inhibitory effects of WAY-100635 could not be explained by the concomitant effects on the reflex respiratory response. WAY-

100635 significantly attenuated the reflex tachypnoea evoked which would tend to increase cardiac vagal outflow during the cardiopulmonary reflex.

In addition to its inhibitory effects on the reflex bradycardia and tachypnoea, WAY-100635 also significantly attenuated the reflex decrease in mean arterial pressure. This may be due in part to the simultaneous reduction of the bradycardia which would tend to reduce the fall in cardiac output during the reflex. However, it is also probably due to the inhibitory effects of WAY-100635 on the reflex renal sympathoinhibition. It is possible that the reduction of the renal sympathoinhibition may be related to the increase in ongoing renal nerve activity that was observed after i.c. administration of WAY-100635. However at certain time points after the administration of WAY-100635, ongoing renal nerve activity had returned below control values whilst the sympathoinhibition was still attenuated.

4.5 Effect of 5-HT$_{1A}$ receptor ligands on the baroreceptor reflex

Stimulation of baroreceptor afferents

Baroreceptor afferents were activated by electrically stimulating the left aortic nerve. The aortic nerve contains both myelinated (A) and non-myelinated (C) baroreceptor afferents fibres (Thoren and Jones, 1977). Previous studies in rabbits have shown that the hypotension, bradycardia and sympathoinhibitory responses evoked by electrical stimulation of the aortic nerve are maximal when both A- and C-fibres are activated simultaneously (Douglas et al., 1956; Kardon et al., 1973; Numao et al., 1983). In the present studies, the stimulus intensities used were sufficient to evoke maximal bradycardias thus suggesting that both fibre types were activated. Also, the stimulus parameters used in this study were similar to those used in previous experiments which were shown to activate both myelinated and non-myelinated fibres in the aortic nerve (Kardon et al., 1973).

Baroreceptor afferents in the aortic nerve were stimulated at increasing frequencies from 5 - 160 Hz. Earlier studies suggest that non-myelinated

afferents exert depressor effects at stimulation frequencies as low as 1 Hz, whereas myelinated fibres do not produce any effects until stimulation frequencies exceed 16 Hz (Douglas et al., 1956; Douglas and Ritchie, 1956) Therefore in these experiments, whilst the responses evoked at stimulation frequencies of 20 Hz and above are probably due to stimulation of both A- and C-fibres, those produced at 5 and 10 Hz are most likely due to activation of non-myelinated C-fibres alone.

The magnitudes of the reflex hypotension, sympathoinhibition and bradycardia evoked by baroreceptor afferent stimulation were frequency dependent. Optimum responses were obtained at 80 Hz which correlates well with findings in other studies in the rabbit (Neil et al., 1949; Douglas and Ritchie, 1956; Kardon et al., 1973; Numao et al., 1983). Unlike in other species, stimulation of the aortic nerve did not produce a reflex apnoea but actually caused a slight increase in respiratory rate. Similar findings have been previously reported in the rabbit (Douglas and Ritchie, 1956; Numao et al., 1983). Whilst this may suggest the presence of chemoreceptor fibres in the aortic nerve, it may also be a secondary effect due to the fall in arterial blood pressure produced by aortic nerve stimulation which would tend to cause a reflex chemoreceptor stimulation of breathing (Heymans and Neil, 1958).

This particular method of stimulating arterial baroreceptors was considered to be preferable to using vasoconstrictor drugs such as phenylephrine as it enabled constant stimuli to be given throughout the protocol and was unaffected by any changes in baseline arterial blood pressure that may occur following administration of 5-HT_{1A} receptor ligands. By stimulating at a variety of frequencies from threshold to maximal levels, this method also provided an entire stimulus response profile and therefore either facilitatory or inhibitory actions of the ligands could be easily identified.

Effects of the 5-HT_{1A} receptor partial agonist buspirone

Central administration of buspirone significantly potentiated the vagal bradycardias evoked by sub-maximal and maximal stimulation of aortic baroreceptor afferents. These effects were significantly attenuated by i.v. pre-treatment with WAY-100635 which itself had no effect on the evoked bradycardias. This suggests that the potentiations of bradycardias evoked by baroreceptor afferent stimulation were due to selective activation of 5-HT_{1A} receptors. The same dose of buspirone administered i.v. had no significant effect on the bradycardias evoked by aortic nerve stimulation at most frequencies. Only at 80 Hz during the first set of stimulation was the increase in R-R interval significantly potentiated, however this was significantly less than the potentiation evoked by buspirone when administered i.c. These results therefore indicate that the 5-HT_{1A} receptors involved were located centrally.

The bradycardia evoked by baroreceptor afferents is known to be modulated by changes in respiration (Potter, 1982; Daly and Kirkman, 1989; Daly, 1991). The effects of buspirone on the vagal bradycardias in the present study do not however appear to be secondary to its effects on respiration. The increase in baseline respiratory frequency evoked following administration of buspirone would tend to inhibit the increase in cardiac vagal outflow evoked by baroreceptor afferent stimulation. Also, buspirone had no significant effect on the slight increases in respiratory frequency evoked by baroreceptor stimulation.

As i.c. administration of buspirone caused a more profound bradycardia and therefore presumably a greater fall in cardiac output during the baroreceptor reflex, it might be expected that the reflex hypotension would also increase. In the present studies however, the magnitude of the reflex hypotensions were reduced following administration of buspirone. Previous studies in rats have shown that the magnitude of the reflex hypotension evoked by stimulating the aortic nerve is related to the initial levels of baseline mean arterial blood pressure (Nosaka et al., 1993). These authors have demonstrated

that the reflex fall in arterial blood pressure increases linearly as the initial levels of baseline arterial blood pressure are raised. It is therefore possible that in the present study, the inhibitory effects of i.c. and i.v. buspirone on the reflex hypotensions were secondary to the concomitant falls in baseline arterial blood pressure that were also evoked. This could not however explain the inhibitory effects of i.c. buspirone on the reflex hypotensions evoked during the first set of aortic nerve stimulations, since at this time point, baseline blood pressure was not significantly different from control levels. At the same time, there was also no significant change in baseline phrenic burst rate suggesting that these effects were not due to any changes in respiration.

During the second and third set of stimulations, the inhibitory effects of i.c. buspirone on the baroreceptor reflex hypotensions were attenuated by pre-treatment with WAY-100635 suggesting that they were 5-HT_{1A} receptor-mediated effects. Previous studies in rabbits have shown that the reflex fall in arterial blood pressure evoked by stimulating the aortic nerves is significantly reduced following i.v. administration of methysergide (Evans, 1981). It is possible that this effect was also due to activation of 5-HT_{1A} receptors since methysergide is known to possess high affinity for this 5-HT receptor subtype (see Zifa and Fillion, 1992). However, in that study, the effects of methysergide on the reflex hypotension could be explained by its simultaneous depressor effects on baseline arterial blood pressure.

During the second and third sets of stimulations the reflex renal sympathoinhibitions also tended to be reduced following i.c. or i.v. administration of buspirone however this effect did not reach significance at all frequencies of stimulation. Pre-treatment with WAY-100635 attenuated the effect of buspirone on the renal sympathoinhibitions suggesting it was due to the activation of 5-HT_{1A} receptors

Effects of the 5-HT_{1A} receptor antagonist WAY-100635

Intracisternal administration of WAY-100635 significantly attenuated the reflex bradycardia evoked by stimulating the aortic baroreceptor afferents at maximal frequencies. The effect on the reflex bradycardia cannot be explained by any concomitant changes in respiratory activity since i.c. WAY-100635 had no effect on the baseline phrenic discharge or the slight increase in phrenic burst rate evoked by baroreceptor stimulation.

The inhibition of the bradycardia caused by WAY-100635 would be expected to cause a smaller fall in cardiac output during the baroreceptor reflex and would therefore tend to reduce the reflex hypotension. In these studies, the reflex hypotension was significantly reduced following WAY-100635, however this may also have been due to the inhibitory effects of WAY-100635 on the reflex sympathoinhibition. The significant attenuation of the renal sympathoinhibition could be due in part to the increase in ongoing renal nerve activity that occurred after i.c. administration of WAY-100635. This seems unlikely since the effects on ongoing renal nerve activity were most obvious prior to the second and third set of baroreceptor stimulation, whereas the effect on the reflex sympathoinhibition occurred only during the first set of stimulations.

The effects of i.c. WAY-100635 can be assumed to be centrally mediated, since in the pre-treatment studies, i.v. administration of WAY-100635 had no significant effect on the reflex responses evoked by maximal baroreceptor stimulation.

4.6 Effect of 5-HT_{1A} receptor ligands on the chemoreceptor reflex

Stimulation of carotid body chemoreceptor afferents

In the present studies, carotid chemoreceptor afferents were stimulated by close arterial injection of NaCN into the region of the carotid bifurcation. This method has been used extensively in a number of species (Matsumoto, 1982; Daly and Kirkman, 1989; Sun and Reis; 1995) since it evokes a rapid-onset

chemoreceptor stimulation whilst avoiding the complex interactions that occur during systemic hypoxia (see Marshall, 1994). The volume of NaCN solution injected in these studies was kept low (<0.25 ml) so as to eliminate the possibility of stimulating carotid baroreceptors. In a number of experiments, injection of equivalent volumes of saline into the carotid artery were found to have no significant effects on baseline cardiorespiratory variables. NaCN has also been previously shown to have no direct stimulatory effects on carotid baroreceptor afferents (McQueen, 1980).

It is unlikely that at the doses used, the responses evoked by intra-arterial NaCN were the result of any direct toxic effects. In a similar study in rabbits, injections of NaCN (5-400 $\mu g\ kg^{-1}$) had no effects on respiratory variables following bilateral peripheral chemoreceptor denervation (Schramm and Grunstein, 1987). Whilst NaCN has been shown to directly excite medullary sympathoexcitatory neurones in rats (Sun et al., 1992), other studies in cats have shown that the sympathoexcitation evoked by similar doses of NaCN as those used in this study can be eliminated by bilateral section of the carotid sinus nerves (Matsumoto et al., 1987). Direct toxic effects on the heart are unlikely due to the high cardiac content of cytochrome oxidase (Olson, 1962).

Effects of the 5-HT_{1A} receptor partial agonist buspirone

Central administration of buspirone significantly potentiated the reflex bradycardia evoked by chemoreceptor afferent stimulation. This effect could be attenuated by pre-treatment with WAY-100635 suggesting that it was due to the selective activation of 5-HT_{1A} receptors.

As a result of the increased bradycardia following administration of buspirone, the reflex hypertension might be expected to be reduced due to a greater fall in cardiac output during the reflex. In these studies however, i.c. buspirone significantly potentiated the reflex hypertension. This effect was probably due in part to the increased sympathetic outflow since an increased renal sympathoexcitation was observed after administration of buspirone. Both

of these effects could be significantly attenuated by WAY-100635 indicating that they were due to the selective activation of 5-HT_{1A} receptors.

Ten minutes after administration of the same dose of buspirone i.v., the vagal bradycardia, reflex hypertension and sympathoexcitation evoked by chemoreceptor stimulation were also significantly potentiated. These effects were not significantly different to those obtained following i.c. buspirone. Both the bradycardia and the vasoconstriction evoked by chemoreceptor stimulation are known to be attenuated during periods of increased respiratory drive (Daly et al., 1986; Daly and Kirkman, 1989; Daly, 1991). It is therefore possible that at least some of the effects of i.v. buspirone after 10 minutes could have been due to the significant reduction of the reflex increase in phrenic nerve activity which also occurred at this time. This effect did not occur at any of the other stimulation times and in most of these cases, the responses produced by i.v. buspirone tended to be less than those caused when the drug was given i.c. suggesting that they were due to activation of central 5-HT_{1A} receptors.

Effects of the 5-HT_{1A} receptor antagonist WAY-100635

When all the data were taken together, WAY-100635 administered either i.v. or i.c. had no overall significant effects on the reflex responses evoked by carotid body chemoreceptor stimulation. However, in three out of six experiments, the reflex bradycardia was attenuated 5 minutes after i.c. administration of WAY-100635, whilst in the remaining three experiments, WAY-100635 had no effect. The reason for this variation is unknown, however it does not appear to be due to any drug delivery problems, since in the same experiments in which the chemoreceptor reflex was unaffected, WAY-100635 was found to significantly inhibit the baroreceptor reflex. It is possible that a higher dose of WAY-100635 is required to consistently attenuate the chemoreceptor-evoked bradycardia. Alternatively, it is possible that these differences may have been due to varying levels of anaesthesia between animals within the group since serotonergic pathways are known to be affected

by anaesthetics (Kubin et al., 1992). In the experiments in which the bradycardia was reduced, there appeared to be no obvious effects on the other reflex responses evoked.

4.7 Modulatory effects of buspirone

The present studies have shown that central application of buspirone potentiates the vagal bradycardias evoked by stimulating cardiopulmonary, baroreceptor and chemoreceptor afferents. In the case of the cardiopulmonary and baroreceptor reflex bradycardias, the potentiating effects of buspirone have been shown to be mediated centrally. Although the present results tend to suggest that the effect of buspirone on the chemoreceptor reflex bradycardia is also due to actions at central site, this has not been proved unequivocally and requires further investigation. When administered i.v., the selective 5-HT_{1A} receptor antagonist WAY-100635 had no effect on the reflex bradycardias but attenuated the potentiating effects of i.c. buspirone. This demonstrated that that buspirone is selectively activating 5-HT_{1A} receptors to facilitate the reflex bradycardias. Since the present studies have also shown that activation of 5-HT_{1A} receptors are involved in the central pathways mediating the reflex bradycardias (see 4.7 for discussion), it is perhaps surprising that i.v. WAY-100635 can block the potentiating effects of buspirone and yet have no effect on the reflex itself. A possible explanation could be that when administered i.v., the brainstem concentration of WAY-100635 is high enough to block the effects of exogenously applied 5-HT_{1A} agonist, but is not great enough to affect the synaptic transmission involved in these reflex pathways.

Possible sites of action

Since in the present studies, buspirone was administered intracisternally, it is not possible to determine the exact central sites at which it acts to cause its modulatory effects. The experiment using the dye Pontamine sky blue suggests that the most likely site of action of intracisternally injected drugs is the

brainstem. Several possible sites exist within the brainstem and are discussed below.

The nucleus tractus solitarius

One possible site at which the modulatory effects of buspirone are mediated is the nucleus tractus solitarius. This nucleus is the main site of termination of baroreceptor, chemoreceptor and cardiopulmonary afferents and is located in the dorsomedial aspect of the medulla. This area showed heaviest staining after i.c. administration of Pontamine sky blue and in the rat, has been shown to contain 5-HT_{1A} receptor binding sites (Thor et al., 1992b).

It is possible that buspirone may excite NTS neurones involved in mediating the cardiovagal components of the reflex pathways. However, since the activation of the 5-HT_{1A} receptors has been shown to cause neuronal inhibition via the activation of an inwardly rectifying K^+ conductance (Colino and Halliwell, 1987), it is unlikely that buspirone would excite these NTS neurones directly via actions on postsynaptic 5-HT_{1A} receptors. In hippocampal neurones, activation of 5-HT_{1A} receptors has been shown to increase cell excitability indirectly by reducing the magnitude of inhibitory inputs onto the neurone, a process known as disinhibition (Segal, 1990). Neurones in the NTS involved in mediating the baroreceptor reflex bradycardia are known to be tonically inhibited by GABAergic inputs since microinjection of the GABA receptor antagonist bicuculline into the NTS of rats potentiates the bradycardic effects evoked by aortic nerve stimulation (Kubo and Kihara, 1988b). This tonic inhibition probably arises from a population of intrinsic GABA-containing neurones that have been identified in the NTS (Meeley et al., 1985). In the dentate gyrus of the guinea pig, 5-HT_{1A} receptors are thought to be located either pre- or postsynaptically on GABAergic interneurones and activation of these receptors has been shown to negatively modulate the release of GABA (Matsuyama et al., 1997). If 5-HT_{1A} receptors are also located on inhibitory GABAergic neurones of the NTS then their activation following i.c.

administration of buspirone could explain the facilitatory effects observed on the baroreceptor reflex bradycardia.

Although the majority of 5-HT_{1A} receptors in the NTS are known to be located postsynaptically on non-serotonergic cells (Thor et al., 1992b), whether 5-HT_{1A} receptors are located on intrinsic GABAergic neurones in the NTS remains to be determined. It is also unknown whether NTS neurones mediating the cardiovagal components of the cardiopulmonary or chemoreceptor reflexes are tonically inhibited by GABAergic inputs. However, if similar inputs exist, it is possible that they could also be inhibited via activation of 5-HT_{1A} receptors.

In the rat, the hypotension evoked by stimulation of the aortic nerve was also potentiated following microinjection of bicuculline into the NTS (Kubo and Kihara, 1988b). This may suggest that the NTS neurones mediating the sympathetic component of the baroreceptor reflex are also tonically inhibited by GABAergic neurones or it could indicate that the tonic inhibition occurs at an early stage in the reflex pathway prior to the divergence of baroreceptor inputs into the separate sympathetic and vagal components. Therefore, if the effects of buspirone on baroreceptor bradycardia were due to the inhibition of tonic GABAergic inputs in the NTS then the sympathetic responses evoked by baroreceptor afferent stimulation might also have been expected to be potentiated. In the present study however, the hypotension and sympathoinhibition evoked by both baroreceptor and cardiopulmonary afferent stimulation were attenuated following i.c. administration of the 5-HT_{1A} agonist. It is possible that these effects may have been due to the direct inhibition of NTS neurones involved in the sympathetic limb of these reflexes via the activation of postsynaptic 5-HT_{1A} receptors.

Iontophoretic studies in rats have shown that 5-HT_{1A} receptor agonists have both excitatory and inhibitory effects on neurones in the NTS (Wang et al., 1997). These results could fit in with the hypothesis described above. For example, if the activity of a neurone that expresses 5-HT_{1A} receptors is recorded, iontophoretic application of 5-HT_{1A} receptor ligands would be

expected to result in neuronal inhibition. Alternatively, if the neurone recorded received tonic inputs from a local inhibitory neurone that expresses 5-HT_{1A} receptors, then iontophoretically applied 5-HT_{1A} receptor agonists may spread to and inhibit these inhibitory neurones thus increasing the excitability of the recorded neurone. Whilst in the study by Wang et al (1997), the majority of NTS neurones studied were not functionally identified, it is of interest that those which were activated by stimulation of cardiac vagal afferents were all excited by the application of the 5-HT_{1A} receptor agonist 8-OH-DPAT.

In rats, cats and dogs, microinjection of the 5-HT_{1A} receptor agonist 8-OH-DPAT into the NTS has been shown to have no significant effect on baseline cardiovascular parameters (Shvaloff and Laguzzi, 1986; Vayssettes-Courchay et al., 1993; Laubie et al., 1989). However, the effects of such microinjections on the reflex responses evoked by stimulating baroreceptor, chemoreceptor or cardiopulmonary afferents has not been established.

The nucleus ambiguus and dorsal vagal nucleus

Several lines of evidence from studies in the rat and cat suggest that the modulatory effects of buspirone on reflex vagal bradycardias may be due to actions at the nucleus ambiguus (NA) and dorsal vagal nucleus (DVN) where the majority of cardiac vagal preganglionic neurones are located. Both nuclei have been shown to contain 5-HT_{1A} receptor bindings sites (Dashwood et al., 1988b; Manaker and Verderame, 1990) and iontophoretic studies have shown that activation of this receptor subtype can excite vagal preganglionic neurones in the DVN (Wang et al., 1995b). Microinjection of 5-HT or the 5-HT_{1A} receptor agonist 8-OH-DPAT into either the DVN or the NA has been shown to evoke a bradycardia (Izzo et al., 1988; Sporton et al., 1991) thus suggesting that activation of 5-HT_{1A} receptors can cause excitation of cardiac vagal preganglionic neurones. It is not known whether 5-HT_{1A} receptors are present in the DVN or NA of the rabbit, however previous studies in this species have also shown that central application of 5-HT_{1A} receptor agonists can evoke a

vagal bradycardia suggesting that similar mechanisms may exist (Futuro-Neto et al., 1993; Dando et al., 1998).

The DVN is located in the dorsomedial medulla. Since this area was heavily stained following intracisternal injection of the dye Pontamine sky blue it seems highly likely that buspirone may have accessed the DVN following its injection into the cisterna magna. Whilst the NA is located deeper in the medullary reticular formation and might be less accessible to intracisternally applied drugs, anatomical studies in the rat have shown that the dendritic fields of the CVPNs within this nucleus extend to the ventral surface of the medulla (Izzo et al., 1993), an area which was also stained following injection of the dye. Therefore, it is possible that the modulatory effects of buspirone on the reflex bradycardias were due to actions on 5-HT_{1A} receptors within these areas.

In the present studies activation of central 5-HT_{1A} receptors caused a fall in baseline heart rate. It is possible that this was due to the increased sensitivity of the cardiovagal component of the baroreceptor reflex that occurred following administration of buspirone, since CVPNs are known to receive tonic inputs from arterial baroreceptors (McAllen and Spyer, 1978b; Jordan et al., 1982). However, this is unlikely, since the ongoing activity of baroreceptor afferents is relatively low in anaesthetised rabbits with baseline mean arterial blood pressures similar to those in the present studies (Thoren and Jones, 1977). It is more likely that in the present studies, buspirone directly increased the ongoing activity or excitability of cardiac vagal preganglionic neurones via actions on 5-HT_{1A} receptors resulting in a fall in baseline heart rate. The increased excitability would presumably increase the efficacy of excitatory reflex inputs onto the CVPNs such as those arising from cardiopulmonary, baroreceptor and chemoreceptor afferents thus resulting in a potentiation of the evoked bradycardias.

The exact mechanism by which 5-HT_{1A} receptor agonists excite CVPNs is not known. Since activation of 5-HT_{1A} receptors causes neuronal inhibition (Colino and Halliwell, 1987), it unlikely that the receptors are located

postsynaptically on the CVPNs themselves. CVPNs in the NA and DVN are known to receive tonic GABAergic inhibitory inputs (see Jordan and Spyer, 1987; Wang et al., 1995a). If 5-HT_{1A} receptors are located presynaptically on the GABAergic terminals in the vagal nuclei as has been shown to occur in GABAergic interneurones of the hippocampus (Matsuyama et al, 1997), it could provide one explanation for the excitatory effects of 5-HT_{1A} receptor agonists on cardiac vagal outflow.

Medullary raphe nuclei

Shortly after its administration, buspirone caused an increase in ongoing renal nerve activity. This effect was due to activation of 5-HT_{1A} receptors since it was attenuated by pre-treatment with WAY-100635 Similar changes in ongoing renal nerve activity have been observed in rats following i.c.v. injection of 5-HT_{1A} receptors agonists (Anderson et al., 1992). These effects however, are thought to be mediated in the forebrain at sites close to the third ventricle. Such brain areas would not be readily accessible to a drug injected intracisternally and thus could not explain the present findings. One possibility is that the sympathoexcitation was due to actions in the raphe obscurus since a study in rats has shown that microinjection of flesinoxan or 8-OH-DPAT into the raphe obscurus causes a pressor effect presumably mediated by an increase in sympathetic activity (Dreteler et al., 1991).

Actions at the medullary raphe could also provide another explanation for the inhibitory effects of buspirone on the reflex hypotensions and sympathoinhibitions evoked by cardiopulmonary and baroreceptor afferent stimulation. Microinjections of 8-OH-DPAT into the raphe pallidus/obscurus of the rat has been shown to decrease the gain of the of the baroreceptor induced sympathoinhibition, whilst the gain was unaffected by injections into the RVLM (Nosjean and Guyenet, 1991). Since the majority of 5-HT_{1A} receptors in the raphe pallidus and obscurus are located presynaptically on serotonergic neurones (Thor et al., 1992a) and since activation of these receptors results in

neuronal inhibition, it seems reasonable to speculate that neurones of the raphe pallidus may contribute to the overall effectiveness of the baroreceptor reflex sympathoinhibition. Similar mechanisms may also contribute to the reflex sympathoinhibition evoked by stimulating cardiopulmonary afferents. It is of interest that in the rabbit, Fos-positive neurones were located in the raphe pallidus following baroreceptor and cardiopulmonary afferent stimulation (Li and Dampney, 1992; Gieroba et al., 1995).

The nucleus raphe obscurus is thought to send serotonergic projections to the dorsolateral aspect of the periaqueductal grey matter (PAG) in the midbrain (Clements et al., 1985) and electrophysiological studies have shown that 5-HT has inhibitory effects on neuronal activity within this midbrain region (Lovick, 1993). The PAG is thought to be a major structure involved in the integration of the hypothalamic defence reaction since chemical stimulation within this region evokes the full pattern of visceral responses associated with behavioural alerting (Hilton and Redfern, 1986). Chemical stimulation of the raphe obscurus has recently been shown to inhibit the defence response evoked by electrically stimulating the PAG (Lovick and Schenberg, 1996). If this inhibitory serotonergic projection from the raphe obscurus is tonically active, it is possible that it may be inhibited by administration of 5-HT_{1A} receptor agonists via activation of somatodendritic 5-HT_{1A} receptors which would lead to an increase in the activity of PAG neurones mediating defensive behaviour. Since stimulation of defence areas has been previously shown to excite NTS neurones receiving chemoreceptor input (Silva-Carvalho et al., 1993), this proposed mechanism may explain the excitatory effects of buspirone in the present studies on the reflex responses evoked by chemoreceptor afferent stimulation. Defence area stimulation has also been shown to inhibit baroreceptor and cardiopulmonary reflexes (Coote et al., 1979; Jones et al., 1994b). Therefore this could also provide an explanation for the inhibitory effects of buspirone on the reflex hypotensions and sympathoinhibitions evoked by stimulation these afferents.

The rostral ventrolateral medulla

It is possible that the fall in baseline arterial pressure evoked by buspirone was due in part to actions in the raphe pallidus and rostral ventromedial medulla. These areas, which are located relatively near to the ventral surface, contain serotonergic neurones that are thought to provide tonic excitatory inputs to SPNs in the spinal cord (Loewy and McKellar, 1981; McCall, 1984). Autoradiographic studies show high densities of 5-HT_{1A} receptor binding sites in these regions the majority of which occur on serotonergic neurones (Thor et al., 1990; Thor et al, 1992a). Microinjection of 8-OH-DPAT into the raphe pallidus or RVMM has also been shown to evoke a hypotension (Nosjean and Guyenet, 1991; Helke et al., 1993). The fact that the decreases in blood pressure and ongoing sympathetic nerve activity evoked by intravenous administration of 8-OH-DPAT are not significantly affected following lesions in the raphe or chemical destruction of serotonergic neurones has suggested however that neurones in these regions play a minor role in mediating the depressor effects of 5-HT_{1A} receptor agonists (McCall et al., 1989; Helke et al., 1993).

It is therefore more likely, that the reduction in baseline arterial pressure evoked by buspirone in the present study is due to effects in the rostral ventrolateral medulla. Autoradiographic studies have shown that this area also contains 5-HT_{1A} receptor binding sites (Thor et al., 1992a) and microinjections of 8-OH-DPAT or buspirone into this region have been shown to evoke a hypotension in the rat and dog (Laubie et al., 1989; Lovick, 1989; Nosjean and Guyenet, 1991; Kubo et al., 1995). This response appears to be due to direct effects on spinally-projecting sympathoexcitatory neurones that are present within this region since the ongoing activity of these neurones has been shown to be inhibited by iontophoretic application of these agents (Wang and Lovick, 1992; Kubo et al., 1995).

It is highly likely that buspirone applied intracisternally may gain access to the RVLM via diffusion from the ventral surface. Previous studies in rats pre-

treated with the serotonergic neurotoxin (5,7-DHT) have shown that 8-OH-DPAT applied to the ventral surface of the medulla causes a hypotension which was ascribed to actions on non-serotonergic neurones in the RVLM (Helke et al., 1993).

Although 5-HT_{1A} are present in the IML (Dashwood et al., 1988a), in this study, effects of buspirone at the level of the SPNs seem unlikely since staining did not extend past the higher cervical regions of the spinal cord following intracisternal administration of Pontamine sky blue. Previous studies have also shown that iontophoretically applied 8-OH-DPAT has no effect on the firing rate of SPNs (Clement and McCall, 1990) and intrathecal administration of 8-OH-DPAT also has no effect on baseline blood pressure or renal nerve activity (Yusof and Coote, 1988).

Brainstem respiratory groups

The increase in baseline respiratory frequency evoked by activation of central 5-HT_{1A} receptors in the present study could result from direct effects on respiratory neurones in the dorsal and/or ventral brainstem respiratory groups. Iontophoretic studies have shown that 5-HT_{1A} receptor agonists have inhibitory effects on all classes of respiratory neurones (Lalley, 1994; Lalley et al., 1994). In the anaesthetised cat, the increases in respiratory frequency evoked by i.v. administration of the 5-HT_{1A} receptor agonists 5-MeODMT or 8-OH-DPAT are thought to be due to the shortening of the inspiratory phase of the respiratory cycle resulting from inhibitory effects on early inspiratory neurones (Lalley, 1994; Lalley et al., 1994).

In the present study, the reflex tachypnoea evoked by cardiopulmonary afferent stimulation was attenuated 5 minutes after i.c. or i.v. administration of buspirone. This pattern of respiratory activity evoked by stimulating cardiopulmonary afferents is thought to be due to increases in activity of post-inspiratory neurones (Richter, 1982). It is therefore possible that the effects of buspirone on the reflex tachypnoea resulted from direct effects on post-

inspiratory neurones since this type of neurone has been shown to be inhibited by iontophoretic application of 5-MeODMT (Lalley, 1994).

The reflex respiratory responses evoked by stimulating chemoreceptor afferents are thought to be due increases in activity of pre-inspiratory, post-inspiratory and expiratory neurones (Lawson et al., 1989; Dogas et al., 1995; Sun and Reis, 1996). It is therefore possible that the inhibitory effects of buspirone on the reflex increase in phrenic burst rate evoked by stimulating chemoreceptor afferents were also due to direct effects on these respiratory neurones since they have all been shown to be inhibited by iontophoretic application of 5-HT_{1A} receptor agonists (Lalley, 1994; Lalley et al., 1994).

Stimulation of the raphe obscurus in the cat has been shown to cause increases in respiratory frequency and phrenic nerve amplitude (Holtman et al., 1986; Millhorn, 1986) which can be blocked by i.v. pre-treatment with methysergide (Millhorn, 1986). In addition, stimulation of the raphe pallidus has been shown to activate respiratory neurones (Lalley, 1986). These respiratory effects are thought to be produced via serotonergic projections from the medullary raphe to the brainstem respiratory groups. Fos studies in the rat have shown that neurones in the medullary raphe are activated by stimulation of the carotid sinus nerve or hypoxia (Erickson and Millhorn, 1994). This and previous findings have therefore prompted speculation that activation of medullary raphe neurones may contribute in part to the respiratory responses evoked by stimulating chemoreceptor afferents (Millhorn, 1986). If this is the case, it may provide an alternative explanation for the slight inhibitory effects on the chemoreceptor-evoked respiratory responses evoked by i.v. buspirone, since the medullary raphe neurones involved may be inhibited by activation of somatodendritic 5-HT_{1A} receptors.

4.8 Modulatory effects of WAY-100635

In the present studies, exogenous application of the 5-HT_{1A} receptor partial agonist buspirone has been shown to modulate the cardiorespiratory

responses evoked by stimulating cardiopulmonary, baroreceptor and chemoreceptor afferents. Whilst these findings are of pharmacological interest, these results alone do not determine whether 5-HT_{1A} receptors are tonically activated during these cardiorespiratory reflexes. By blocking central 5-HT_{1A} receptors using the silent 5-HT_{1A} receptor antagonist WAY-100635, the involvement of these receptors in the neural pathways mediating the baroreceptor, cardiopulmonary and chemoreceptor reflexes have been investigated. Although central application of WAY-100635 had no overall effect on the responses evoked by stimulating chemoreceptor afferents, the present studies have shown that in the presence of central 5-HT_{1A} receptor blockade the reflex responses evoked by stimulating cardiopulmonary and baroreceptor afferents are markedly attenuated.

In a previous study in rats, WAY-100635 has been shown to act as an antagonist at α_1-adrenoceptors when administered at high doses (Martin et al., 1999). It could therefore be argued that the effects of WAY-100635 observed in the present studies are due to actions at α_1-adrenoceptors rather than 5-HT_{1A} receptors. Blockade of α_1-adrenoceptors causes a fall in arterial blood pressure due to effects at the level of the vasculature and also via a centrally-mediated sympathoinhibition (Ramage, 1984). Since neither of these effects were observed following i.v. or i.c. administration of WAY-100635 in the present studies, it seems unlikely that any α_1-adrenoceptor blockade occurred.

Thus, it appears that endogenous serotonergic pathways are activated during the baroreceptor and cardiopulmonary reflex which, via effects on 5-HT_{1A} receptors, facilitate the reflex responses evoked. The origin of these serotonergic pathways and the sites at which the endogenously released 5-HT activates 5-HT_{1A} receptors are not clear from this study. Several possibilities exist however and are discussed below.

Possible sites of action

Actions at the level of the efferent outflow

Previous studies have shown that 5-HT or 5-HT_{1A} receptor agonists evoke sympathoinhibitory and depressor responses when microinjected into the RVLM (Laubie et al., 1989; Lovick, 1989; Kubo et al., 1995) and a vagal bradycardia when microinjected into the NA or DVN (Izzo et al., 1988; Sporton et al, 1990). In addition, intravenous or central administration of 5-HT_{1A} receptor agonists have been shown to modulate the activity of neurones in the brainstem respiratory groups resulting in an increase in respiratory rate (Lalley 1994; Rose et al., 1995). These brainstem sites are known to be involved in mediating the alterations in sympathetic, parasympathetic and respiratory outflows evoked by stimulating cardiopulmonary and baroreceptor afferents and have all been shown to receive serotonergic inputs (Nicholas and Hancock, 1988; Izzo et al., 1993; Holtman et al., 1990; Voss et al., 1990). If these serotonergic inputs are activated by stimulation of cardiopulmonary and baroreceptor afferents, the subsequent activation of 5-HT_{1A} receptors in the RVLM, vagal nuclei and brainstem respiratory groups would tend to facilitate the reflex responses evoked.

The sources of the serotonergic inputs to the RVLM, vagal nuclei and the brainstem respiratory groups have not been clearly defined, however they are thought to arise predominantly from the medullary raphe nuclei (Nicholas and Hancock, 1988; Brodin et al., 1990; Connelly et al., 1989). Whilst Fos studies have shown that neurones in the medullary raphe are activated during the cardiopulmonary and baroreceptor reflex (Li and Dampney, 1992; Gieroba et al., 1995) their exact role remains uncertain. Electrical or chemical stimulation of the raphe magnus has been shown to potentiate the hypotension and bradycardia evoked by stimulation of the aortic nerve (Inui et al., 1994). This nucleus is known to be responsible for mediating the baroreceptor facilitation that has been shown to be evoked by stimulation of the ventrolateral part of the midbrain periaqueductal grey matter or the pre-optic area of the hypothalamus

(Inui et al., 1995). Since the baroreceptor reflex bradycardia and hypotension are attenuated following lesions in the pre-optic area (Hilton and Spyer, 1971; Miyajima and Bunag, 1985) it is possible that these descending inputs act tonically via the raphe magnus to reinforce baroreceptor function. However, whether this tonic facilitation of the baroreceptor reflex occurs is mediated by serotonergic projections from the raphe magnus remains to be determined.

If serotonergic mechanisms such as those described above do facilitate the responses evoked by cardiopulmonary and baroreceptor afferent stimulation via the activation of 5-HT_{1A} receptors into the RVLM, vagal nuclei and respiratory groups, it could provide an explanation for the inhibitory effects of WAY-100635 on all the reflex responses evoked by cardiopulmonary and baroreceptor stimulation since all the brainstem areas are likely to be accessed.

Actions at the nucleus tractus solitarius

The fact that WAY-100635 in the present study inhibited all components of the baroreceptor and cardiopulmonary reflex shortly after its intracisternal administration might however suggest that the effects were mediated at an early stage in the reflex pathways at a site relatively close to cisterna magna such as the NTS. The inhibitory effects of the 5-HT_{1A} receptor antagonist WAY-100635 on the baroreceptor and cardiopulmonary reflexes in the present experiments suggests that stimulation of these afferents causes the release of 5-HT which, via actions on 5-HT_{1A} receptors, facilitates the evoked responses.

One possible source of 5-HT is from the afferent neurones themselves. Studies in the rat have shown that some vagal afferent fibres that project to the NTS contain 5-HT (Nosjean et al., 1990). It is possible that this population of 5-HT-containing afferents includes baroreceptor and cardiopulmonary receptor afferents. In this respect, neurones in the aortic nerve of the cat have been shown to accumulate exogenously applied 5-HT suggesting that they may be serotonergic (Gaudin-Chazal et al., 1983). A more detailed study in rats has provided indirect evidence to suggest however, that in this species, 5-HT is not

present in baroreceptor or cardiopulmonary afferents (Sykes et al., 1994). This study revealed that vagal afferents immunoreactive for 5-HT terminate predominantly in the ventral subnucleus of the NTS but not in areas that receive afferent inputs from cardiopulmonary and baroreceptor afferents such as the dorsolateral, medial and commissural subnuclei. Receptor binding studies might also suggest that 5-HT_{1A} receptors are not activated at the first central synapse of the baroreceptor or cardiopulmonary reflex. In the rat, high levels of 5-HT_{1A} receptor binding are found on neurones of the NTS in the central and interstitial subnuclei while only moderate and low levels are found in the commissural and other subnuclei (Manaker and Verderame, 1990; Thor et al., 1992b). Therefore, from data in the rat, it seems unlikely that 5-HT is released at the first synapse of cardiopulmonary and baroreceptor reflex however, since similar studies have not been performed in the rabbit, this possibility cannot be completely discounted.

The NTS is known to receive serotonergic inputs from the area postrema and the medullary raphe (Steinbusch, 1981; Thor and Helke, 1987). Fos expression studies in the rabbit have demonstrated that neurones in these areas are activated in response to baroreceptor and cardiopulmonary afferent input respectively. (Li and Dampney et al., 1992; Gieroba et al., 1995). Whilst it was not determined whether the specific neurones activated were serotonergic, it seems reasonable to suggest that these pathways may result in the release of 5-HT into the NTS.

Another possibility is that baroreceptor and cardiopulmonary afferent stimulation may result in the release of 5-HT from neurones within the NTS itself. Relatively little is known about the local neural circuits in the NTS which are involved in processing baroreceptor and cardiopulmonary afferent inputs, however, it is possible that they involve the activation of intrinsic serotonergic interneurones which have been located in the medial subnucleus of the NTS (Calza et al., 1985). Assuming that 5-HT is released into the NTS via one or more of the above mechanisms, it is possible that it may facilitate the

baroreceptor and cardiopulmonary reflex via actions on 5-HT_{1A} receptors located on local inhibitory GABAergic neurones as described in the previous section.

4.9 Future studies

Since in the present studies, only one dose of either buspirone or WAY-100635 were tested, it is not clear whether the drugs were reaching maximally effective concentrations. It may therefore be of interest to investigate the dose-related effects of the 5-HT_{1A} receptor ligands on baseline cardiorespiratory parameters and on the evoked reflex responses. Additional doses could be administered in separate groups of animals or, alternatively, cumulative doses could be administered in the same animal and the effects on baseline and evoked reflex responses measured. This would enable dose reponse profiles to be constructed and the maximally effective doses to be determined.

The above experiments may also clarify whether 5-HT_{1A} receptor mechanisms are involved in the neural pathways that mediate the chemoreceptor reflex. In the present experiments, WAY-100635 attenuated the bradycardia evoked by stimulation of carotid chemoreceptor afferents in some animals, whilst in others, this response was unaffected. It has been suggested that these differences may have been due to the use of an insufficient dose of WAY-100635 or due to varying levels of anaesthesia between the animals in the group. Further experiments could therefore be performed using a higher dose of WAY-100635 or a slightly lower dose of anaesthetic.

The present studies have shown however that central 5-HT_{1A} receptor mechanisms are involved in the neural pathways that mediate the cardiopulmonary and baroreceptor reflexes. The next step would be to identify more precisely the brainstem sites at which these receptors are activated during the reflexes. Possible areas have been discussed and these include the NTS, NA, DVN, RVLM and brainstem respiratory nuclei. Microinjecting WAY-100635 into these brainstem regions and examining the subsequent effects on

the reflex responses would be one method to identify the area of the brainstem involved. Iontophoretic techniques could also be used to investigate more discretely the effects of 5-HT_{1A} receptor ligands on individual neurones in the above brainstem sites. The effects of iontophoretically applied 5-HT_{1A} receptor agonists on NTS, DVN and RVLM neurones have been examined in previous studies in the rat, however, there is no data available from studies in rabbits. It is still also uncertain from the previous studies whether 5-HT_{1A} receptor are tonically active in these areas therefore it may be of interest to examine the effects of iontophoretically applied WAY-100635 on the ongoing and reflexly-evoked activity of neurones in these brainstem regions.

The present studies have shown that 5-HT_{1A} receptor mechanisms play a facilitatory role in control of reflex cardiac vagal activity and similar mechanisms have also been shown to be involved in the vagal control of the airways (Bootle et al., 1998). It would therefore be of interest to examine whether these facilitatory serotonergic mechanisms are also involved in the control of vagal outflow to other areas such as the gastrointestinal system or the pancreas.

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5 Appendix

5.1 Spike 2 script for measuring changes in mean arterial blood pressure

```
numvar 7                                    sets variables
var ana bsy bdi
var psy pdi bap sbd
normal; clear; view 1; window 0 0 100 95
yrange 1 0 200
repeat
mark
setup
setdi
peak
pdia
roun
calc
END

PROC MARK                                   locates mark
view 2; window 0 0 100 95
off all; on 7
draw 0 maxtime
cursors 1
print 0 "Find marker"
interact
print 0 "%d" c1
ana:=c1
return

PROC SETUP                                  measures baseline
view 1                                      systolic blood pressure
off all; on 1
yrange 1 0 200
cursors 1
setc 1 ana
print 0 "scanning in"
draw ana-5 ana+60
hcursor 1
printto 0
```

```
print "Choose baseline systolic BP."
interact
bsy:=hc
return

PROC SETDI                                  measures baseline
hcursor 1                                   diastolic blood pressure
printto 0
print "Choose baseline diastolic BP."
interact
bdi:=hc
return

PROC PEAK                                   measures highest or
hcursor 1                                   lowest systolic blood
print 0 "Choose peak systolic BP."          pressure
interact
psy:=hc
return

PROC PDIA                                   measures highest or
hcursor 1                                   lowest diastolic blood
print 0 "Choose peak diastolic BP."         pressure
interact
pdi:=hc
return

PROC ROUN
bsy:=round(bsy)
bdi:=round(bdi)
psy:=round(psy)
pdi:=round(pdi)
bap:=round(bap)
sbd:=round(sbd)
return

PROC CALC                                   calculates baseline mean
moveto 10 10                                arterial blood pressure
printto 1                                   and highest or lowest
print "   Baseline systolic  =%d " bsy       mean arterial blood pressure
```

```
print "          diastolic =%d mmHg." bdi
print "Pk response systolic  =%d " psy
print "          diastolic =%d mmHg." pdi
moveto 70 10
bap:=bdi+((bsy-bdi)/3)
bap:=round(bap)
print "Base MAP= %d" bap
sbd:=pdi+((psy-pdi)/3)
sbd:=round(sbd)
print "Peak MAP= %d" sbd                    calculates maximum reflex
print "P cg MAP= %d" sbd-bap                change in mean arterial
return                                      blood pressure
```

5.2 Spike 2 script for measuring changes in R-R interval

```
VAR minint maxint events echan oldch bas res        sets variables

NORMAL;clear
view 2 "R-R interval plot";window 0 0 100 95;ON TITLE
view 1;window 0 0 100 95;ON LABELS
oldch:=0
REPEAT
SCATTER
END

PROC SCATTER
MENU 0 "Scatter plot of event times" 4
MENU 1 1 "Event channel to analyse" 9
MENU 1 2 "start time to plot" 1 0 1500
MENU 1 3 "finish plot" 1 0 5000
MENU 1 4 "      Esc=Exit, Enter=Go" 0
MENU 2 e echan minint maxint x
IF (e=0)|(echan=0);END;ENDIF

newevent 0
newevent 1 16000
newevent 6 echan minint maxint
setaxes 2
view 2                                      plots a graphs of R-R
yrange 1 0.15 0.35                          intervals against time
draw minint maxint
```

```
PRINT 0 "Drawing scatter plot for %d events" EVENT
for i:=2 EVENT
x:=event[i]-event[i-1]
mover 1 event[i] x
drawr 1 event[i] x
next i
hcursor 1
print 0 "Choose baseline"                    measures baseline
interact                                     R-R interval
bas:=hc
hcursor 1
print 0 "Choose response"                    measures peak
interact                                     R-R interval
res:=hc
hcursor 1
print 0 "Set level of R-R interval."
cursors 2 1

moveto 15 10                                 calculates maximum
print 1 "Baseline R-R interval is %d s." bas reflex change in
moveto 15 15                                 R-R interval
print 1 "Peak response R-R interval is %d s." res
moveto 15 20
print 1 "Maximum change in R-R interval is %d s." res-bas
RETURN
```

5.3 Spike 2 script for measuring changes in integrated renal nerve activity (cardiopulmonary and chemoreceptor reflex)

```
numvar 4
var bas res ans ana                          sets variables
MARK
MEAS

PROC MARK                                    locates marker
view 2; window 0 0 100 95
normal
off all; on 7
draw 0 maxtime
cursors 1
print 0 "Find marker"
```

```
interact
print 0 "%d" c1
ana:=c1
return

PROC MEAS                                   measures integrated renal
view 1;window 0 0 100 95                    nerve activity 30 sec before
off all                                     and 30 sec after the mark
on 6
yrange 6 -0.2 1

draw 0 maxtime
count 6 ana ana+30 b
count 6 ana-30 ana a

moveto 20 20
Print 1 "PBG/NACN"
print 1 "baseline 30s= %d" a
print 1 "response 30s= %d" b
print 1 "reflex change = %d" b-a
ans:=(b/a)*100

ans:=round(ans)
print 1 "response RNA= %d % the size of baseline RNA." ans
hcursor 0
end
```

5.4 Spike 2 script for measuring changes in integrated renal nerve activity (baroreceptor reflex)

```
numvar 4
var bas res ans ana                         sets variables
MARK
MEAS

PROC MARK                                   locates marker
view 2; window 0 0 100 95
normal
off all; on 7
draw 0 maxtime
cursors 1
```

```
print 0 "Find marker"
interact
print 0 "%d" c1
ana:=c1
return

PROC MEAS
view 1;window 0 0 100 95
off all
on 6
yrange 6 -0.2 1

draw 0 maxtime
count 6 ana ana+10 b
count 6 ana-10 ana a

moveto 20 20
print 1 "AORTIC"
print 1 "baseline 10s= %d" a
print 1 "response 10s= %d" b
print 1 "reflex change = %d" b-a
ans:=(b/a)*100

ans:=round(ans)
print 1 "response RNA= %d % the size of baseline RNA." ans
hcursor 0
end
```

measures integrated renal nerve activity 10 sec before and 10 sec after the mark

5.5 Tables of results

Table 5.1

The effects of saline (20 μl i.c.; n=5) on baseline mean arterial blood pressure (MABP), R-R interval, phrenic burst rate (PBR) and integrated renal nerve activity (IRNA). The table shows mean control baseline values measured before administration of saline and baseline values measured at time points after administration of saline immediately prior to stimulation of the cardiopulmonary afferents (see table 5.2). Values in parenthesis show changes (Δ) from the control values. All values are mean ± s.e. mean.

Time after drug (min)	MABP (mmHg)	R-R interval (ms)	PBR (bursts min^{-1})	IRNA (%)
CONTROL	82 ± 9	221 ± 12	66 ± 4	100
5	78 ± 10 **(-5 ± 3)**	220 ± 15 **(-1 ± 4)**	71 ± 5 **(5 ± 2)**	103 ± 5 **(3 ± 5)**
20	80 ± 10 **(-3 ± 1)**	211 ± 14 **(-10 ± 7)**	72 ± 5 **(5 ± 3)**	101 ± 8 **(1 ± 8)**
35	80 ± 10 **(-3 ± 3)**	210 ± 15 **(-11 ± 7)**	69 ± 4 **(3 ± 3)**	102 ± 6 **(2 ± 6)**
50	76 ± 9 **(-6 ± 3)**	210 ± 15 **(-11 ± 6)**	72 ± 3 **(6 ± 4)**	90 ± 9 **(-10 ± 9)**

Table 5.2

The effects of saline (20 μl i.c.; n=5) on the reflex changes in mean arterial blood pressure (MABP), R-R interval, phrenic burst rate (PBR) and integrated renal nerve activity (IRNA) evoked by stimulating cardiopulmonary afferents with PBG. The table shows mean control reflex changes evoked before administration of saline and reflex changes evoked at time points after administration of saline. Values in parenthesis show changes (Δ) from the control responses. All values are mean ± s.e. mean.

Time after drug (min)	Decrease MABP (mmHg)	Increase R-R interval (ms)	Increase PBR (bursts min^{-1})	Decrease IRNA (%)
CONTROL	35 ± 4	32 ± 8	41 ± 5	100
5	33 ± 4 **(-2 ± 2)**	35 ± 9 **(3 ± 2)**	39 ± 6 **(-2 ± 1)**	91 ± 19 **(-9 ± 19)**
20	32 ± 4 **(-3 ± 1)**	33 ± 9 **(2 ± 2)**	39 ± 4 **(-2 ± 2)**	113 ± 17 **(13 ± 17)**
35	34 ± 3 **(-1 ± 2)**	36 ± 9 **(4 ± 2)**	38 ± 3 **(-3 ± 2)**	122 ± 17 **(22 ± 17)**
50	32 ± 3 **(-3 ± 2)**	34 ± 9 **(3 ± 5)**	38 ± 3 **(-3 ± 3)**	122 ± 19 **(22 ± 19)**

Table 5.3

The effects of buspirone (200 μg kg^{-1} i.c.; n=5) on baseline mean arterial blood pressure (MABP), R-R interval, phrenic burst rate (PBR) and integrated renal nerve activity (IRNA). The table shows mean control baseline values measured before administration of buspirone and baseline values measured at time points after administration of buspirone immediately prior to stimulation of the cardiopulmonary afferents (see table 5.4). Values in parenthesis show changes (Δ) from the control values. All values are mean ± s.e. mean.

Changes caused by buspirone i.c. have been compared with those caused by saline i.c. using two-way ANOVA and the least significant difference test.
*p<0.05, **p<0.01 compared to saline i.c.

Time after drug (min)	MABP (mmHg)	R-R interval (ms)	PBR (bursts min^{-1})	IRNA (%)
CONTROL	74 ± 9	232 ± 12	84 ± 8	100
5	80 ± 5 **(6 ± 7*)**	238 ± 13 **(6 ± 5)**	99 ± 14* **(16 ± 15)**	170 ± 38** **(70 ± 38**)**
20	57 ± 6 **(-17 ± 4**)**	272 ± 15** **(39 ± 11**)**	110 ± 11** **(26 ± 7*)**	94 ± 20 **(-6 ± 20)**
35	57 ± 7 **(-17 ± 2**)**	268 ± 15** **(36 ± 9**)**	113 ± 12** **(30 ± 6**)**	69 ± 11 **(-31 ± 11)**
50	60 ± 8 **(-14 ± 2)**	260 ± 12* **(28 ± 7**)**	108 ± 12** **(24 ± 5)**	63 ± 8 **(-37 ± 8)**

Table 5.4

The effects of buspirone (200 μg kg^{-1} i.c.; n=5) on the reflex changes in mean arterial blood pressure (MABP), R-R interval, phrenic burst rate (PBR) and integrated renal nerve activity (IRNA) evoked by stimulating cardiopulmonary afferents with PBG. The table shows mean control reflex changes evoked before administration of buspirone and reflex changes evoked at time points after administration of buspirone. Values in parenthesis show changes (Δ) from the control responses. All values are mean ± s.e. mean.

Changes caused by buspirone i.c. have been compared with those caused by saline i.c. using two-way ANOVA and the least significant difference test. *p<0.05, **p<0.01 compared to saline i.c.

Time after drug (min)	Decrease MABP (mmHg)	Increase R-R interval (ms)	Increase PBR (bursts min^{-1})	Decrease IRNA (%)
CONTROL	29 ± 3	38 ± 8	31 ± 9	100
5	17 ± 2** **(-12 ± 4**)**	44 ± 39 **(7 ± 34)**	8 ± 5** **(-23 ± 7**)**	31 ± 22* **(-69 ± 22*)**
20	19 ± 1** **(-9 ± 2*)**	161 ± 55** **(124 ± 56**)**	24 ± 8 **(-7 ± 1)**	69 ± 16 **(-31 ± 16)**
35	19 ± 2** **(-9 ± 2*)**	107 ± 39 **(70 ± 41)**	30 ± 8 **(-1 ± 2)**	55 ± 19** **(-45 ± 19*)**
50	22 ± 2* **(-7 ± 1)**	65 ± 17 **(27 ± 19)**	32 ± 8 **(1 ± 2)**	52 ± 18** **(-48 ± 18*)**

Table 5.5

The effects of WAY-100635 pre-treatment (100 μg kg^{-1} i.v.; n=5) on baseline mean arterial blood pressure (MABP), R-R interval, phrenic burst rate (PBR) and integrated renal nerve activity (IRNA). The table shows mean control baseline values measured before administration of WAY-100635 and baseline values measured 5 min after administration of WAY-100635 immediately prior to stimulation of the cardiopulmonary afferents (see table 5.6). Values in parenthesis show changes **(Δ)** from the control values. All values are mean ± s.e. mean.

Changes in baseline values caused by WAY-100635 i.v. have been compared with those caused by saline i.c. using two-way ANOVA and the least significant difference test. Changes from control caused by WAY-100635 i.v. have been compared with those caused by saline i.c. using one-way ANOVA.
*p<0.05, **p<0.01 compared to saline i.c.

Time after drug (min)	**MABP (mmHg)**	**R-R interval (ms)**	**PBR (bursts min^{-1})**	**IRNA (%)**
CONTROL	83 ± 6	230 ± 6	67 ± 3	100
5	87 ± 8 **(4 ± 3)**	223 ± 6 **(-6 ± 3)**	64 ± 3 **(-2 ± 1*)**	124 ± 6** **(24 ± 6*)**

Table 5.6

The effects of WAY-100635 pre-treatment (100 μg kg^{-1} i.v.; n=5) on the reflex changes in mean arterial blood pressure (MABP), R-R interval, phrenic burst rate (PBR) and integrated renal nerve activity (IRNA) evoked by stimulating cardiopulmonary afferents with PBG. The table shows mean control reflex changes evoked before administration of WAY-100635 and reflex changes evoked 5 min after administration of WAY-100635. Values in parenthesis show changes **(Δ)** from the control responses. All values are mean ± s.e. mean.

Changes in reflex values caused by WAY-100635 i.v. have been compared with those caused by saline i.c. using two-way ANOVA and the least significant difference test. Changes from control caused by WAY-100635 i.v. have been compared with those caused by saline i.c. using one-way ANOVA.
*p<0.05, **p<0.01 compared to saline i.c.

Time after drug (min)	**Decrease MABP (mmHg)**	**Increase R-R interval (ms)**	**Increase PBR (bursts min^{-1})**	**Decrease IRNA (%)**
CONTROL	30 ± 4	28 ± 7	24 ± 9	100
5	30 ± 4 **(0 ± 1)**	27 ± 5 **(-2 ± 3)**	16 ± 7* **(-8 ± 3)**	104 ± 23 **(4 ± 23)**

Table 5.7

The effects of buspirone (200 μg kg^{-1} i.c.) 20 min after pre-treatment with WAY-100635 (100 μg kg^{-1} i.v.; n=5) on baseline mean arterial blood pressure (MABP), R-R interval, phrenic burst rate (PBR) and integrated renal nerve activity (IRNA). The table shows mean control baseline values measured before administration of buspirone and baseline values measured at time points after administration of buspirone immediately prior to stimulation of the cardiopulmonary afferents (see table 5.8). Values in parenthesis show changes (Δ) from the control values. All values are mean ± s.e. mean.

Changes caused by buspirone i.c. after pre-treatment with WAY-100635 i.v. have been compared with those caused by saline i.c. and those caused by buspirone i.c. alone using two-way ANOVA and the least significant difference test.

*p<0.05, **p<0.01 compared to saline i.c.

†p<0.05, ††p<0.01 compared to buspirone i.c. alone.

Time after drug (min)	MABP (mmHg)	R-R interval (ms)	PBR (bursts min^{-1})	IRNA (%)
CONTROL	87 ± 8	223 ± 6	64 ± 3	100
5	99 ± 10 **(12 ± 8**)**	232 ± 8 **(9 ± 9)**	66 ± 6† **(2 ± 6)**	123 ± 17 **(23 ± 17)**
20	68 ± 5 **(-19 ± 3**)**	234 ± 6† **(10 ± 4* ††)**	76 ± 4† **(12 ± 3)**	63 ± 13* **(-37 ± 13)**
35	73 ± 6 **(-14 ± 4*)**	226 ± 5†† **(3 ± 5††)**	77 ± 5†† **(13 ± 3)**	89 ± 21 **(-11 ± 21)**
50	85 ± 7† **(-2 ± 3)**	220 ± 5†† **(-3 ± 5††)**	76 ± 5† **(11 ± 5)**	100 ± 20 **(0 ± 20)**

Table 5.8

The effects of buspirone (200 μg kg^{-1} i.c.) 20 min after pre-treatment with WAY-100635 (100 μg kg^{-1} i.v.; n=5) on the reflex changes in mean arterial blood pressure (MABP), R-R interval, phrenic burst rate (PBR) and integrated renal nerve activity (IRNA) evoked by stimulating cardiopulmonary afferents with PBG. The table shows mean control reflex changes evoked before administration of buspirone and reflex changes evoked at time points after administration of buspirone. Values in parenthesis show changes (Δ) from the control responses. All values are mean ± s.e. mean.

Changes caused by buspirone i.c. after pre-treatment with WAY-100635 i.v. have been compared with those caused by saline i.c. and those caused by buspirone i.c. alone using two-way ANOVA and the least significant difference test.

*p<0.05, **p<0.01 compared to saline i.c.

†p<0.05, ††p<0.01 compared to buspirone i.c. alone.

Time after drug (min)	Decrease MABP (mmHg)	Increase R-R interval (ms)	Increase PBR (bursts min^{-1})	Decrease IRNA (%)
CONTROL	30 ± 4	27 ± 5	16 ± 7**	100
5	28 ± 4† **(-1 ± 4††)**	22 ± 6 **(-5 ± 5)**	12 ± 5** **(-3 ± 2††)**	91 ± 20† **(-9 ± 20)**
20	22 ± 4 **(-7 ± 2)**	51 ± 13†† **(24 ± 9** †)**	17 ± 8* **(2 ± 1)**	94 ± 24 **(-6 ± 24)**
35	25 ± 5 **(-4 ± 3)**	32 ± 8† **(6 ± 4)**	16 ± 7* **(0 ± 2)**	121 ± 32† **(21 ± 32†)**
50	29 ± 5 **(0 ± 2)**	29 ± 7 **(2 ± 3)**	16 ± 8* **(1 ± 2)**	119 ± 27† **(19 ± 27†)**

Table 5.9

The effects of buspirone (200 μg kg^{-1} i.v.; n=4) on baseline mean arterial blood pressure (MABP), R-R interval, phrenic burst rate (PBR) and integrated renal nerve activity (IRNA). The table shows mean control baseline values measured before administration of buspirone and baseline values measured at time points after administration of buspirone immediately prior to stimulation of the cardiopulmonary afferents (see table 5.10). Values in parenthesis show changes (Δ) from the control values. All values are mean ± s.e. mean.

Changes caused by buspirone i.v. have been compared with those caused by saline i.c. and those caused by buspirone i.c. using two-way ANOVA and the least significant difference test.

*p<0.05, **p<0.01 compared to saline i.c.

†p<0.05, ††p<0.01 compared to buspirone i.c. alone.

Time after drug (min)	MABP (mmHg)	R-R interval (ms)	PBR (bursts min^{-1})	IRNA (%)
CONTROL	88 ± 6	213 ± 12	70 ± 2	100
5	73 ± 4 **(-15 ± 2* ††)**	217 ± 12 **(4 ± 2)**	73 ± 1 **(3 ± 2)**	80 ± 7* †† **(-20 ± 7††)**
20	77 ± 4† **(-11 ± 2)**	214 ± 11†† **(0 ± 3††)**	66 ± 1†† **(-4 ± 1* †)**	68 ± 7** **(-32 ± 7**)**
35	78 ± 2† **(-10 ± 4)**	212 ± 11†† **(-2 ± 4††)**	68 ± 2†† **(-2 ± 0†)**	70 ± 7** **(-30 ± 7*)**
50	82 ± 2† **(-6 ± 4)**	210 ± 10† **(-4 ± 4††)**	73 ± 4† **(3 ± 2)**	69 ± 12 **(-31 ± 12)**

Table 5.10

The effects of buspirone (200 μg kg^{-1} i.v.; n=4) on the reflex changes in mean arterial blood pressure (MABP), R-R interval, phrenic burst rate (PBR) and integrated renal nerve activity (IRNA) evoked by stimulating cardiopulmonary afferents with PBG. The table shows mean control reflex changes evoked before administration of buspirone and reflex changes evoked at time points after administration of buspirone Values in parenthesis show changes (Δ) from the control responses. All values are mean ± s.e. mean.

Changes caused by buspirone i.v. have been compared with those caused by saline i.c. and those caused by buspirone i.c. using two-way ANOVA and the least significant difference test.

*p<0.05, **p<0.01 compared to saline i.c.

†p<0.05, ††p<0.01 compared to buspirone i.c. alone.

Time after drug (min)	Decrease MABP (mmHg)	Increase R-R interval (ms)	Increase PBR (bursts min^{-1})	Decrease IRNA (%)
CONTROL	36 ± 3†	23 ± 6	37 ± 10	100
5	26 ± 3† **(-10 ± 1**)**	40 ± 2 **(17 ± 5*)**	23 ± 2* **(-14 ± 8*)**	103 ± 9† **(3 ± 9†)**
20	27 ± 3† **(-9 ± 2*)**	32 ± 2† **(9 ± 5†)**	29 ± 5 **(-8 ± 5)**	81 ± 7 **(-19 ± 7)**
35	27 ± 2† **(-9 ± 2**)**	29 ± 3 **(6 ± 3)**	28 ± 4 **(-9 ± 6)**	75 ± 7* **(-25 ± 7)**
50	29 ± 2† **(-7 ± 2)**	31 ± 4 **(7 ± 4)**	26 ± 5 **(-11 ± 5)**	64 ± 5* **(-36 ± 5*)**

Table 5.11

The effects of WAY-100635 (100 μg kg^{-1} i.c.; n=6) on baseline mean arterial blood pressure (MABP), R-R interval, phrenic burst rate (PBR) and integrated renal nerve activity (IRNA). The table shows mean control baseline values measured before administration of WAY-100635 and baseline values measured after administration of WAY-100635 immediately prior to stimulation of the cardiopulmonary afferents (see table 5.12). Values in parenthesis show changes (Δ) from the control values. All values are mean ± s.e. mean.

Changes caused by WAY-100635 i.c. have been compared with those caused by saline i.c. using two-way ANOVA and the least significant difference test. *p<0.05, **p<0.01 compared to saline i.c.

Time after drug (min)	MABP (mmHg)	R-R interval (ms)	PBR (bursts min^{-1})	IRNA (%)
CONTROL	85 ± 7	244 ± 7	61 ± 3*	100
5	75 ± 7 **(-10 ± 6)**	245 ± 9 **(2 ± 3)**	63 ± 2 **(2 ± 2)**	129 ± 14 **(29 ± 14)**
15	76 ± 8 **(-9 ± 2)**	247 ± 10 **(4 ± 5)**	65 ± 4* **(3 ± 5)**	84 ± 8 **(-16 ± 8)**

Table 5.12

The effects of WAY-100635 (100 μg kg^{-1} i.c.; n=6) on the reflex changes in mean arterial blood pressure (MABP), R-R interval, phrenic burst rate (PBR) and integrated renal nerve activity (IRNA) evoked by stimulating cardiopulmonary afferents with PBG. The table shows mean control reflex changes evoked before administration of WAY-100635 and the reflex changes evoked at time points after administration of WAY-100635. Values in parenthesis show changes (Δ) from the control responses. All values are mean ± s.e. mean.

Changes caused by WAY-100635 i.c. have been compared with those caused by saline i.c. using two-way ANOVA and the least significant difference test. *p<0.05, **p<0.01 compared to saline i.c.

Time after drug (min)	**Decrease MABP (mmHg)**	**Increase R-R interval (ms)**	**Increase PBR (bursts min^{-1})**	**Decrease IRNA (%)**
CONTROL	34 ± 4	56 ± 6	34 ± 3	100
5	12 ± 3** **(-22 ± 4**)**	9 ± 2** **(-46 ± 6**)**	18 ± 10 **(-16 ± 8*)**	52 ± 11* **(-48 ± 11)**
15	24 ± 5 **(-9 ± 1*)**	40 ± 11 **(-15 ± 8)**	28 ± 7 **(-5 ± 6)**	65 ± 12* **(-35 ± 12*)**

Table 5.13

The effects of saline (20 μl i.c.; n=5) on baseline mean arterial blood pressure (MABP), R-R interval, phrenic burst rate (PBR) and integrated renal nerve activity (IRNA). The table shows mean control baseline values measured before administration of saline and baseline values measured at time points after administration of saline immediately prior to the stimulation of baroreceptor afferents (see table 5.14). Values in parenthesis show changes (Δ) from the control values. All values are mean ± s.e. mean.

Time after drug (min)	MABP (mmHg)	R-R interval (ms)	PBR (bursts min^{-1})	IRNA (%)
CONTROL	81 ± 8	225 ± 11	83 ± 5	100
7.5	73 ± 8 **(-8 ± 2)**	215 ± 14 **(-10 ± 6)**	92 ± 9 **(9 ± 6)**	105 ± 12 **(5 ± 12)**
22.5	79 ± 9 **(-2 ± 3)**	212 ± 15 **(-13 ± 9)**	91 ± 7 **(8 ± 3)**	109 ± 10 **(9 ± 10)**
37.5	76 ± 10 **(-5 ± 3)**	211 ± 15 **(-14 ± 9)**	88 ± 4 **(5 ± 2)**	112 ± 10 **(12 ± 10)**

Table 5.14

The effects of saline (20 μl i.c.; n=5) on the reflex changes in mean arterial blood pressure (MABP), R-R interval, phrenic burst rate (PBR) and integrated renal nerve activity (IRNA) evoked by stimulating the left aortic nerve at six frequencies. The tables show the mean control reflex changes evoked before administration of saline followed by the reflex changes evoked during the sets of stimulations performed 7.5, 22.5 and 37.5 min after administration of saline. Values in parenthesis show changes (Δ) from the corresponding control responses. All values are mean ± s.e. mean.

Control stimulations

Aortic stimulation freq (Hz)	**Decrease MABP (mmHg)**	**Increase R-R interval (ms)**	**Increase PBR (bursts min^{-1})**	**Decrease IRNA (%)**
5	11 ± 3	4 ± 1	0 ± 1	100
10	18 ± 5	5 ± 1	2 ± 2	100
20	24 ± 5	9 ± 2	3 ± 1	100
40	31 ± 5	15 ± 2	3 ± 1	100
80	33 ± 5	24 ± 4	5 ± 2	100
160	32 ± 5	25 ± 5	5 ± 2	100

7.5 min after saline i.c.

Aortic stimulation freq (Hz)	Decrease MABP (mmHg)	Increase R-R interval (ms)	Increase PBR (bursts min^{-1})	Decrease IRNA (%)
5	11 ± 3 **(0 ± 2)**	4 ± 1 **(0 ± 1)**	-4 ± 2 **(-3 ± 2)**	69 ± 44 **(-31 ± 44)**
10	19 ± 7 **(1 ± 1)**	5 ± 2 **(0 ± 1)**	1 ± 1 **(-1 ± 1)**	131 ± 26 **(31 ± 26)**
20	25 ± 5 **(1 ± 1)**	9 ± 2 **(0 ± 2)**	3 ± 2 **(1 ± 2)**	110 ± 10 **(10 ± 10)**
40	31 ± 6 **(0 ± 2)**	16 ± 5 **(1 ± 4)**	0 ± 2 **(-4 ± 1)**	100 ± 11 **(0 ± 11)**
80	32 ± 5 **(0 ± 1)**	22 ± 6 **(-2 ± 4)**	3 ± 1 **(-2 ± 2)**	90 ± 6 **(-10 ± 6)**
160	35 ± 5 **(2 ± 2)**	26 ± 8 **(0 ± 4)**	4 ± 2 **(-1 ± 3)**	128 ± 31 **(28 ± 31)**

22.5 min after saline i.c.

5	15 ± 7 **(5 ± 5)**	4 ± 1 **(-1 ± 0)**	-1 ± 1 **(0 ± 2)**	114 ± 16 **(14 ± 16)**
10	21 ± 6 **(3 ± 1)**	5 ± 1 **(0 ± 0)**	1 ± 2 **(-1 ± 2)**	132 ± 33 **(32 ± 33)**
20	28 ± 5 **(4 ± 2)**	9 ± 1 **(-1 ± 1)**	1 ± 2 **(-1 ± 2)**	167 ± 56 **(67 ± 56)**
40	31 ± 4 **(0 ± 1)**	16 ± 4 **(1 ± 3)**	3 ± 2 **(0 ± 2)**	129 ± 28 **(29 ± 28)**
80	34 ± 5 **(1 ± 1)**	22 ± 5 **(-2 ± 3)**	6 ± 2 **(2 ± 2)**	120 ± 16 **(20 ± 16)**
160	33 ± 5 **(1 ± 1)**	25 ± 6 **(-1 ± 3)**	4 ± 2 **(-1 ± 2)**	123 ± 35 **(23 ± 35)**

37.5 min after saline i.c.

5	14 ± 4 **(3 ± 3)**	4 ± 1 **(0 ± 1)**	0 ± 2**(0 ± 2)**	128 ± 63 **(28 ± 63)**
10	18 ± 6 **(0 ± 2)**	6 ± 1 **(1 ± 1)**	1 ± 2 **(-1 ± 2)**	150 ± 47 **(50 ± 47)**
20	26 ± 5 **(2 ± 2)**	10 ± 2 **(1 ± 3)**	3 ± 2 **(0 ± 2)**	199 ± 82 **(99 ± 82)**
40	32 ± 5 **(1 ± 2)**	16 ± 5 **(1 ± 4)**	4 ± 1 **(1 ± 1)**	129 ± 40 **(29 ± 40)**
80	33 ± 4 **(0 ± 1)**	25 ± 5 **(1 ± 3)**	4 ± 1 **(-1 ± 2)**	108 ± 26 **(8 ± 26)**
160	33 ± 5 **(1 ± 1)**	24 ± 7 **(-2 ± 4)**	5 ± 2 **(1 ± 2)**	125 ± 35 **(25 ± 35)**

Table 5.15

The effects of buspirone (200 μg kg^{-1} i.c.; n=5) on baseline mean arterial blood pressure (MABP), R-R interval, phrenic burst rate (PBR) and integrated renal nerve activity (IRNA). The table shows mean control baseline values measured before administration of buspirone and baseline values measured at time points after administration of buspirone immediately prior to the stimulation of baroreceptor afferents (see table 5.16). Values in parenthesis show changes (Δ) from the control values. All values are mean ± s.e. mean.

Changes caused by buspirone i.c. have been compared with those caused by saline i.c. using two-way ANOVA and the least significant difference test. *p<0.05, **p<0.01 compared to saline i.c.

Time after drug (min)	MABP (mmHg)	R-R interval (ms)	PBR (bursts min^{-1})	IRNA (%)
CONTROL	73 ± 8	232 ± 12	100 ± 12	100
7.5	70 ± 5 **(-3 ± 4)**	289 ± 38** **(58 ± 36**)**	108 ± 15 **(8 ± 12)**	176 ± 35** **(76 ± 35**)**
22.5	53 ± 6* **(-20 ± 4**)**	273 ± 16** **(42 ± 10**)**	128 ± 15** **(28 ± 6*)**	78 ± 10 **(-22 ± 10)**
37.5	56 ± 8 **(-17 ± 2**)**	270 ± 15** **(38 ± 8**)**	133 ± 18** **(33 ± 9**)**	73 ± 9* **(-27 ± 9*)**

Table 5.16

The effects of buspirone (200 μg kg^{-1} i.c.; n=5) on the reflex changes in mean arterial blood pressure (MABP), R-R interval, phrenic burst rate (PBR) and integrated renal nerve activity (IRNA) evoked by stimulating the left aortic nerve at six frequencies. The tables show the mean control reflex changes evoked before administration of buspirone followed by the reflex changes evoked during the sets of stimulations performed 7.5, 22.5 and 37.5 min after administration of buspirone. Values in parenthesis show changes (Δ) from the corresponding control responses. All values are mean ± s.e. mean.

Changes caused by buspirone i.c. have been compared with those caused by saline i.c. using three-way ANOVA and the least significant difference test.

*p<0.05, **p<0.01 compared to saline i.c.

Control stimulations

Aortic stimulation freq (Hz)	Decrease MABP (mmHg)	Increase R-R interval (ms)	Increase PBR (bursts min^{-1})	Decrease IRNA (%)
5	15 ± 2	7 ± 2	-1 ± 2	100
10	19 ± 4	8 ± 3	0 ± 2	100
20	25 ± 3	14 ± 1	1 ± 1	100
40	26 ± 4	20 ± 4	3 ± 2	100
80	29 ± 4	34 ± 8	4 ± 3	100
160	29 ± 4	50 ± 19	2 ± 1	100

7.5 min after buspirone i.c.

Aortic stimulation freq (Hz)	Decrease MABP (mmHg)	Increase R-R interval (ms)	Increase PBR (bursts min^{-1})	Decrease IRNA (%)
5	9 ± 2 **(-5 ± 3)**	14 ± 9 **(8 ± 10)**	0 ± 2 **(1 ± 3)**	101 ± 10 **(1 ± 10)**
10	12 ± 2 **(-7 ± 4**)**	25 ± 17 **(17 ± 17)**	0 ± 1 **(0 ± 2)**	77 ± 15 **(-23 ± 15)**
20	18 ± 2 **(-7 ± 3**)**	45 ± 25* **(31 ± 24*)**	2 ± 3 **(1 ± 3)**	104 ± 16 **(4 ± 16)**
40	20 ± 3* **(-6 ± 1*)**	78 ± 31** **(58 ± 29**)**	-1 ± 2 **(-4 ± 3)**	105 ± 12 **(5 ± 12)**
80	20 ± 3* **(-9 ± 2**)**	124 ± 33** **(89 ± 29**)**	0 ± 1 **(-3 ± 3)**	115 ± 11 **(15 ± 11)**
160	19 ± 3** **(-10 ± 2**)**	150 ± 30** **(100 ± 25**)**	0 ± 1 **(-2 ± 1)**	82 ± 13 **(-18 ± 13)**

22.5 min after buspirone i.c.

5	7 ± 2 **(-7 ± 3**)**	12 ± 3 **(6 ± 2)**	-1 ± 1 **(0 ± 3)**	108 ± 48 **(8 ± 48)**
10	13 ± 3 **(-6 ± 2**)**	19 ± 3 **(11 ± 3)**	0 ± 1 **(1 ± 3)**	60 ± 13 **(-40 ± 13)**
20	15 ± 2* **(-10 ± 2**)**	30 ± 3 **(16 ± 3)**	-2 ± 1 **(-3 ± 2)**	62 ± 17** **(-38 ± 17*)**
40	17 ± 3* **(-9 ± 2**)**	63 ± 11** **(43 ± 11**)**	1 ± 1 **(-2 ± 3)**	61 ± 15 **(-39 ± 15)**
80	18 ± 3** **(-11 ± 2**)**	96 ± 19** **(62 ± 14**)**	0 ± 1** **(-4 ± 2)**	64 ± 19 **(-36 ± 19)**
160	19 ± 3* **(-10 ± 2**)**	102 ± 20** **(52 ± 14**)**	-2 ± 1* **(-4 ± 2)**	55 ± 17 **(-45 ± 17)**

37.5 min after buspirone i.c.

5	7 ± 2 **(-8 ± 2**)**	7 ± 2 **(0 ± 1)**	-3 ± 1 **(-2 ± 1)**	72 ± 27 **(-28 ± 27)**
10	13 ± 3 **(-6 ± 2*)**	13 ± 3 **(5 ± 2)**	-3 ± 2 **(-3 ± 2)**	62 ± 19* **(-38 ± 19*)**
20	17 ± 3 **(-8 ± 2**)**	24 ± 4 **(10 ± 4)**	1 ± 2 **(0 ± 2)**	59 ± 20** **(-41 ± 20**)**
40	18 ± 3* **(-7 ± 1**)**	46 ± 10 **(26 ± 9)**	-1 ± 1* **(-4 ± 1)**	60 ± 18 **(-40 ± 18)**
80	21 ± 4* **(-8 ± 2**)**	74 ± 18** **(40 ± 13*)**	-3 ± 1** **(-6 ± 3)**	71 ± 21 **(-29 ± 21)**
160	22 ± 3 **(-7 ± 2*)**	74 ± 16** **(24 ± 9)**	-1 ± 1** **(-3 ± 2)**	48 ± 15* **(-52 ± 15)**

Table 5.17

The effects of WAY-100635 pre-treatment (100 μg^{-1} i.v.; n=5) on baseline mean arterial blood pressure (MABP), R-R interval, phrenic burst rate (PBR) and integrated renal nerve activity (IRNA). The table shows mean control baseline values measured before administration of WAY-100635 and baseline values measured 7.5 min after administration of WAY-100635 immediately prior to the stimulation of baroreceptor afferents (see table 5.18). Values in parenthesis show changes **(Δ)** from the control values. All values are mean ± s.e. mean.

Changes in baseline values caused by WAY-100635 i.v. have been compared with those caused by saline i.c. using two-way ANOVA and the least significant difference test. Changes from control caused by WAY-100635 i.v. have been compared with those caused by saline i.c. using one-way ANOVA.
*p<0.05, **p<0.01 compared to saline i.c.

Time after drug (min)	**MABP (mmHg)**	**R-R interval (ms)**	**PBR (bursts min^{-1})**	**IRNA (%)**
CONTROL	84 ± 5	235 ± 6	78 ± 5	100
7.5	83 ± 7 **(-1 ± 3)**	224 ± 6 **(-11 ± 4)**	73 ± 4* **(-4 ± 3)**	113 ± 5 **(13 ± 5)**

Table 5.18

The effects of WAY-100635 (100 μg kg^{-1} i.v.; n=5) on the reflex changes in mean arterial blood pressure (MABP), R-R interval, phrenic burst rate (PBR) and integrated renal nerve activity (IRNA) evoked by stimulating the left aortic nerve at six frequencies. The tables show the mean control reflex changes evoked before administration of WAY-100635 followed by the reflex changes evoked during the set of stimulations performed 7.5 min after administration of WAY-100635. Values in parenthesis show changes (Δ) from the corresponding control responses. All values are mean ± s.e. mean.

Changes in the reflex responses caused by WAY-100635 i.v. have been compared with those caused by saline i.c. using three-way ANOVA and the least significant difference test. Changes from control caused by WAY-100635 i.v. have been compared with those caused by saline i.c. using two-way ANOVA and the least significant difference test.
*$p<0.05$, **$p<0.01$ compared to saline i.c.

Control stimulations

Aortic stimulation freq (Hz)	Decrease MABP (mmHg)	Increase R-R interval (ms)	Increase PBR (bursts min^{-1})	Decrease IRNA (%)
5	16 ± 2	5 ± 1	0 ± 1	100
10	23 ± 2	8 ± 2	3 ± 1	100
20	30 ± 2	13 ± 3	4 ± 1	100
40	30 ± 3	16 ± 4	4 ± 2	100
80	31 ± 3	21 ± 4	5 ± 1	100
160	31 ± 3	22 ± 4	3 ± 1	100

7.5 min after WAY-100635 i.v.

5	11 ± 3 **(-6 ± 2*)**	4 ± 1 **(-2 ± 1)**	-1 ± 1 **(-1 ± 1)**	64 ± 15 **(-36 ± 15)**
10	18 ± 3 **(-5 ± 2*)**	6 ± 1 **(-3 ± 1)**	2 ± 1 **(-1 ± 1)**	101 ± 24 **(1 ± 24)**
20	25 ± 3 **(-4 ± 2*)**	11 ± 3 **(-2 ± 1)**	2 ± 2 **(-2 ± 1)**	96 ± 21 **(-4 ± 21)**
40	27 ± 4 **(-3 ± 2)**	12 ± 2 **(-4 ± 1)**	2 ± 1 **(-3 ± 2)**	93 ± 15 **(-7 ± 15)**
80	29 ± 3 **(-2 ± 2)**	18 ± 4 **(-3 ± 2)**	3 ± 1 **(-1 ± 1)**	97 ± 19 **(-3 ± 19)**
160	30 ± 2 **(-1 ± 1)**	19 ± 2 **(-4 ± 2)**	4 ± 2 **(1 ± 2)**	101 ± 14 **(1 ± 14)**

Table 5.19

The effects of buspirone (200 μg kg^{-1} i.c.) 20 min after pre-treatment with WAY-100635 (100 μg kg^{-1} i.c.; n=5) on baseline mean arterial blood pressure (MABP), R-R interval, phrenic burst rate (PBR) and integrated renal nerve activity (IRNA). The table shows mean control baseline values measured before administration of buspirone and baseline values measured at time points after administration of buspirone immediately prior to the stimulation of baroreceptor afferents (see table 5.20). Values in parenthesis show changes **(Δ)** from the control values. All values are mean ± s.e. mean.

Changes caused by buspirone i.c. after pre-treatment with WAY-100635 i.c. have been compared with those caused by saline i.c. and those caused by buspirone i.c. alone using two-way ANOVA and the least significance difference test.

*p<0.05, **p<0.01 compared to saline i.c.

†p<0.05, ††p<0.01 compared to buspirone i.c. alone.

Time after drug (min)	MABP (mmHg)	R-R interval (ms)	PBR (bursts min^{-1})	IRNA (%)
CONTROL	83 ± 7	224 ± 6	73 ± 4†	100
7.5	87 ± 8 **(4 ± 6**)**	239 ± 11†† **(14 ± 10** ††)**	73 ± 8** †† **(0 ± 4)**	123 ± 28†† **(23 ± 28†)**
22.5	67 ± 5 **(-16 ± 3**)**	235 ± 6† **(11 ± 5* †)**	87 ± 7†† **(14 ± 4)**	62 ± 9** **(-38 ± 9**)**
37.5	76 ± 7† **(-7 ± 3†)**	226 ± 5† **(2 ± 6†)**	86 ± 7†† **(13 ± 4†)**	91 ± 13 **(-9 ± 13)**

Table 5.20

The effects of buspirone (200 μg kg^{-1} i.c.) 20 min after pre-treatment with WAY-100635 (100 μg kg^{-1} i.v.; n=5) on the reflex changes in mean arterial blood pressure (MABP), R-R interval, phrenic burst rate (PBR) and integrated renal nerve activity (IRNA) evoked by stimulating the left aortic nerve at six frequencies. The tables show the mean control reflex changes evoked before administration of buspirone followed by the reflex changes evoked during the sets of stimulations performed 7.5, 22.5 and 37.5 min after administration of buspirone. Values in parenthesis show changes (Δ) from the corresponding control responses. All values are mean ± s.e. mean.

Changes caused by buspirone i.c. after pre-treatment with WAY-100635 i.v. have been compared with those caused by saline i.c. and those caused by buspirone i.c. alone using three-way ANOVA and the least significant difference test.

*p<0.05, **p<0.01 compared to saline i.c.

†p<0.05, ††p<0.01 compared to buspirone i.c. alone.

Control stimulations

Aortic stimulation freq (Hz)	Decrease MABP (mmHg)	Increase R-R interval (ms)	Increase PBR (bursts min^{-1})	Decrease IRNA (%)
5	11 ± 3	4 ± 1	-1 ± 1	100
10	18 ± 3	6 ± 1	2 ± 1	100
20	25 ± 3	11 ± 3	2 ± 2	100
40	27 ± 4	12 ± 2	2 ± 1	100
80	29 ± 3	18 ± 4	3 ± 1	100
160	30 ± 2	19 ± 2	4 ± 2	100

7.5 min after buspirone i.c. in the presence of WAY-100635 i.v.

Aortic stimulation freq (Hz)	Decrease MABP (mmHg)	Increase R-R interval (ms)	Increase PBR (bursts min^{-1})	Decrease IRNA (%)
5	12 ± 1 **(1 ± 2)**	8 ± 3 **(4 ± 2)**	1 ± 1* **(2 ± 1*)**	214 ± 79** †† **(114 ± 79** ††)**
10	17 ± 3 **(-1 ± 1)**	13 ± 4 **(7 ± 3)**	2 ± 1 **(-1 ± 1)**	156 ± 29† **(56 ± 29†)**
20	24 ± 2 **(-2 ± 3)**	27 ± 11** **(16 ± 8**)**	4 ± 1 **(2 ± 2)**	143 ± 22 **(43 ± 22)**
40	21 ± 2 **(-6 ± 3)**	35 ± 11** †† **(22 ± 9** †)**	2 ± 0 **(1 ± 1)**	126 ± 29 **(26 ± 29)**
80	22 ± 3 **(-7 ± 3)**	53 ± 11** †† **(35 ± 7** ††)**	3 ± 1 **(-1 ± 0)**	111 ± 22 **(11 ± 22)**
160	23 ± 4* **(-7 ± 2**)**	61 ± 12** †† **(42 ± 10** ††)**	2 ± 1 **(-2 ± 2)**	98 ± 23 **(-2 ± 23)**

22.5 min after buspirone i.c. in the presence of WAY-100635 i.v.

5	11 ± 2 **(0 ± 2†)**	6 ± 1 **(2 ± 1)**	0 ± 2 **(1 ± 1)**	151 ± 63 **(51 ± 63)**
10	18 ± 2 **(0 ± 2)**	11 ± 3 **(5 ± 2)**	0 ± 2 **(-2 ± 1)**	124 ± 26 **(24 ± 26)**
20	24 ± 3† **(-2 ± 3†)**	21 ± 6 **(10 ± 3*)**	3 ± 2† **(1 ± 1)**	108 ± 20 **(8 ± 20)**
40	24 ± 4 **(-3 ± 4)**	27 ± 6† **(15 ± 4*)**	4 ± 3 **(3 ± 3)**	120 ± 22 **(20 ± 22)**
80	28 ± 3† **(-2 ± 3†)**	38 ± 6* †† **(20 ± 3** ††)**	3 ± 1 **(-1 ± 1)**	107 ± 17 **(7 ± 17)**
160	26 ± 4 **(-4 ± 3)**	39 ± 6* †† **(20 ± 5** †)**	2 ± 2† **(-1 ± 2)**	103 ± 10 **(3 ± 10)**

37.5 min after buspirone i.c. in the presence of WAY-100635 i.v.

5	13 ± 3 **(2 ± 3††)**	5 ± 2 **(2 ± 2)**	0 ± 2 **(1 ± 1)**	229 ± 56* †† **(129 ± 56* ††)**
10	19 ± 3 **(1 ± 4†)**	10 ± 3 **(4 ± 3)**	1 ± 2† **(-1 ± 2)**	158 ± 27†† **(58 ± 27†)**
20	29 ± 3†† **(4 ± 5††)**	15 ± 3 **(4 ± 3)**	6 ± 3† **(3 ± 3)**	147 ± 21† **(47 ± 21†)**
40	28 ± 4† **(1 ± 2†)**	19 ± 4 **(6 ± 2)**	2 ± 2 **(0 ± 3)**	125 ± 19 **(25 ± 19)**
80	32 ± 4†† **(3 ± 3††)**	25 ± 4†† **(7 ± 2†)**	5 ± 3†† **(2 ± 2††)**	146 ± 21† **(46 ± 21)**
160	28 ± 5 **(-2 ± 4)**	28 ± 3† **(9 ± 3)**	2 ± 0 **(-1 ± 2)**	115 ± 16† **(15 ± 16)**

Table 5.21

The effects of buspirone (200 μg kg^{-1} i.v.; n=4) on baseline mean arterial blood pressure (MABP), R-R interval, phrenic burst rate (PBR) and integrated renal nerve activity (IRNA). The table shows mean control baseline values measured before administration of buspirone and baseline values measured at time points after administration of buspirone immediately prior to the stimulation of baroreceptor afferents (see table 5.22). Values in parenthesis show changes (Δ) from the control values. All values are mean ± s.e. mean.

Changes caused by buspirone i.v. have been compared with those caused by saline i.c. and those caused by buspirone i.c. using two-way ANOVA and the least significant difference test.

*p<0.05, **p<0.01 compared to saline i.c.

†p<0.05, ††p<0.01 compared to buspirone i.c. alone.

Time after drug (min)	MABP (mmHg)	R-R interval (ms)	PBR (bursts min^{-1})	IRNA (%)
CONTROL	88 ± 3	221 ± 8	87 ± 3	100
7.5	73 ± 4 **(-14 ± 1††)**	219 ± 9†† **(-2 ± 3††)**	85 ± 3 **(-1 ± 3**)**	78 ± 14* †† **(-22 ± 14* ††)**
22.5	76 ± 2†† **(-12 ± 2**)**	219 ± 9†† **(-2 ± 4††)**	82 ± 2†† **(-5 ± 2** ††)**	75 ± 13** **(-25 ± 13*)**
37.5	78 ± 3†† **(-10 ± 2)**	215 ± 9†† **(-5 ± 4††)**	86 ± 3†† **(-1 ± 1††)**	76 ± 10** **(-24 ± 10**)**

Table 5.22

The effects of buspirone (200 μg kg^{-1} i.v.; n=4) on the reflex changes in mean arterial blood pressure (MABP), R-R interval, phrenic burst rate (PBR) and integrated renal nerve activity (IRNA) evoked by stimulating the left aortic nerve at six frequencies. The tables show the mean control reflex changes evoked before administration of buspirone followed by the reflex changes evoked during the sets of stimulations performed 7.5, 22.5 and 37.5 min after administration of buspirone. Values in parenthesis show changes (Δ) from the corresponding control responses. All values are mean ± s.e. mean.

Changes caused by buspirone i.v. have been compared with those caused by saline i.c. and those caused by buspirone i.c. using three-way ANOVA and the least significant difference test.

*$p<0.05$, **$p<0.01$ compared to saline.i.c.

†$p<0.05$, ††$p<0.01$ compared to buspirone i.c. alone.

Control stimulations

Aortic stimulation freq (Hz)	Decrease MABP (mmHg)	Increase R-R interval (ms)	Increase PBR (bursts min^{-1})	Decrease IRNA (%)
5	14 ± 5	5 ± 1	0 ± 2	100
10	19 ± 6	8 ± 2	2 ± 3	100
20	26 ± 4	10 ± 3	5 ± 4	100
40	26 ± 3	13 ± 2	4 ± 1	100
80	27 ± 3	23 ± 5	4 ± 3	100
160	28 ± 3	28 ± 9	5 ± 4	100

7.5 min after buspirone i.v.

Aortic stimulation freq (Hz)	Decrease MABP (mmHg)	Increase R-R interval (ms)	Increase PBR (bursts min^{-1})	Decrease IRNA (%)
5	10 ± 3 **(-4 ± 2)**	7 ± 2 **(2 ± 1)**	-1 ± 1 **(-1 ± 2)**	77 ± 13 **(-23 ± 13)**
10	15 ± 4 **(-4 ± 3)**	9 ± 3 **(2 ± 1)**	0 ± 1 **(-2 ± 1)**	89 ± 14 **(-11 ± 14)**
20	18 ± 2 **(-8 ± 3**)**	15 ± 3 **(5 ± 1)**	-1 ± 3 **(-6 ± 2* †)**	79 ± 10 **(-21 ± 10)**
40	21 ± 1 **(-5 ± 3)**	25 ± 8†† **(12 ± 7†)**	3 ± 2 **(-1 ± 1)**	92 ± 18 **(-8 ± 18)**
80	22 ± 1 **(-5 ± 3)**	44 ± 15* †† **(21 ± 11** ††)**	3 ± 2 **(-1 ± 1)**	74 ± 17 **(-26 ± 17)**
160	22 ± 1* **(-6 ± 3*)**	40 ± 11†† **(12 ± 6††)**	1 ± 1 **(-4 ± 3)**	81 ± 7 **(-19 ± 7)**

22.5 min after buspirone i.v.

5	9 ± 3 **(-4 ± 3**)**	6 ± 2 **(1 ± 1)**	-1 ± 2 **(0 ± 1)**	67 ± 9 **(-33 ± 9)**
10	14 ± 3 **(-5 ± 3*)**	8 ± 3 **(1 ± 2)**	1 ± 1 **(-2 ± 2)**	62 ± 10 **(-38 ± 10)**
20	20 ± 3 **(-6 ± 2**)**	14 ± 4 **(4 ± 2)**	4 ± 2† **(-1 ± 2)**	61 ± 10** **(-39 ± 10*)**
40	20 ± 2 **(-6 ± 3)**	20 ± 8† **(7 ± 7†)**	3 ± 1 **(-1 ± 1)**	62 ± 14 **(-38 ± 14)**
80	21 ± 2* **(-5 ± 3*)**	31 ± 15†† **(8 ± 14††)**	3 ± 1 **(-1 ± 2)**	61 ± 13 **(-39 ± 13)**
160	22 ± 1* **(-6 ± 2*)**	33 ± 13†† **(5 ± 10††)**	1 ± 1 **(-4 ± 4)**	75 ± 10 **(-25 ± 10)**

37.5 min after buspirone i.v.

5	11 ± 3 **(-3 ± 2)**	6 ± 2 **(1 ± 1)**	0 ± 1 **(0 ± 1)**	72 ± 10 **(-28 ± 10)**
10	15 ± 3 **(-4 ± 3)**	8 ± 3 **(1 ± 1)**	2 ± 3 **(-1 ± 2)**	74 ± 19* **(-26 ± 19)**
20	21 ± 2 **(-5 ± 3*)**	13 ± 4 **(3 ± 2)**	3 ± 3 **(-2 ± 2)**	74 ± 12** **(-26 ± 12**)**
40	23 ± 2 **(-3 ± 3)**	18 ± 8 **(5 ± 7)**	3 ± 1 **(-1 ± 1)**	67 ± 12 **(-33 ± 12)**
80	23 ± 2 **(-3 ± 3)**	28 ± 12† **(5 ± 11)**	3 ± 2† **(-1 ± 1)**	70 ± 16 **(-30 ± 16)**
160	25 ± 2 **(-3 ± 4)**	31 ± 13† **(3 ± 10)**	4 ± 1† **(-2 ± 4)**	84 ± 13 **(-16 ± 13)**

Table 5.23

The effects of WAY-100635 (100 μg kg^{-1} i.c.; n=6) on baseline mean arterial blood pressure (MABP), R-R interval, phrenic burst rate (PBR) and integrated renal nerve activity (IRNA). The table shows mean control baseline values measured before administration of WAY-100635 and baseline values measured at time points after administration of WAY-100635 immediately prior to the stimulation of baroreceptor afferents (see table 5.24). Values in parenthesis show changes (Δ) from the control values. All values are mean ± s.e. mean.

Changes caused by WAY-100635 i.c. have been compared with those caused by saline i.c. using two-way ANOVA and the least significant difference test. *p<0.05, **p<0.01 compared to saline i.c.

Time after drug (min)	MABP (mmHg)	R-R interval (ms)	PBR (bursts min^{-1})	IRNA (%)
CONTROL	88 ± 9	241 ± 8	70 ± 2	100
1.5	79 ± 10 **(-9 ± 3)**	240 ± 10 **(-2 ± 4)**	61 ± 2 **(-9 ± 2)**	128 ± 9* **(28 ± 9)**
11.5	79 ± 6 **(-9 ± 6)**	245 ± 7* **(3 ± 5)**	71 ± 9 **(1 ± 10)**	144 ± 17** **(44 ± 17*)**
21.5	83 ± 6 **(-5 ± 4)**	241 ± 9 **(-1 ± 4)**	70 ± 5 **(0 ± 6)**	128 ± 8* **(28 ± 8*)**

Table 5.24

The effects of WAY-100635 (100 μg kg^{-1} i.c.; n=6) on the reflex changes in mean arterial blood pressure (MABP), R-R interval, phrenic burst rate (PBR) and integrated renal nerve activity (IRNA) evoked by the stimulation of the left aortic nerve at 80 and 160 Hz. The tables show the mean control reflex changes evoked before administration of WAY-100635 followed by the reflex changes evoked during the sets of stimulations performed 1.5, 11.5 and 21.5 min after administration of WAY-100635. Values in parenthesis show changes (Δ) from the corresponding control responses. All values are mean ± s.e. mean.

Changes caused by WAY-100635 i.c. have been compared with those caused by saline i.c. using three-way ANOVA and the least significant difference test.

*p<0.05, **p<0.01 compared to saline i.c.

Control stimulations

Aortic stimulation freq (Hz)	**Decrease MABP (mmHg)**	**Increase R-R interval (ms)**	**Increase PBR (bursts min^{-1})**	**Decrease IRNA (%)**
80	28 ± 4	33 ± 6	3 ± 1	100
160	28 ± 4	34 ± 6	2 ± 1	100

1.5 min after WAY-100635 i.c.

Aortic stimulation freq (Hz)	Decrease MABP (mmHg)	Increase R-R interval (ms)	Increase PBR (bursts min^{-1})	Decrease IRNA (%)
80	15 ± 4* **(-13 ± 3**)**	6 ± 1 **(-27 ± 6**)**	1 ± 1 **(-1 ± 1)**	81 ± 16 **(-19 ± 16)**
160	9 ± 3** **(-19 ± 3**)**	5 ± 1* **(-29 ± 6**)**	1 ± 0 **(-1 ± 1)**	27 ± 14** **(-73 ± 14**)**

11.5 min after WAY-100635 i.c.

80	17 ± 7* **(-11 ± 4**)**	24 ± 8 **(-9 ± 10)**	2 ± 1* **(0 ± 1)**	82 ± 27 **(-19 ± 27)**
160	19 ± 7* **(-9 ± 4**)**	23 ± 8 **(-11 ± 11)**	3 ± 1 **(1 ± 1)**	76 ± 24 **(-25 ± 24)**

21.5 min after WAY-100635 i.c.

80	27 ± 5 **(-1 ± 3)**	28 ± 7 **(-5 ± 3)**	4 ± 1 **(2 ± 0)**	89 ± 16 **(-11 ± 16)**
160	27 ± 4 **(-1 ± 2)**	30 ± 6 **(-5 ± 4)**	3 ± 1 **(1 ± 1)**	66 ± 23* **(-34 ± 23)**

Table 5.25

The effects of saline (20 μl i.c.; n=5) on baseline mean arterial blood pressure (MABP), R-R interval, phrenic burst rate (PBR) and integrated renal nerve activity (IRNA). The table shows mean control baseline values measured before administration of saline and baseline values measured at time points after administration of saline immediately prior to the stimulation of chemoreceptor afferents (see table 5.26). Values in parenthesis show changes (Δ) from the control values. All values are mean ± s.e. mean.

Time after drug (min)	MABP (mmHg)	R-R interval (ms)	PBR (bursts min^{-1})	IRNA (%)
CONTROL	79 ± 5	259 ± 9	82 ± 9	100
10	78 ± 6 **(-1 ± 2)**	254 ± 10 **(-5 ± 3)**	82 ± 10 **(0 ± 1)**	113 ± 10 **(13 ± 10)**
20	78 ± 5 **(-1 ± 1)**	254 ± 11 **(-5 ± 4)**	84 ± 10 **(2 ± 2)**	94 ± 17 **(-6 ± 17)**
30	77 ± 6 **(-2 ± 2)**	256 ± 12 **(-3 ± 6)**	85 ± 10 **(4 ± 1)**	93 ± 16 **(-7 ± 16)**
40	82 ± 6 **(4 ± 2)**	261 ± 15 **(2 ± 9)**	86 ± 11 **(4 ± 2)**	110 ± 22 **(10 ± 22)**

Table 5.26

The effects of saline (20 μl i.c.; n=5) on the reflex changes in mean arterial blood pressure (MABP), R-R interval, phrenic burst rate (PBR) and integrated renal nerve activity (IRNA) evoked by stimulating chemoreceptor afferents with NaCN. The table shows mean control reflex changes evoked before administration of saline and reflex changes evoked at time points after administration of saline. Values in parenthesis show changes (Δ) from the control responses. All values are mean ± s.e. mean.

Time after drug (min)	Increase MABP (mmHg)	Increase R-R interval (ms)	Increase PBR (bursts min^{-1})	Increase IRNA (%)
CONTROL	9 ± 2	30 ± 2	13 ± 3	100
10	8 ± 1 **(-1 ± 1)**	24 ± 4 **(-6 ± 3)**	13 ± 3 **(0 ± 1)**	108 ± 15 **(8 ± 15)**
20	9 ± 2 **(0 ± 1)**	30 ± 5 **(0 ± 3)**	12 ± 2 **(-1 ± 2)**	101 ± 17 **(1 ± 17)**
30	9 ± 2 **(-1 ± 1)**	34 ± 6 **(5 ± 5)**	11 ± 2 **(-2 ± 2)**	113 ± 14 **(13 ± 14)**
40	5 ± 1 **(-4 ± 2)**	32 ± 5 **(2 ± 3)**	14 ± 4 **(1 ± 4)**	102 ± 26 **(2 ± 26)**

Table 5.27

The effects of buspirone (200 μg kg^{-1} i.c.; n=5) on baseline mean arterial blood pressure (MABP), R-R interval, phrenic burst rate (PBR) and integrated renal nerve activity (IRNA). The table shows mean control baseline values measured before administration of buspirone and baseline values measured at time points after administration of buspirone immediately prior to the stimulation of chemoreceptor afferents (see table 5.28). Values in parenthesis show changes (Δ) from the control values. All values are mean ± s.e. mean.

Changes caused by buspirone i.c. have been compared with those caused by saline i.c. using two-way ANOVA and the least significant difference test.
*p<0.05, **p<0.01 compared to saline i.c.

Time after drug (min)	MABP (mmHg)	R-R interval (ms)	PBR (bursts min^{-1})	IRNA (%)
CONTROL	77 ± 4	253 ± 17	87 ± 8	100
10	74 ± 5 **(-2 ± 6)**	335 ± 50* **(82 ± 36**)**	99 ± 10 **(13 ± 15)**	274 ± 72** **(174 ± 72**)**
20	63 ± 3* **(-13 ± 2**)**	311 ± 39 **(59 ± 26*)**	103 ± 8 **(16 ± 14)**	135 ± 47 **(35 ± 47)**
30	64 ± 4 **(-13 ± 3**)**	303 ± 34 **(50 ± 20*)**	104 ± 8 **(17 ± 12)**	70 ± 14 **(-30 ± 14)**
40	66 ± 5* **(-11 ± 3**)**	292 ± 28 **(39 ± 14)**	108 ± 8 **(21 ± 8)**	58 ± 14 **(-42 ± 14)**

Table 5.28

The effects of buspirone (200 μg kg^{-1} i.c.; n=5) on the reflex changes in mean arterial blood pressure (MABP), R-R interval, phrenic burst rate (PBR) and integrated renal nerve activity (IRNA) evoked by stimulating chemoreceptor afferents with NaCN. The table shows mean control reflex changes evoked before administration of buspirone and reflex changes evoked at time points after administration of buspirone. Values in parenthesis show changes (Δ) from the control responses. All values are mean ± s.e. mean.

Changes caused by buspirone i.c. have been compared with those caused by saline i.c. using two-way ANOVA and the least significant difference test.
*p<0.05, **p<0.01 compared to saline i.c.

Time after drug (min)	Increase MABP (mmHg)	Increase R-R interval (ms)	Increase PBR (bursts min^{-1})	Increase IRNA (%)
CONTROL	5 ± 2	31 ± 7	12 ± 2	100
10	9 ± 3 **(5 ± 4*)**	86 ± 24** **(55 ± 23*)**	7 ± 4 **(-5 ± 3)**	329 ± 110 **(229 ± 110)**
20	12 ± 1 **(7 ± 2**)**	104 ± 24** **(73 ± 23**)**	6 ± 5 **(-7 ± 5)**	926 ± 304** **(826 ± 304**)**
30	7 ± 1 **(2 ± 1)**	75 ± 26 **(44 ± 24)**	7 ± 4 **(-6 ± 3)**	565 ± 128** **(465 ± 128*)**
40	5 ± 1 **(0 ± 1)**	63 ± 20 **(32 ± 17)**	7 ± 5 **(-6 ± 6)**	414 ± 115 **(314 ± 115)**

Table 5.29

The effects of WAY-100635 pre-treatment (100 μg kg^{-1} i.v.; n=5) on baseline mean arterial blood pressure (MABP), R-R interval, phrenic burst rate (PBR) and integrated renal nerve activity (IRNA). The table shows mean control baseline values measured before administration of WAY-100635 and baseline values measured 5 min after administration of WAY-100635 immediately prior to stimulation of chemoreceptor afferents (see table 5.30). Values in parenthesis show changes (Δ) from the control values. All values are mean ± s.e. mean.

Changes in baseline values caused by WAY-100635 i.v. have been compared with those caused by saline i.c. using two-way ANOVA and the least significant difference test. Changes from control caused by WAY-100635 i.v. have been compared with those caused by saline i.c. using one-way ANOVA.
*p<0.05, **p<0.01 compared to saline i.c.

Time after drug (min)	MABP (mmHg)	R-R interval (ms)	PBR (bursts min^{-1})	IRNA (%)
CONTROL	73 ± 4	243 ± 9	74 ± 1	100
10	75 ± 4 **(2 ± 1)**	240 ± 8 **(-3 ± 2)**	70 ± 1 **(-4 ± 1*)**	130 ± 11 **(30 ± 11)**

Table 5.30

The effects of WAY-100635 pre-treatment (100 μg kg^{-1} i.v.; n=5) on the reflex changes in mean arterial blood pressure (MABP), R-R interval, phrenic burst rate (PBR) and integrated renal nerve activity (IRNA) evoked by stimulating chemoreceptor afferents with NaCN. The table shows mean control reflex changes evoked before administration of WAY-100635 and reflex changes evoked 5 min after administration of WAY-100635. Values in parenthesis show changes (Δ) from the control responses. All values are mean ± s.e. mean.

Changes in reflex responses caused by WAY-100635 i.v. have been compared with those caused by saline i.c. using two-way ANOVA and the least significant difference test. Changes from control caused by WAY-100635 i.v. have been compared with those caused by saline i.c. using one-way ANOVA.
*p<0.05, **p<0.01 compared to saline i.c.

Time after drug (min)	Increase MABP (mmHg)	Increase R-R interval (ms)	Increase PBR (bursts min^{-1})	Increase IRNA (%)
CONTROL	7 ± 1	23 ± 5	17 ± 3	100
10	4 ± 1 **(-2 ± 1)**	14 ± 2 **(-9 ± 3)**	18 ± 3 **(1 ± 3)**	87 ± 13 **(-13 ± 13)**

Table 5.31

The effects of buspirone (200 μg kg^{-1} i.c.) 20 min after pre-treatment with WAY-100635 (100 μg kg^{-1} i.v.; n=5) on baseline mean arterial blood pressure (MABP), R-R interval, phrenic burst rate (PBR) and integrated renal nerve activity (IRNA). The table shows mean control baseline values measured before administration of buspirone and baseline values measured at time points after administration of buspirone immediately prior to the stimulation of chemoreceptor afferents (see table 5.32). Values in parenthesis show changes (Δ) from the control values. All values are mean ± s.e. mean.

Changes caused by buspirone i.c. after pre-treatment with WAY-100635 i.v. have been compared with those caused by saline i.c. and those caused by buspirone i.c.alone using two-way ANOVA and the least significant difference test.

*p<0.05, **p<0.01 compared to saline i.c.

†p<0.05, ††p<0.01 compared to buspirone i.c. alone.

Time after drug (min)	MABP (mmHg)	R-R interval (ms)	PBR (bursts min^{-1})	IRNA (%)
CONTROL	75 ± 4	240 ± 8	66 ± 2†	100
10	74 ± 4 **(-1 ± 4)**	256 ± 8† **(16 ± 7† *)**	56 ± 3†† * **(-10 ± 3**)**	119 ± 27†† **(19 ± 27††)**
20	67 ± 4 **(-8 ± 1*)**	256 ± 8 **(16 ± 7*)**	72 ± 3†† **(6 ± 2)**	69 ± 8 **(-31 ± 8)**
30	70 ± 4 **(-5 ± 1)**	256 ± 10 **(17 ± 9)**	78 ± 5†† **(13 ± 4**)**	58 ± 5 **(-42 ± 5)**
40	75 ± 4 **(1 ± 1†)**	255 ± 10 **(15 ± 9)**	76 ± 4†† **(11 ± 3*)**	71 ± 9 **(-29 ± 9)**

Table 5.32

The effects of buspirone (200 μg kg^{-1} i.c.) 20 min after pre-treatment with WAY-100635 (100 μg kg^{-1} i.v.; n=5) on the reflex changes in mean arterial blood pressure (MABP), R-R interval, phrenic burst rate (PBR) and integrated renal nerve activity (IRNA) evoked by stimulating chemoreceptor afferents with NaCN. The table shows mean control reflex changes evoked before administration of buspirone and reflex changes evoked at time points after administration of buspirone. Values in parenthesis show changes (Δ) from the control responses. All values are mean ± s.e. mean.

Changes caused by buspirone i.c. after pre-treatment with WAY-100635 i.v. have been compared with those caused by saline i.c. and those caused by buspirone i.c. alone using two-way ANOVA and the least significant difference test.

*$p<0.05$, **$p<0.01$ compared to saline i.c.

†$p<0.05$, ††$p<0.01$ compared to buspirone i.c. alone.

Time after drug (min)	Increase MABP (mmHg)	Increase R-R interval (ms)	Increase PBR (bursts min^{-1})	Increase IRNA (%)
CONTROL	4 ± 1	14 ± 2*	17 ± 4	100
10	8 ± 3 **(4 ± 3*)**	21 ± 12†† **(7 ± 13†)**	15 ± 3 **(-2 ± 3)**	110 ± 24 **(10 ± 24)**
20	5 ± 2†† **(1 ± 2†)**	30 ± 4†† **(16 ± 5†)**	14 ± 2 **(-3 ± 3)**	148 ± 10†† * **(48 ± 10††)**
30	4 ± 1* **(-1 ± 2)**	27 ± 3† **(13 ± 3)**	12 ± 2 **(-5 ± 3)**	138 ± 11† **(38 ± 11†)**
40	3 ± 1 **(-2 ± 1)**	28 ± 4 **(14 ± 4)**	14 ± 4 **(-2 ± 2)**	119 ± 14 **(19 ± 14)**

Table 5.33

The effects of buspirone (200 μg kg^{-1} i.v.; n=4) on baseline mean arterial blood pressure (MABP), R-R interval, phrenic burst rate (PBR) and integrated renal nerve activity (IRNA). The table shows mean control baseline values measured before administration of buspirone and baseline values measured at time points after administration of buspirone immediately prior to the stimulation of chemoreceptor afferents (see table 5.34). Values in parenthesis show changes (Δ) from the control values. All values are mean ± s.e. mean.

Changes caused by buspirone i.v. have been compared with those caused by saline i.c. and those caused by buspirone i.c. using two-way ANOVA and the least significant difference test.

*p<0.05, **p<0.01 compared to saline i.c.

†p<0.05, ††p<0.01 compared to buspirone i.c. alone.

Time after drug (min)	MABP (mmHg)	R-R interval (ms)	PBR (bursts min^{-1})	IRNA (%)
CONTROL	80 ± 4	253 ± 7	73 ± 7	100
10	68 ± 2 **(-13 ± 2** †)**	264 ± 7 **(11 ± 4* †)**	80 ± 8 **(6 ± 3*)**	77 ± 2* †† **(-23 ± 2††)**
20	69 ± 2 **(-11 ± 2**)**	260 ± 7 **(8 ± 4)**	77 ± 9† **(3 ± 3)**	74 ± 4 **(-26 ± 4)**
30	70 ± 2 **(-10 ± 3**)**	256 ± 8 **(4 ± 3)**	78 ± 8† **(5 ± 2)**	74 ± 5 **(-26 ± 5)**
40	71 ± 3 **(-9 ± 3**)**	254 ± 7 **(1 ± 3)**	80 ± 9† **(6 ± 3)**	70 ± 8* **(-30 ± 8*)**

Table 5.34

The effects of buspirone (200 μg kg^{-1} i.v.; n=4) on the reflex changes in mean arterial blood pressure (MABP), R-R interval, phrenic burst rate (PBR) and integrated renal nerve activity (IRNA) evoked by stimulating chemoreceptor afferents with NaCN. The table shows mean control reflex changes evoked before administration of buspirone and reflex changes evoked at time points after administration of buspirone. Values in parenthesis show changes (Δ) from the control responses. All values are mean ± s.e. mean.

Changes caused by buspirone i.v. have been compared with those caused by saline i.c. and those caused by buspirone i.c. using two-way ANOVA and the least significant difference test.

*p<0.05, **p<0.01 compared to saline i.c.

†p<0.05, ††p<0.01 compared to buspirone i.c. alone.

Time after drug (min)	Increase MABP (mmHg)	Increase R-R interval (ms)	Increase PBR (bursts min^{-1})	Increase IRNA (%)
CONTROL	8 ± 2	21 ± 5	18 ± 6	100
10	13 ± 4 **(5 ± 2**)**	70 ± 17** **(49 ± 13**)**	7 ± 5 **(-11 ± 1**)**	548 ± 367** **(448 ± 367*)**
20	12 ± 3 **(4 ± 1*)**	43 ± 10† **(22 ± 5*)**	16 ± 7 **(-2 ± 1)**	128 ± 23†† **(28 ± 23††)**
30	12 ± 4 **(4 ± 2*)**	42 ± 16 **(21 ± 13)**	14 ± 6 **(-4 ± 1)**	115 ± 22 **(15 ± 22)**
40	12 ± 3† **(4 ± 1**)**	38 ± 9 **(17 ± 5)**	15 ± 7 **(-3 ± 3)**	229 ± 87 **(129 ± 87)**

Table 5.35

The effects of WAY-100635 (100 μg kg^{-1} i.c.; n=5) on baseline mean arterial blood pressure (MABP), R-R interval, phrenic burst rate (PBR) and integrated renal nerve activity (IRNA). The table shows mean control baseline values measured before administration of WAY-100635 and baseline values measured after administration of WAY-100635 immediately prior to the stimulation of chemoreceptor afferents (see table 5.36). Values in parenthesis show changes (Δ) from the control values. All values are mean ± s.e. mean.

Changes caused by WAY-100635 i.c. have been compared with those caused by saline i.c. using two-way ANOVA and the least significant difference test. *p<0.05, **p<0.01 compared to saline i.c.

Time after drug (min)	MABP (mmHg)	R-R interval (ms)	PBR (bursts min^{-1})	IRNA (%)
CONTROL	89 ± 6	229 ± 12	73 ± 4	100
5	86 ± 7 **(-3 ± 3)**	226 ± 13 **(-3 ± 3)**	72 ± 4 **(-1 ± 3)**	141 ± 16 **(41 ± 16)**
15	80 ± 6 **(-9 ± 5)**	229 ± 11 **(0 ± 5)**	79 ± 10 **(7 ± 10)**	109 ± 10 **(9 ± 10)**
25	84 ± 5 **(-5 ± 3)**	226 ± 12 **(-3 ± 3)**	76 ± 6 **(3 ± 6)**	107 ± 7 **(7 ± 7)**

Table 5.36

The effects of WAY-100635 (100 μg kg^{-1} i.c.; n=5) on the reflex changes in mean arterial blood pressure (MABP), R-R interval, phrenic burst rate (PBR) and integrated renal nerve activity (IRNA) evoked by stimulating chemoreceptor afferents with NaCN. The table shows mean control reflex changes evoked before administration of WAY-100635 and the reflex changes evoked at time points after administration of WAY-100635 Values in parenthesis show changes (Δ) from the control responses. All values are mean ± s.e. mean.

Changes caused by WAY-100635 i.c. have been compared with those caused by saline i.c. using two-way ANOVA and the least significant difference test. *$p<0.05$, **$p<0.01$ compared to saline i.c.

Time after drug (min)	Increase MABP (mmHg)	Increase R-R interval (ms)	Increase PBR (bursts min^{-1})	Increase IRNA (%)
CONTROL	11 ± 2	36 ± 7	12 ± 4	100
5	15 ± 3 **(4 ± 3)**	26 ± 7 **(-10 ± 7)**	8 ± 2 **(-4 ± 4)**	195 ± 83 **(95 ± 83)**
15	11 ± 3 **(0 ± 2)**	39 ± 7 **(3 ± 4)**	10 ± 6 **(-2 ± 3)**	25 ± 101 **(-75 ± 101)**
25	10 ± 4 **(-1 ± 1)**	37 ± 6 **(1 ± 4)**	10 ± 6 **(-2 ± 3)**	92 ± 29 **(-8 ± 29)**