THE ROLE OF 5-HYDROXYTRYPTAMINE RECEPTORS
IN CENTRAL CARDIOVASCULAR REGULATION
IN RATS AND CATS

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by

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ABSTRACT

Administration of 5-hydroxytryptamine (5-HT) to the lateral cerebral ventricle of the rat and cat causes a pressor response. The physiological mechanism(s) and the nature of the 5-HT receptor(s) mediating this response are unknown. Using novel agonists and antagonists for 5-HT receptors, the subtypes of 5-HT receptors mediating the cardiovascular effects of intracerebroventricular (i.c.v.) administration of 5-HT were investigated in anaesthetized rats and cats, and in conscious Long-Evans (normal) and Brattleboro (vasopressin deficient) rats. Measurements were made of blood pressure, heart rate, sympathetic nerve activity and respiratory variables in anaesthetized preparations, while in conscious preparations (used to avoid anaesthetic-induced cardiovascular depression) blood pressure and heart rate were measured along with blood flows in the mesenteric, renal and hindlimb vascular beds using Doppler flow probes.

In anaesthetized rats, 5-HT (i.c.v.) produced a pressor response associated with biphasic changes in heart rate and sympathetic nerve activity; bradycardia and sympathoinhibition were followed by tachycardia and sympathoexcitation. The sympathoinhibitory effects of 5-HT were blocked by 5-HT$_2$/5-HT$_1_C$ receptor antagonists (i.c.v.) and a vasopressin V$_1$-receptor antagonist (i.v.) which unmasked immediate sympathoexcitation. 5-HT$_1_A$ receptor agonists (i.c.v.) caused immediate increases in blood pressure, tachycardia and sympathoexcitation which were blocked by 5-HT$_1_A$ receptor antagonists (i.c.v.). The response to 5-HT was abolished by combined 5-HT$_2$/5-HT$_1_C$ and 5-HT$_1_A$ receptor blockade. In conscious rats, 5-HT caused a pressor response, bradycardia, mesenteric vasoconstriction and hindquarters vasodilatation. The mesenteric vasoconstriction and bradycardia were attenuated by a vasopressin V$_1$-receptor antagonist (i.v.). In Brattleboro rats the mesenteric vasoconstriction caused by 5-HT was reduced compared to normal rats.

In anaesthetized cats, 5-HT and a 5-HT$_2$/5-HT$_1_C$ receptor agonist (i.c.v.) caused an increase in blood pressure associated with femoral vasoconstriction, tachycardia and sympathoexcitation. The response to 5-HT was blocked by a 5-HT$_2$/5-HT$_1_C$ receptor antagonist (i.c.v.).

In conclusion, in rats 5-HT caused a pressor response which was mediated in part, by the release of vasopressin following activation of central 5-HT$_2$/5-HT$_1_C$ receptors. This resulted in an initial bradycardia and sympathoinhibition due to activation of the baroreceptor reflex. 5-HT also caused sympathoexcitation following activation of central 5-HT$_1_A$ receptors. In anaesthetized cats evidence suggests that the 5-HT-induced pressor response was mediated by activation of forebrain 5-HT$_2$/5-HT$_1_C$ receptors.
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Abbreviations

The standard abbreviations used by the British Journal of Pharmacology have been adopted throughout this thesis [see Instructions to Authors, (1991) Br. J. Pharmacol., 102, 7-9].

The following abbreviations have also been used:-

BP blood pressure
MAP mean arterial pressure
HR heart rate
RNA renal nerve activity
ANA adrenal nerve activity
SNA splanchnic nerve activity
CNA cardiac nerve activity
PNA phrenic nerve activity
REN renal
MES mesenteric
HQ hindquarters
FF femoral flow
FAC femoral arterial conductance
TP tracheal pressure
Insp. rate inspiratory rate

Chemical names and their abbreviations are given in the methods sections.
CHAPTER 1

GENERAL INTRODUCTION

1.1 The Serotonergic System

Rapport et al. (1948) isolated a vasoconstrictor substance from the serum which was termed serotonin. Independently, Ersparmer and colleagues identified a substance located in the enterochromaffin cell system and named it enteramine. Enteramine was shown to be identical to serotonin and this substance was later identified as 5-hydroxytryptamine (5-HT; Rapport et al., 1948; Rapport, 1949; see Ersparmer, 1954). Twarog & Page (1953) demonstrated the presence of 5-HT in the mammalian brain and regional variations in 5-HT concentration within the CNS were subsequently reported (Amin et al., 1954). This led to the suggestion that 5-HT may act as a neurotransmitter in the CNS (Brodie & Shore, 1957).

1.2 Synthesis and metabolism of 5-HT

The precursor of 5-HT is the essential amino acid L-tryptophan. The initial, rate-limiting step in 5-HT synthesis is the hydroxylation of tryptophan to 5-hydroxytryptophan and this is catalysed by the enzyme tryptophan hydroxylase. 5-hydroxytryptophan is then converted to 5-hydroxytryptamine by the enzyme aromatic L-amino acid decarboxylase. With the exception of platelets, 5-HT is synthesized in the tissues in which it is stored and appreciable amounts of 5-HT are found in the enterochromaffin cells in the gastrointestinal mucosa, enteric neurones, mast cells (of rodents) and in lung, kidney, spleen and heart tissues. 5-HT is also synthesized in the pineal gland where it serves as a precursor for melatonin production. 5-HT is accumulated and stored in blood platelets, which release their 5-HT content upon aggregation. However, synthesis of 5-HT has not been detected within these particles and this pool of 5-HT
originates largely from the enterochromaffin cell system. Furthermore, 5-HT can be taken up by, stored in, and released by sympathetic nerves. The synthesis and storage and metabolism of 5-HT in peripheral tissue has been reviewed in more detail elsewhere and the reader is referred to Verbeuren (1989). In the brain 5-HT is localized in nerve cells and fibres in specific brain regions (Dahlstrom and Fuxe, 1965; Steinbusch, 1981). As 5-HT does not readily cross the blood brain barrier (Lexchin et al., 1977) synthesis of this amine occurs within specific neurones. The enzyme tryptophan hydroxylase has been located within the serotonergic neuronal system and has been used as a specific marker for these neurones (Palkovits et al., 1977).

The main metabolic pathway of 5-HT is oxidative deamination by monoamine oxidase and the principle product is 5-hydroxyindole acetic acid. The conjugative processes of sulphation and glucuronidation can also inactivate 5-HT. The principal sites of inactivation of 5-HT in the circulation are the endothelial cells of the liver and lungs (see Verbeuren, 1989). Within the CNS inactivation of released 5-HT occurs via a high affinity active transport mechanism which transports 5-HT into the presynaptic neurone (see Ross, 1982; see Tamir & Gershon, 1990). Following reuptake, 5-HT is either transported into synaptic vesicles or metabolized to 5-hydroxyindole acetic acid by intraneuronal monoamine oxidase.

1.3 Anatomy of the serotonergic pathways

Initial studies in the rat used a formaldehyde-induced fluorescence technique to map serotonergic pathways. The cell bodies of serotonergic neurones (designated B1-B9 cell groups) are located in the midline areas of the caudal midbrain and brainstem and are associated with the raphé nuclei (Dahlstrom and Fuxe, 1964). A more sensitive immunohistochemical technique (Steinbusch et al., 1978) was used to confirm and advance these initial
studies and a more widespread distribution of both serotonergic cell bodies and serotonin-containing terminals has been demonstrated (Steinbusch, 1981). Further immunohistochemical studies have extended this to include other species (eg. cat; Jacobs et al., 1984). These studies have been comprehensively reviewed (see Jacobs & Azmitia, 1992). The serotonergic cell groups have both ascending and descending projections and have target regions in the forebrain and spinal cord (Fuxe, 1965; Azmitia, 1978; Steinbusch, 1981; see Jacobs & Azmitia, 1992). The ascending projections arise from the rostral raphe nuclei, the dorsal (B7) and median (B8) nuclei. Fibres arising in these nuclei travel to the forebrain in the medial forebrain bundle which can be subdivided into two main neuronal systems; a) the dorsal raphe forebrain tract which innervates lateral forebrain structures (eg. the basal ganglia, amygdala, accumbens, thalamus and piriform cortex); b) the median raphe forebrain tract which innervates medial forebrain structure (eg. the olfactory bulbs, preoptic area, septum, hippocampus and cingulate cortex; Azmitia, 1978; Azmitia & Segal, 1978). Some of the ascending projections from the dorsal raphe nucleus do not run in the median forebrain bundle but in separate tracts which innervate the cortex (cortical tract), the periventricular region of the thalamus and hypothalamus (periventricular tract) and the substantia nigra, ventrolateral geniculate body nuclei and the suprachiasmatic nucleus of the hypothalamus (arcuate tract; Azmitia, 1978). Descending axons, mainly originating from the caudal raphe nuclei, the raphe obscurus (B2), pallidus (B1) and magnus (B3) and the ventrolateral medulla [rostral ventrolateral medulla (B3), lateral paragigantocellular recticular nucleus (B3), caudal ventrolateral medulla (B1)], were found to innervate the dorsal and lateral horns of the spinal cord and the intermediolateral cell column (Dahlstrom & Fuxe, 1964; Loewy, 1981; for reviews see Tork, 1990 and Jacobs & Azmitia, 1992).
1.4 5-HT Receptors

The first evidence for the existence of distinct 5-HT receptor types came from a study by Gaddum & Picarelli (1957) using isolated guinea pig ileum. Two distinct 5-HT receptors were defined on the basis of differential antagonism of the contractile effects of exogenously applied 5-HT by dibenzyline or morphine. 5-HT mediates contraction of the ileum through two separate mechanisms; a direct action on smooth muscle which is blocked by dibenzyline, hence the D-receptor and an indirect action through the release of acetylcholine from parasympathetic nerve endings which is prevented by morphine, termed the M-receptor. Further progress was made in the classification of 5-HT receptors following the development of radioligand binding techniques, some 20 years later. A selective high affinity and saturable binding site was demonstrated for \(^{3}\text{H}\) 5-HT in the brain (Bennett & Snyder, 1976; Fillion et al., 1976). \(^{3}\text{H}\) Spiperone was also shown to label a low affinity 5-HT binding site (Leysen et al., 1978). This led Peroutka & Snyder (1979) to propose that there were two types of 5-HT binding sites in the rat brain; 5-HT\(_{1}\) (labelled with high affinity by \(^{3}\text{H}\) 5-HT) and 5-HT\(_{2}\) (labelled with low affinity by \(^{3}\text{H}\) 5-HT and high affinity by \(^{3}\text{H}\) spiperone). They also suggested that the 5-HT\(_{1}\) recognition site was heterogeneous because the inhibition of \(^{3}\text{H}\) 5-HT binding caused by spiperone was biphasic. Pedigo et al. (1981) confirmed that there were indeed two high affinity binding sites for \(^{3}\text{H}\) 5-HT which they termed 5-HT\(_{1A}\) and 5-HT\(_{1B}\); 5-HT\(_{1A}\) recognition sites were shown to have a high affinity for spiperone and 5-HT\(_{1B}\) sites a low affinity for spiperone. The aminotetralin 8-OH-DPAT (Arvidson et al., 1981), was subsequently found to have high affinity and selectivity for the 5-HT\(_{1A}\) subtype and is now considered as an archetypal 5-HT\(_{1A}\) receptor agonist (Middlemiss & Fozard, 1983). The 5-HT\(_{1B}\) recognition site was found to have high affinity for certain \(\beta\)-adrenoreceptor antagonists, thus Hoyer et al. (1985a) demonstrated that the 5-HT\(_{1B}\) site can be labelled with
iodocyanopindolol in the presence of isoprenaline to prevent specific binding to $\beta$-adrenoreceptors. Interestingly, 5-HT$_{1B}$ receptors could only be detected in rodents (rat and mouse) and were not detected in other species including man (Hoyer et al., 1986a; see Hoyer, 1991). A further binding site for [³H] 5-HT was subsequently identified in the choroid plexus on the basis of high affinity displacement of [³H] 5-HT by mesulergine (Pazos et al., 1984; Hoyer et al., 1985a). This site, the 5-HT$_{1C}$ receptor, was initially classified as a subtype of the 5-HT$_1$ group due to its high affinity for 5-HT. However, this receptor has been shown to have similar pharmacology, second messenger coupling and structural homology to the 5-HT$_2$ receptor and has recently been reclassified as a subtype of the 5-HT$_2$ class, termed 5-HT$_{2C}$ (see below). The existence of a fourth site, the 5-HT$_{1D}$ binding site, in bovine brain was proposed by Heuring & Perutka (1987). The existence of this site was demonstrated following the displacement of [³H] 5-HT in the presence of 8-OH-DPAT and mesulergine to mask the 5-HT$_{1A}$ and 5-HT$_{1C}$ binding sites, respectively. The 5-HT$_{1D}$ binding site has been found in non-rodent species which lack the 5-HT$_{1B}$ binding site (eg. guinea pig, pig, cat, dog, and man; Waeberefa/., 1988a, 1988b; 1989a, 1889b; Hoyer et al., 1988). However, it has been suggested that the 5-HT$_{1D}$ binding site is also present in the rat brain (Herrick-Davis & Titeler, 1988).

Following the identification of these binding sites for 5-HT, attempts were made to determine whether or not they correlated with functional 5-HT receptors identified in the brain or the periphery. This ultimately led to the unifying scheme for naming and classifying 5-HT receptors and binding sites in which three principle 5-HT receptor classes were recognized; "5-HT$_1$-like", 5-HT$_2$ and 5-HT$_3$ (Bradley et al., 1986). The term "5-HT$_1$-like" was proposed for the heterogeneous group of receptors exhibiting high affinity for 5-HT and the close analogue 5-carboxamidotryptamine (5-CT). The criteria defining this receptor class were; 1. 5-CT
should mimic the effects of 5-HT at an equal or lower concentration,
2. responses should be blocked by methiothepin (a nonselective
"5-HT₁-like"/5-HT₂ receptor antagonist) and methysergide (a non-selective
antagonist with 5-HT₁ receptor partial agonist ability) and 3. responses
should be resistant to antagonism by selective 5-HT₂ and 5-HT₃ receptor
antagonists such as ketanserin and MDL72222 (see Bradley et al., 1986).
The 5-HT₁-like class not only included the 5-HT₁ₐ, 5-HT₁₈, 5-HT₁₆ and
5-HT₁ᵌ binding sites, but also encompassed, for instance, the pre-junctional
5-HT receptor responsible for inhibiting sympathetic noradrenaline release
(Feniuk et al., 1979), the post-junctional 5-HT receptors effecting
contraction (Feniuk, 1984; Feniuk et al., 1985; Humphrey et al., 1988) and
relaxation of smooth muscle (Feniuk et al., 1983; Trevethick et al., 1984)
and the 5-HT receptor mediating tachycardia in vivo (Connor et al., 1986).

5-HT₂ receptors were recognised to be the same as Gaddum & Picarrelli's
D-receptor (Humphrey et al., 1982). Previous studies with more selective
ligands for the 5-HT₂ binding site (for instance, ketanserin (Leysen et al.,
1981; 1982), mianserin (Peroutka & Snyder, 1981), cyproheptadine,
(Leysen et al., 1982)) clearly demonstrated that the pharmacology of the
5-HT₂ site closely correlated (with the exception of the rat stomach fundus
receptor) with that of the D-receptor measured in functional studies in
vascular and intestinal smooth muscle (Cohen et al., 1983b; Humphrey et
al., 1982; Leysen et al., 1984; Engel et al., 1984; Maayani et al., 1984;
see Humphrey, 1984). The criteria defining the 5-HT₂ receptor class were;
1. responses should be susceptible to potent antagonism by ketanserin,
methysergide and other archetypal 'D' receptor antagonists and the pA₂
values for these antagonists should be similar to their pKₐ values
determined from ligand binding studies and 2. responses should be
resistant to antagonism by selective "5-HT₁-like" and 5-HT₃ receptor
antagonists (Bradley et al., 1986).
The 5-HT\textsubscript{3} receptor was the third class of receptors defined by Bradley \textit{et al.} (1986) and encompassed the M-receptor described by Gaddum & Picarelli (1957). Characterisation of this receptor was made possible following the discovery of the selective antagonists MDL72222 (Fozard, 1984a) and ICS 205-930 (tropisetron; Richardson \textit{et al.}, 1985). Agonists with some selectivity for this receptor were also discovered, for instance phenlybiguanide and 2-methyl-5-HT (Richardson \textit{et al.}, 1985; see Fozard, 1990; Kilpatrick & Tyers, 1992). The criteria defining this receptor class were: 1. responses should be blocked by selective 5-HT\textsubscript{3} receptor antagonists such as MDL72222 and tropisetron, 2. responses should be resistant to blockade by "5-HT\textsubscript{1}-like" and 5-HT\textsubscript{2} receptor antagonists such as methiothepin and ketanserin and 3. the 5-HT\textsubscript{3} receptor agonist 2-methyl-5-HT should mimic the effects of 5-HT at a similar concentration. The 5-HT\textsubscript{3} receptor is widely distributed throughout the peripheral nervous system and mediates the depolarizing actions of 5-HT (Fozard, 1984b).

More recently, ligand binding studies have shown the presence of 5-HT\textsubscript{3} receptors in brain tissue (Kilpatrick \textit{et al.}, 1987).

Recently, a non classical 5-HT receptor (i.e. outside the classification proposed by Bradley \textit{et al.}, 1986) was discovered and designated 5-HT\textsubscript{4} (Dumuis \textit{et al.}, 1988b). This receptor was found to be positively linked to adenylate cyclase and differs from 5-HT\textsubscript{1}-like receptors which, when activated, inhibit this enzyme.

In addition to the 5-HT receptor subtypes mentioned above, there are several potential receptors characterized either by binding or by functional studies that do not fit in any of the proposed categories. This is the case for the 5-HT\textsubscript{1E} receptor, a high affinity binding site in human cortical membranes at which 5-CT and ergotamine have low affinity (Leonhardt \textit{et al.})
Further, Gershon and colleagues have described the presence of a peripheral $5\text{-HT}_1\text{P}$ receptor which mediates slow excitatory postsynaptic potentials in many enteric neurones. A high affinity binding site for $[{}^{3}\text{H}]5\text{-HT}$ in myenteric plexus membranes correlates with the functional effects of the $5\text{-HT}_1\text{P}$ receptor (Mawe et al., 1986; see Gershon et al., 1989). Other 5-HT receptors which appear to be "orphans" of the current classification scheme have also been described (see Hoyer et al., 1993).

There is evidence for the subdivision of the $5\text{-HT}_2$, $5\text{-HT}_3$ and $5\text{-HT}_1\text{D}$ receptor subtypes and this as well as other aspects of 5-HT receptor classification have been extensively reviewed (for reviews see Zifa & Fillion, 1992; Humphrey et al., 1993; Hoyer et al., 1993). As an alternative to the classification of 5-HT receptors based on binding data or functional studies, Hartig (1989) proposed a simple receptor classification on the basis of structural homology (established by molecular biology) and the transduction mechanism. This classification system identified two receptor superfamilies; the G-protein super-family ($5\text{-HT}_2$, $5\text{-HT}_{1\text{C}}$, $5\text{-HT}_{1\text{A}}$, $5\text{-HT}_{1\text{B}}$, $5\text{-HT}_{1\text{D}}$, $5\text{-HT}_{1\text{E}}$ and $5\text{-HT}_4$) and the ligand-gated ion channel super-family ($5\text{-HT}_3$). The G-protein-coupled receptors have been subdivided on the basis of their second messenger system; the $5\text{-HT}_{1\text{A}}$, $5\text{-HT}_{1\text{B}}$, $5\text{-HT}_{1\text{D}}$, $5\text{-HT}_{1\text{E}}$ and $5\text{-HT}_4$ receptors are associated with adenylate cyclase, whereas the $5\text{-HT}_{1\text{C}}$ and $5\text{-HT}_2$ receptors are coupled to phosphoinositide turnover. Recently a new classification scheme has been proposed by the Serotonin Receptor Nomenclature Committee to extend that of Bradley et al. (1986) and this classification uses three types of criteria to characterize a receptor; operational, structural and transductional. The new nomenclature proposed for 5-HT receptors has been outlined in two reviews (see Humphrey et al., 1993; Hoyer et al., 1993). Four receptor types have been identified in the proposed scheme; $5\text{-HT}_1$, $5\text{-HT}_2$, $5\text{-HT}_3$ and $5\text{-HT}_4$. The $5\text{-HT}_1$ class has been subdivided into $5\text{-HT}_{1\text{A}}$, $5\text{-HT}_{1\text{B}}$, $5\text{-HT}_{1\text{D}}$, $5\text{-HT}_{1\text{E}}$ and $5\text{-HT}_{1\text{F}}$ subtypes,
which have distinct pharmacology but have similar gene structure and are negatively coupled to adenylate cyclase. The 5-HT$_2$ receptor class has three subtypes; 5-HT$_{2A}$ (the classical 5-HT$_2$ receptor), 5-HT$_{2B}$ (rat fundus receptor) and 5-HT$_{2C}$ (previously the 5-HT$_{1C}$ receptor). The subtypes of the 5-HT$_2$ receptor have been cloned and have been shown to have a similar structure and stimulate phospholipase C. The 5-HT$_3$ receptor is a cation channel and is therefore distinct from the other 5-HT receptor subtypes. Although no structural information is available for the 5-HT$_4$ receptor, it is considered to constitute another G-protein coupled receptor. The operational characteristics of this receptor are distinct from the 5-HT$_1$ and 5-HT$_2$ receptors and 5-HT$_4$ receptors (unlike 5-HT$_1$ receptors) are positively coupled to adenylate cyclase.

The pharmacology and characteristics of the main 5-HT receptors will be considered in more detail. Three classes of the 5-HT$_1$ receptor subgroup will be considered, the 5-HT$_{1A}$, 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors. 5-HT$_3$ and 5-HT$_4$ receptors will also be briefly reviewed. The 5-HT$_{1C}$ receptor will be discussed with the 5-HT$_2$ receptor, according to the proposal of the Serotonin Receptor Nomenclature Committee (Humphrey et al., 1993; Hoyer et al., 1993). Under the new scheme 5-HT$_{1C}$ receptors have been reclassified and are now termed 5-HT$_{2C}$ receptors. As this change has been proposed towards the completion of this thesis the old term (5-HT$_{1C}$ receptor) will be used to maintain continuity with published data.

1.4.1 5-HT$_1$ Receptors

5-HT$_{1A}$ Receptors

There are at present several groups of compounds that act as agonists, partial agonists or antagonists at the 5-HT$_{1A}$ receptor. 8-OH-DPAT is the prototypical 5-HT$_{1A}$ agonist with marked potency and selectivity. However, many indoles activate 5-HT$_{1A}$ receptors including, 5-CT, DP-5-CT and
RU24969. These compounds have good potency at this receptor but only DP-5-CT has both the required potency and selectivity (Mir et al., 1987; Schoeffter & Hoyer, 1988; Doods et al., 1988; Van Wijngaarden et al., 1990; see Hoyer et al., 1991). Flesinoxan is also a potent and selective agonist (Wouters et al., 1988a, 1988b; Van Wijngaarden et al., 1990).

Urapidil activates 5-HT\textsubscript{1A} receptors as well as \(\alpha_1\)-adrenoreceptors (Gross et al., 1987; Sanders et al., 1988), however a derivative, 5-methyl-urapidil is more selective. Buspirone, ipsapirone and gepirone (pyrimidinylpiperazines) are active at 5-HT\textsubscript{1A} receptors (Gozlan et al., 1983; Dompert et al., 1985; see Dourish et al., 1986). These compounds have been shown to be partial agonists (Smith & Peroutka, 1986; Segal et al., 1989). Some ergot derivatives have affinity for 5-HT\textsubscript{1A} receptors eg. \(d\)-LSD and metergoline (see Hoyer et al., 1988a), although these compounds are not selective and also interact with other 5-HT receptors, \(\alpha\)-adrenoreceptors and dopamine receptors. The 5-HT\textsubscript{1A} receptor antagonists currently available are not selective and most display partial agonist behaviour in certain test systems. Spiperone (Lum & Piercey, 1988; Schoeffter & Hoyer, 1988; Hoyer, 1991) and spiroxatrine (Nelson & Taylor, 1986; Hoyer, 1991) also bind to 5-HT\textsubscript{2} and dopamine receptors. Cyanopindolol, propranolol and pindolol (Middlemiss et al., 1977; Engel et al., 1986; Fozard et al., 1987; see Hoyer, 1991) bind to 5-HT\textsubscript{1B} receptors and \(\beta\)-adrenoreceptors.

Methiothepin and metergoline will block 5-HT\textsubscript{1A} receptor mediated effects (Fozard et al., 1987) but are non selective antagonists at 5-HT receptors (see Hoyer, 1991). Methiothepin also interacts with histamine receptors and \(\alpha\)-adrenoreceptors. An analogue of 8-OH-DPAT, 8-MeO-CIEPAT is an antagonist at central 5-HT\textsubscript{1A} receptors (Fozard et al., 1987) but displays agonist activity in forskolin-stimulated adenylate cyclase activity assays (Schoeffter & Hoyer, 1988). MDL 73005EF (Moser et al., 1990), BMY 7378 (Yocca et al., 1987; Chaput & de Montigny, 1988; Sharp et al., 1990) and NAN-190 (Glennon et al., 1988a, 1988b) can block behavioural...
and electro-physiological responses mediated by 5-HT$_{1A}$ receptor activation. However, these compounds are partial agonists at 5-HT$_{1A}$ receptors in other test systems (Sprouse, 1991; Yocca et al., 1987; Stubbs et al., 1991; Hjorth & Sharp, 1990). Recently, potent and selective antagonists for 5-HT$_{1A}$ receptors have been reported. These include WAY 100135 (Fletcher et al., 1991), S-14063 (Dabire et al., 1991), SDZ 216-525 (Schoeffter et al., 1993) and (S)-UH-301 (Bjork et al., 1991a, 1991b). These antagonists have only recently been made available and confirmation of their selective antagonist effects, without significant agonist ability is required.

Interestingly, the intrinsic activity and potency of the compounds acting at 5-HT$_{1A}$ receptors depend on the brain region and functional model used (see Zifa & Pillion, 1992) and this difference may be related to the location of the 5-HT$_{1A}$ receptor i.e. pre- or post-synaptic.

The distribution of 5-HT$_{1A}$ binding sites in the CNS has been studied in several species using autoradiography (eg. rat and human; Pazos & Palacios, 1985; Hoyer et al., 1986a; Pazos et al., 1987a) and a similar distribution has been reported in all species tested. 5-HT$_{1A}$ receptors are densely localized in the limbic system (hippocampus, septum, amygdala and the cortical limbic area) and in the dorsal and median raphé nuclei. The 5-HT receptors are located both postsynaptically in the serotonergic projection regions and presynaptically on the soma and dendrites of serotonergic neurones in the raphé nuclei where they function as autoreceptors (somatodendritic autoreceptors). This was demonstrated by the finding that destruction of serotonergic neurones with 5,7-dihydroxytryptamine results in the reduction of 5-HT$_{1A}$ receptor binding in the raphé nuclei (i.e. destruction of somatodendritic receptors; Weissman-Nanopoulos et al., 1985; Verge et al., 1985, 1986) but not in the serotonergic nerve terminals (eg. hippocampal pyramidal cells) (Verge et al., 1986). Tonic activity in
serotonergic dorsal raphé neurones can be inhibited by 5-HT via activation of somatodendritic autoreceptors (see Haigler & Aghajanian, 1977; Wang & Aghajanian, 1978). Selective 5-HT₁A receptor agonists, such as 8-OH-DPAT, ipsapirone and buspirone, suppress the spontaneous firing of serotonergic neurones (Sprouse & Aghajanian, 1986, 1988; VanderMaelen et al., 1986; Sinton & Fallon, 1988). The non-selective 5-HT₁A receptor antagonists, spiperone and (-)propranolol have been shown to block these suppressant effects (Sprouse & Aghajanian, 1986; Lum & Piercey, 1988). These functional studies confirm that the autoreceptors in the raphé nuclei are 5-HT₁A receptors (see Aghajanian et al., 1990).

Further, local administration of 5-HT₁A receptor agonists to the raphé nuclei inhibits the synthesis and release of 5-HT (Hjorth & Magnusson, 1988; Hillegaart et al., 1990) following activation of the somatodendritic autoreceptor. Behavioural studies also provide support for an agonist action at autoreceptors (see Dourish et al., 1986).

The 5-HT₁A receptor has been cloned (Fargin et al., 1988; see Hartig, 1989) and is similar to other G-protein linked receptors, in that it has seven membrane-spanning domains. 5-HT₁A receptors have been demonstrated to be negatively coupled to adenylate cyclase in hippocampal membranes from several species and the inhibition of forskolin stimulated cAMP production in the calf hippocampus has been shown to be correlated to the affinity at central 5-HT₁A binding sites (Schoeffter & Hoyer, 1988). Although it is well established that 5-HT₁A receptors are negatively coupled to adenylate cyclase, it has been demonstrated that 5-HT₁A receptors are positively coupled to adenylate cyclase in some preparations. For instance, 5-HT₁A agonists were also found to activate adenylate cyclase in rat (Markstein et al., 1986) and guinea pig (Shenker et al., 1985; 1987) hippocampus. This controversy is discussed elsewhere (for review see Zifa & Fillion, 1992).
There are several functional correlates for the 5-HT$_{1A}$ receptor including physiological, neuroendocrine and behavioral responses. Activation of central 5-HT$_{1A}$ receptors causes a reduction in blood pressure and sympathetic outflow in many species (see Ramage, 1990). 8-OH-DPAT causes a centrally mediated increase in plasma ACTH (Gilbert et al., 1988), corticosterone (Koenig et al., 1987) and adrenaline (Chaouloff et al., 1990a, 1990b) levels presumably through a central mechanism involving the hypothalamus (see Van de Kar, 1991). Activation of 5-HT$_{1A}$ receptors also induces hypothermia (Goodwin & Green, 1985; Hjorth, 1985), hyperphagia (Dourish et al., 1985) and certain aspects of the '5-HT behavioural syndrome' including hyperlocomotion, flat body posture, and forepaw treading (Hjorth et al., 1982; Tricklebank et al., 1984, 1985). Furthermore, certain partial agonists at 5-HT$_{1A}$ receptors (e.g. buspirone, gepirone and ipsapirone) have been shown to possess anxiolytic properties in animals and humans (see Traber & Glaser, 1987).

5-HT$_{1B}$ Receptors
Several agonists bind to 5-HT$_{1B}$ receptors with high (nM) affinity; these include 5-CT, RU 24969 and TFMPP. However, none of these compounds are selective for 5-HT$_{1B}$ receptors (see Schoeffter & Hoyer, 1988; Van Wijngaarden et al., 1990). An analogue of RU 24969, CP-93,129 is the first potent (rat cortex IC$_{50}$: 15nM) and selective 5-HT$_{1B}$ receptor agonist (200-fold and 150-fold selective for 5-HT$_{1B}$ vs 5-HT$_{1A}$ and 5-HT$_{1D}$ receptors, respectively) yet described (Macor et al., 1990). At present there are no selective 5-HT$_{1B}$ receptor antagonists, however, methiothepin and certain $\beta$-adrenoreceptor antagonists (e.g. cyanopindolol) block 5-HT$_{1B}$ receptors as well as other 5-HT$_{1}$-like receptors. Other compounds have affinity for 5-HT$_{1B}$ receptors (see Zifa & Fillion, 1992) including sumatriptan which has affinity for 5-HT$_{1D}$ and 5-HT$_{1A}$ receptors (Schoeffter & Hoyer, 1989b). 5-HT$_{1B}$ receptors are found in rat and mouse brain and the highest
densities are found in the substantia nigra, globus pallidus, dorsal subiculum and superior colliculi (Pazos & Palacios, 1985; Hoyer et al., 1985b). These receptors are only found in rodents.

The 5-HT$_{1B}$ receptor has been cloned in rats (Voigt et al., 1991) and has seven transmembrane domains. The 5-HT$_{1B}$ receptor has a high degree of homology with a recently described human 5-HT$_{1D}$ receptor, a subtype of the 5-HT$_{1D}$ receptor. Structural evidence suggests that the human 5-HT$_{1D}$ receptor and the rat 5-HT$_{1B}$ receptor are species homologues, although their pharmacological properties are distinct (Adham et al., 1992; see Hartig et al., 1992).

5-HT$_{1B}$ receptors are negatively coupled to adenylate cyclase and 5-HT$_{1B}$ receptor agonists inhibit forskolin-stimulated adenylate cyclase activity in rat substantia nigra (Bouhelal et al., 1988; Schoeffter & Hoyer, 1989a; Macor et al., 1990).

5-HT$_{1B}$ receptors function as presynaptic autoreceptors and control the release of 5-HT from serotonergic terminals (Middlemiss, 1984; Engel et al., 1986). The autoreceptor controlling the release of 5-HT from terminals in rat brain slices containing the suprachiasmatic nucleus has also been characterized as a 5-HT$_{1B}$ receptor, using fast cyclic voltammetry (O'Connor & Kruk, 1992). However, their postsynaptic location as heteroreceptors is also suggested by the lack of effect of serotonergic neuron lesions on the number of 5-HT$_{1B}$ receptors (Verge et al., 1986). On non serotonergic terminals, 5-HT$_{1B}$ receptors have been shown to inhibit the release of other transmitters. For example, 5-HT$_{1B}$ receptors will inhibit the release of ACh in rat hippocampus (Maura & Raiteri, 1986). Furthermore, 5-HT has been reported to inhibit the release of noradrenaline from rat vena cava via presynaptic 5-HT$_{1B}$
receptors on sympathetic terminals (Molderings et al., 1987). 5-HT$_{1B}$ receptors have been shown to mediate potent contraction in rat caudal artery (Craig & Martin, 1993). It has been suggested that the inhibitory role of 5-HT on feeding behaviour in rats is due to activation of 5-HT$_{1B}$ receptors (Kennett et al., 1987). Further evidence for the participation of 5-HT$_{1B}$ receptors in this response was provided by Macor et al. (1990) by demonstrating that feeding behaviour was inhibited in rats by the potent 5-HT$_{1B}$ receptor agonist, CP-93, 129.

5-HT$_{1D}$ Receptors

The 5-HT$_{1D}$ receptor is characterised by its high affinity (<10 nM) for 5-HT, 5-CT and some ergot derivatives e.g. metergoline (see Hoyer, 1991; see Van Wijngaarden et al., 1990). However, to date there are no selective ligands for the 5-HT$_{1D}$ receptors. Sumatriptan (GR43175), a potent and selective agonist for the 5-HT$_{1}$-like receptors present in dog saphenous vein and carotid artery, cat arteriovenous anastomoses and primate and dog basilar artery (Perren et al., 1989; Humphrey et al., 1988, 1989; Connor et al., 1989), does have affinity for 5-HT$_{1D}$ receptors. However, this compound is not selective for 5-HT$_{1D}$ receptors and is also a full agonist at 5-HT$_{1B}$ and 5-HT$_{1A}$ receptors (Schoeffter & Hoyer, 1989b).

5-HT$_{1D}$ receptors are found in the brain of pigeon, hamster, guinea pig, rabbit, dog, pig, calf, monkey and man (Bruinvels et al., 1992; Waehler et al., 1989a, 1989b). In these species 5-HT$_{1B}$ receptors are absent and it has been proposed that 5-HT$_{1D}$ receptors play a similar role in these species to 5-HT$_{1B}$ receptors in rodents. This proposal was based on the similarity in pharmacological properties, transduction mechanisms, regional distribution and function of these receptor subtypes (see Hoyer & Middlemiss, 1989). 5-HT$_{1D}$ receptors are widely distributed within the brain and highest densities are found in the substantia nigra and basal ganglia. Lower
concentrations are found in the hippocampus, cortex and raphé (Waeber et al., 1988a, 1989a; Hoyer et al., 1990; Palacios et al., 1992).

There is pharmacological and molecular biological evidence to suggest that 5-HT_{1D} receptors are heterogeneous. Two distinct human 5-HT_{1D} receptors have been cloned to date; these have been termed 5-HT_{1Dα} and 5-HT_{1Dβ}. The receptors have surprisingly similar pharmacological properties even though they are structurally dissimilar. Furthermore, the human 5-HT_{1Dβ} receptor and the rat 5-HT_{1B} receptor share a similar structure yet their pharmacological properties are distinct. These findings have been reviewed in detail elsewhere (see Hartig et al., 1992).

5-HT_{1D} receptors are negatively coupled to adenylate cyclase in calf substantia nigra and inhibit forskolin stimulated adenylate cyclase activity (Hoyer & Schoeffter, 1988; Schoeffter et al., 1988).

5-HT_{1D} receptors are located on 5-HT terminals where they function as autoreceptors and inhibit the release of 5-HT. This has been demonstrated in guinea pigs (Middlemiss et al., 1988) and pigs (Schlicker et al., 1989). It is difficult to evaluate physiological and behavioural correlates of the 5-HT_{1D} receptor due to the lack of selective agonists and antagonists. However, it is interesting to note that sumatriptan is effective in the treatment of migraine (Doenicke et al., 1988; Perrin et al., 1989). The antimigraine mechanism of action of sumatriptan is presently unknown but may be due to its ability to selectively constrict cerebral blood vessels. Alternatively, or possibly in addition to a selective craniovascular action, sumatriptan may abort headache by inhibiting neuropeptide release and blocking activation of perivascular sensory afferent terminals of the trigeminal nerve (Moskowitz, 1992).
1.4.2 5-HT\textsubscript{2} and 5-HT\textsubscript{1C} Receptors

5-HT\textsubscript{2} receptors have been defined as having high affinity for serotonergic antagonists (e.g. ketanserin, spiperone) and relatively low affinity for 5-HT (Peroutka and Snyder, 1979). Responses mediated by 5-HT\textsubscript{2} receptors are characterized by: 1. the susceptibility to antagonism by ketanserin, methysergide and other archetypal 'D' receptor antagonists, 2. resistance to antagonism by selective 5-HT\textsubscript{1}-like and 5-HT\textsubscript{3} receptor antagonists (Bradley \textit{et al.}, 1986).

5-HT\textsubscript{1C} receptors have been identified in the choroid plexus on the basis of high affinity displacement of \[^{3}H\] 5-HT by mesulergine (Pazos \textit{et al.}, 1984; Hoyer \textit{et al.}, 1985a; see Hoyer, 1988b) and were placed in the 5-HT\textsubscript{1} receptor category due to their high affinity for 5-HT. In general, compounds claimed to be selective for 5-HT\textsubscript{2} receptors also bind to 5-HT\textsubscript{1C} receptors with appreciable affinity (see Hoyer, 1991; Van Wijngaarden \textit{et al.}, 1990).

The 5-HT\textsubscript{2} receptor class is now considered to be heterogeneous and 3 distinct subtypes have been described. The 5-HT\textsubscript{2} receptor described by Bradley \textit{et al.} (1986) (see above) is now termed the 5-HT\textsubscript{2A} receptor. The 5-HT receptor mediating contraction of the rat fundus (previously described as a 5-HT\textsubscript{1}-like receptor; Bradley \textit{et al.}, 1986) has recently been cloned (Foguet \textit{et al.}, 1992) and allocated a 5-HT\textsubscript{2B} appellation (Humphrey \textit{et al.}, 1993). The 5-HT\textsubscript{1C} receptor is now considered to be the third 5-HT\textsubscript{2} receptor subtype (5-HT\textsubscript{2C}). As there are no agonists or antagonists which can be described as selective for either receptor subtype, it is difficult, with the tools presently available, to distinguish between 5-HT\textsubscript{2} and 5-HT\textsubscript{1C} receptor mediated responses. For this reason these receptors will be described as 5-HT\textsubscript{2}/5-HT\textsubscript{1C} in this thesis.
Chapter 1

The first potent 5-HT$_2$ receptor antagonist to be used was ketanserin. However, this compound, whilst displaying a high affinity for 5-HT$_2$ receptors, also has moderate affinity for 5-HT$_1C$ receptors and is not selective with respect to other neurotransmitters; it has sub-micromolar affinity for histamine and $\alpha_1$-adrenoreceptors (Leysen et al., 1981; 1982; Van Nueten et al., 1981). Several 5-HT$_2$/5-HT$_1C$ receptor antagonists have been described of which LY 53857, cinanserin and ritanserin have been particularly well characterised (Cohen et al., 1983a; 1985; Rubin et al., 1964; Leysen et al., 1985; see Hoyer, 1991). BW501C67 and xylamidine are also useful 5-HT$_2$/5-HT$_1C$ receptor probes, since they block peripheral serotonergic responses but are ionised at physiological pH and hence cannot cross the blood-brain barrier to affect central 5-HT receptors (Mawson & Wittington, 1970; Fuller et al., 1986).

Agonists that have been reported as selective for 5-HT$_2$ receptors include $\alpha$-methyl-5-HT (Feniuk et al., 1985; Dalton et al., 1986) and the hallucinogenic agents DOI, DOB and DOM (Shannon et al., 1984; Glennon et al., 1986, 1988c; Titeler et al., 1985, 1987). However, these compounds, whilst having high affinity for 5-HT$_2$ also have high affinity at 5-HT$_1C$ sites (Van Wijngaarden et al., 1990; see Hoyer, 1991). $\alpha$-methyl-5-HT also has affinity for 5-HT$_1$ receptors and may act as a mixed 5-HT$_1$/5-HT$_2$ agonist (Ismaiel et al., 1990). Quipazine has also been used to stimulate 5-HT$_2$ receptors, but its use is limited by agonist activity 5-HT$_3$ receptors (Vayssettes-Courchay et al., 1990). m-CPP is an agonist at 5-HT$_1C$ receptors as well as having agonist activity at 5-HT$_2$ and 5-HT$_1B$ receptors (Hoyer, 1988b). Selective 5-HT$_1C$ or 5-HT$_2$ receptor agonists are not presently available.

Both 5-HT$_1C$ (Conn et al., 1986; Hoyer et al., 1989b) and 5-HT$_2$ (Conn & Sanders-Bush, 1984, 1985; de Chaffoy de Courcelles et al., 1985)
receptors have been shown to cause an increase in phosphoinositide turnover resulting in an increase in intracellular calcium. Coupling to the same second messenger system is a further similarity between 5-HT\textsubscript{1C} and 5-HT\textsubscript{2} receptors.

The 5-HT\textsubscript{1C} receptor was the first 5-HT receptor to be cloned and functionally expressed (Julius \textit{et al.}, 1988). The amino acid sequence of this receptor reveals that it belongs to the family of G-protein-coupled receptors that are characterized by seven transmembrane domains. The similarities between 5-HT\textsubscript{1C} and 5-HT\textsubscript{2} sites led Pritchett \textit{et al.} (1988) to use oligonucleotides derived from the 5-HT\textsubscript{1C} receptor sequence to clone the rat 5-HT\textsubscript{2} receptor. The 5-HT\textsubscript{2} receptor is homologous (78\% in transmembrane domains) to the 5-HT\textsubscript{1C} receptor and is a member of the G-protein-super family. The human 5-HT\textsubscript{2} receptor has also been cloned and shares a high sequence homology (90\%) with the rat receptor (Kao \textit{et al.}, 1989). The molecular biology of 5-HT\textsubscript{2} and 5-HT\textsubscript{1C} receptors has been reviewed in more detail elsewhere (see Hartig \textit{et al.}, 1989).

A heterogeneous distribution of 5-HT\textsubscript{2} receptor has been reported in rat (Pazos \textit{et al.}, 1985) and human (Hoyer \textit{et al.}, 1986b; Pazos \textit{et al.}, 1987b) brain. 5-HT\textsubscript{2} receptors are found in high concentrations in the cortex and the corpus mammillare of the hypothalamus. In the basal ganglia, the caudate, putamen and nucleus accumbens are labelled with an intermediate density. Intermediate levels are also reported in the hippocampus. A low density of 5-HT\textsubscript{2} receptors occurs in midbrain areas. By contrast, 5-HT\textsubscript{1C} receptors have been demonstrated in the choroid plexus of several species including rat, pig and man (Pazos \textit{et al.}, 1984; Yagaloff & Hartig, 1986; Hoyer \textit{et al.}, 1986b; Hoyer, 1988b). Autoradiographic studies revealed that 5-HT\textsubscript{1C} receptors are present at low levels in other brain areas including
substantia nigra, globus pallidus, cerebral cortex and olfactory tubercles (Pazos and Palacios, 1985; Pazos et al., 1987a).

The availability of 5-HT\(_2\) receptor antagonists has enabled a comprehensive investigation into the functional effects of this receptor subtype. 5-HT\(_2\) receptors have been demonstrated on peripheral vasculature and mediate the constrictor actions of 5-HT in a wide range of arteries and veins in many species (see Martin, 1993 for a comprehensive review). In conscious and anaesthetized rats, 5-HT causes a transient pressor response which is blocked by 5-HT\(_2\) receptor antagonists (Dalton et al., 1986; Dabire et al., 1988). A similar profile is reported in anaesthetized cats (Connor et al., 1986), but in anaesthetized dogs the pressor response appears to be indirect following the release of catecholamines from the adrenal medulla (Feniuk et al., 1981). In conscious rats quipazine and DOI have been reported to cause an increase in blood pressure due to a direct effect on vascular smooth muscle and an indirect action resulting from the release of renin (Alper & Snider, 1987; Alper, 1990). The release of renin may include an action at both peripheral and central 5-HT\(_2\) receptors (Rittenhouse et al., 1991). Central 5-HT\(_2\) receptors are also thought to regulate the release of vasopressin (see Van de Kar, 1991), although this response may be mediated by 5-HT\(_1\)C receptors (Bagdy et al., 1992). 5-HT\(_2\) receptors have also been implicated in the release of \(\beta\)-endorphin, corticosterone and prolactin (see Van de Kar, 1991). Other functional correlates for the 5-HT\(_2\) receptor include 5-HT induced platelet aggregation and contraction of bronchial and some gastrointestinal tissues (see Bradley et al., 1986).

5-HT\(_2\) receptors have been reported to cause behavioural changes including head twitch in rodents, 'wet dog shake', sexual activity and sleep (for review see Zifa & Fillion, 1992). In addition they may play an important role in the mechanism of action of hallucinogenic drugs (Glennon et al.,
1985). However, this action is poorly understood. Due to the location of 5-HT\textsubscript{1C} receptors in the choroid plexus it has been proposed that these receptors are involved in the production of cerebrospinal fluid (Pazos & Palacios, 1985).

1.4.3 5-HT\textsubscript{3} Receptors

5-HT\textsubscript{3} receptors represent the third class of receptors defined by Bradley et al. (1986). These receptors occur on central and peripheral neurones and correspond to the 'M' receptor defined by Gaddum & Picarelli (1957). A response is defined as being mediated through 5-HT\textsubscript{3} receptors if it is 1. susceptible to antagonism by selective antagonists such as MDL72222 (Fozard, 1984a) and tropisetron (Richardson et al., 1985), 2. resistant to antagonism by selective 5-HT\textsubscript{1} and 5-HT\textsubscript{2} receptor antagonists (e.g. methiothepin and ketanserin) and 3. should be mimicked by 2-methyl-5-HT (see Bradley et al., 1986). In addition to MDL72222 and tropisetron, several other highly selective 5-HT\textsubscript{3} receptor antagonists have been described: ondansetron (GR38032F; Butler et al., 1988) and granisetron (BRL 43694; Nelson & Thomas, 1989; Sanger & Nelson, 1989).

No truly selective 5-HT\textsubscript{3} receptor agonists have yet been described. 2-Methyl-5-HT, phenylbiguanide and quipazine have been reported to be agonists (Richardson et al., 1985; Fozard, 1990; Peroutka & Hamick, 1988), but these display only moderate affinity and poor selectivity for this receptor class (Van Wijngaarden et al., 1990; see Kilpatrick & Tyers, 1992).

5-HT\textsubscript{3} receptors are presently considered to be homogenous, although the reported differences in affinities between tissues for these drugs may imply species differences or the existence of 5-HT\textsubscript{3} receptor subtypes (see Kilpatrick & Tyers, 1992).
The 5-HT$_3$ receptor is a ligand gated ion channel, which causes fast depolarizing responses (Peters et al., 1992). This receptor has been cloned and analysis of the sequence showed that this receptor is a new member of the ligand-gated ion channel super family (see Hartig et al., 1989). Activation of the 5-HT$_3$ receptor leads to the opening and closure of an ion channel that is nonselective for monovalent cations (K$^+$ and Na$^+$; Derkach et al., 1989).

5-HT$_3$ receptors have been located in the periphery and in specific central areas of many species including man. The highest density of central 5-HT$_3$ binding sites is found in specific hindbrain regions, followed by intermediate levels in cortical areas (especially the entorhinal cortex), amygdala and hippocampus (Kilpatrick et al., 1987, 1988, 1989; Waeger et al., 1989b; Hoyer et al., 1989a; Barnes et al., 1989, 1990a, 1990b; Pratt et al., 1990). 5-HT$_3$ receptors have been demonstrated in high concentrations in the hindbrain of mouse, rat, cat and man; specifically in the nucleus tractus solitarius (NTS) and in lower concentrations, in the dorsal motor vagal nucleus, the area postrema and the nucleus of the trigeminal nerve (Pratt et al., 1990). In the periphery 5-HT$_3$ receptors are found in the enteric nervous system and on parasympathetic, sympathetic and sensory nerves where activation elicits an excitatory response (Fozard, 1984b). 5-HT$_3$ receptors mediate a number of responses in isolated tissues, e.g. contraction of the guinea pig ileum, depolarization of the vagus nerve, nodose ganglion and superior cervical ganglion and noradrenaline release from the rabbit heart. 5-HT$_3$ receptors also mediate the Von-Bezold-Jarisch reflex and cause contraction of the urinary bladder in vivo (for reviews see Bradley et al., 1986; Fozard, 1990; Kilpaterick & Tyers, 1992). In the CNS, activation of 5-HT$_3$ receptors in the NTS have been shown to cause a rise in blood pressure (see below). 5-HT$_3$ receptors located in the area postrema are thought to be involved in the emetic mechanism and 5-HT$_3$ receptor
antagonists have been shown to protect against nausea and vomiting caused by cancer chemotherapy in animal models and man (e.g. Costall et al., 1986; Miner & Sanger, 1986; Cunningham et al., 1987; Stables et al., 1987). The mechanism of action of these compounds is unknown and may involve a peripheral action. 5-HT₃ receptor antagonists have also been proposed to possess potential anxiolytic and antipsychotic activity, reduce withdrawal signs after chronic treatment with substances of abuse and improve performance in cognition tests (see Costall et al., 1990; Kilpatrick & Tyers, 1992).

1.4.4 5-HT₄ Receptors

Recently, an additional 5-HT receptor was discovered and designated 5-HT₄ (see Clarke et al., 1989). This receptor was found to be positively linked to adenylate cyclase in both embryo colliculi neurones in primary culture and in adult guinea pig hippocampal membranes (Dumuis et al., 1988b; Bockaert et al., 1990). The 5-HT₄ receptor has yet to be cloned.

The gastrointestinal prokinetic benzamide derivatives (e.g. cisapride, renzapride, zacopride, metaclopramide) were found to be agonists at this receptor (Dumuis et al., 1989a) and the 5-HT₃ receptor antagonist, tropisetron subsequently shown to block 5-HT₄ receptor mediated effects at high concentrations (∼3 or 4 orders of magnitude higher than required to block 5-HT₃ receptors: Dumuis et al., 1988a). A neuronal 5-HT receptor on guinea pig ileum with properties similar to 5-HT₄ receptors has been described. The indirect contraction of guinea pig ileum caused by 5-HT was shown to be biphasic and the initial phase of response (high sensitivity to 5-HT) was shown to be mediated by 5-HT₄ receptors (Craig & Clarke, 1990). 5-HT₄ receptors have subsequently been demonstrated in other peripheral systems such as the guinea pig colon (Elswood et al., 1990), the rat oesophagus (Reeves et al., 1991), the sheep pulmonary vein (Cocks &
Arnold, 1992) and in the human (Kaumann et al., 1990, 1991) and pig heart (Villalon et al., 1991). More selective antagonists have recently been reported for this receptor (see Bockaert et al., 1992), of which GR113808 appears to be among the most potent and selective (Grossman et al., 1993). GR113808 is the first 5-HT$_4$ ligand to be radiolabelled and has been shown to label a single site in homogenates of guinea-pig striatum and hippocampus with high affinity ($K_D$ 0.1-0.2 nM). This enabled the distribution of central 5-HT$_4$ receptors to be investigated and autoradiographic studies, in rat and guinea-pig revealed a high concentration of 5-HT$_4$ binding sites in the striatum, substantia nigra and olfactory tubercle (Grossman et al., 1993).

1.5 Peripheral cardiovascular actions of 5-HT

Intravenous administration of 5-HT to conscious (Dalton et al., 1986) and anaesthetized (Salmoiraghi et al., 1956; Kalkman et al., 1984; Saxena & Lawang, 1985) rats causes a complex triphasic effect on blood pressure; an initial depressor response is followed by a pressor response and then a further hypotensive phase. The initial phase of response is caused by activation of the Von-Bezold-Jarisch reflex. 5-HT stimulates 5-HT$_3$ receptors on sensory afferent neurones in the heart to cause this vagovagal reflex, resulting in a marked transient bradycardia and sympathoinhibition. The combination of these effects leads to the transient fall in blood pressure. This reflex can be elicited with 5-HT$_3$ receptor agonists such as phenylbiguanide (e.g. Bogle et al., 1990), 2-methyl-5-HT (Richards et al., 1985; Dalton et al., 1986) and quipazine (Vayssettes-Courchay et al., 1990). The effect is blocked by 5-HT$_3$ receptor antagonists (Fozard, 1984; Dalton et al., 1986). The secondary hypertensive phase of the response is caused by 5-HT$_2$ receptor activation mediating direct vasoconstriction. This phase of response is mimicked by α-methyl-5-HT and is blocked by ketanserin (e.g. Dalton et al., 1986). The ability of 5-HT$_2$ receptor
antagonists to inhibit completely the hypertensive effects of 5-HT in pithed rats indicates that this response is peripherally mediated (e.g. Feniuk et al., 1982; Dabire et al., 1988). The third hypotensive phase of the 5-HT response is mimicked by 5-CT and antagonized by methiothepin. This phase of response is mediated by vascular 5-HT<sub>1</sub> receptors which cause direct vasodilatation (see Dalton et al., 1986; Saxena & Lawang, 1985; Martin et al., 1987). This effect of 5-CT is also observed in pithed rats indicating a predominantly peripheral effect (Dabire et al., 1988).

5-HT has been shown to increase heart rate in the absence of the Von-Bezold-Jarisch reflex, but the receptors mediating this effect are species dependent. For example, 5-HT and 5-CT cause tachycardia in anaesthetized cats through an action at 5-HT<sub>1</sub> receptors (Connor et al., 1986; Saxena et al., 1985), whereas in anaesthetized rats and dogs tachycardia is mediated by 5-HT<sub>2</sub> receptors (Saxena & Lawang, 1985; Feniuk et al., 1981) and in rabbits it is mediated by 5-HT<sub>3</sub> receptors (Fozard, 1984). The peripheral vascular actions of 5-HT receptors have been recently reviewed (see Martin, 1993).

1.6 Central cardiovascular actions of 5-HT

It has been proposed that the central serotonergic neuronal system can regulate cardiovascular function (see Kuhn et al., 1980a). Fluorescence-histochemical and immunohistochemical studies have shown that 5-HT-containing neurones (the cell bodies of which are located within the raphé nuclei) project to forebrain structures as well as to regions in the midbrain, hindbrain and intermediolateral cell column (Dalstrom & Fuxe, 1965; Steinbush, 1981; Loewy & Neil, 1981; Schaffar et al., 1988), which are involved in cardiovascular regulation. Furthermore, autoradiographic mapping studies have shown that 5-HT receptors are also located in brain regions associated with cardiovascular control (Pazos et al., 1985a; Pazos
Activation of these central 5-HT receptors has been attempted using three main approaches. These include:-

1. Stimulation of the raphé nuclei to enhance ascending and descending serotonergic transmission leading to the release of endogenous 5-HT.
2. Peripheral and central administration of L-tryptophan or 5-hydroxytryptophan (5-HP; precursors of 5-HT) to increase brain concentrations of 5-HT.
3. Administration of 5-HT to the CNS. As 5-HT does not readily cross the blood brain barrier (Lexchin et al., 1977), 5-HT must be given directly into the brain. This has been achieved by microinjecting 5-HT into specific brain nuclei and by administration into the cerebral ventricles (i.c.v.).

The cardiovascular effects following the enhancement of central 5-HT levels through peripheral or central administration of the precursors of 5-HT, namely L-tryptophan and 5-HP have been previously investigated (see Kuhn et al., 1980a. Although variable changes in blood pressure have been observed it is difficult to ascribe these effects solely to an indirect action (via the formation of 5-HT) at central 5-HT receptors as both peripheral and central 5-HT levels will be increased. Furthermore, in many instances, precursors were administered in the presence of monoamine oxidase inhibitors and in this situation both 5-HT and catecholamines will accumulate. Furthermore, the actions of 5-HP require more cautious interpretation as 5-HP can be converted to 5-HT in non-serotonergic systems due to the ubiquitous nature of L-aromatic amino acid decarboxylase. For a comprehensive review of the effects of precursors of 5-HT and the problems associated with this approach see Kuhn et al. (1980a).

Central administration of 5-HT, whether administered to the cerebral ventricles or to discrete brain nuclei, has been shown to produce complex
and variable effects on blood pressure, heart rate and sympathetic outflow 
(see Kuhn et al., 1980a; see Coote, 1990). The complexity of the response 
may depend on the dose of 5-HT, the species tested, the site of central 
administration and whether the animal model was conscious or 
aestheticized. Preferential activation of the various receptor subtypes for 
5-HT may also contribute to the variation in response observed.

1.6.1 Intracerebroventricular administration of 5-HT

Intracerebroventricular (i.c.v.) injections of 5-HT to the lateral cerebral 
ventricle in urethane-anaesthetized rats caused a rise in blood pressure 
associated with variable (Krstic & Djurkovic 1976, 1980) or biphasic 
demonstrated that i.c.v. 5-HT induced a pressor response which was 
associated with a consistent bradycardia and splanchnic sympathoinhibition 
in the urethane anaesthetized rat. In conscious rats i.c.v. administration of 
5-HT also caused a pressor effect but in these animals consistently 
produced a bradycardia (Sukamoto et al., 1984; Dalton, 1986; Pergola & 
Alper, 1991). These changes would appeared to be mediated following 
activation of 5-HT receptors since pretreatment (i.c.v.) with the non-
selective 5-HT receptor antagonists methysergide or 2-bromolysergic acid 
diethylamide (BOL) have been shown to prevent the pressor action of 5-HT 
(Lambert et al., 1978; Sukamoto et al., 1984; Inoue & Buñag, 1989). The 
predominant response following i.c.v. administration of 5-HT to the lateral 
ventricle of the rat is a pressor response. However, in certain studies 
prolonged falls in blood pressure associated with bradycardia followed the 
hypertensive phase (Krstic & Djorkovic, 1981; Dalton, 1986). Interestingly, 
these changes in blood pressure appeared to be dose related; low (15-40 
nmol kg⁻¹ i.c.v.) doses of 5-HT appear to favour a pressor response 
whereas high doses of 5-HT (240-1600 nmol kg⁻¹) tended to cause falls in 
blood pressure (doses from Krstic & Djorkovic (1981) and Dalton (1986);
expressed as nmol kg\(^{-1}\) for comparison with the present study). Furthermore, the change in heart rate caused by i.c.v. 5-HT has been demonstrated to be dose related; low doses of 5-HT (1-12 nmol kg\(^{-1}\)) caused tachycardia whereas high doses (40-400 nmol kg\(^{-1}\)) caused bradycardia (Dedeoglu & Fisher, 1991a).

The cardiovascular actions of 5-HT administered i.c.v. also appear to be species dependent. In the anaesthetized dog (Bhargava & Tangri, 1959; McCubbin et al., 1960; Dhawan et al., 1967) and cat (Baum & Shropshire, 1975; Coote et al., 1987) 5-HT administered to the lateral ventricle caused a reduction in blood pressure and heart rate. In the cat and dog the depressor response appeared to result from the inhibition of sympathetic outflow (Bhargava & Tangri, 1959; Baum & Shropshire, 1975; Coote et al., 1987). However, the differential cardiovascular effects of 5-HT observed between species i.e. a pressor response in rats and a depressor response in cats and dogs may be a consequence of dose. Higher doses of 5-HT may diffuse to brain regions inaccessible to lower doses (see below; Coote et al., 1987).

The central and peripheral mechanisms responsible for the pressor response caused by i.c.v. administration of 5-HT in the rat are unclear. It has been reported that the rise in blood pressure produced by 5-HT was attenuated by cervical transection of the spinal cord, adrenalectomy, adrenergic blocking agents and \(\alpha\)-adrenoreceptor antagonists (Krstic & Djurkovic, 1980), suggesting that there was a sympathoexcitatory component to the response of 5-HT. However, it was demonstrated that the rise in blood pressure was associated with a reduction in splanchnic sympathetic nerve activity (Inoue & Buñag, 1989). Furthermore, hexamethonium, at doses shown to block autonomic ganglia, enhanced the 5-HT-induced rise in blood pressure in the anaesthetized rat (Lambert et al., 1978; Krstic & Djurkovic,
1980); similar results were found in the conscious rat pretreated with chlorisondamine (Pergola & Alper, 1991). Taken together this data suggests that sympathoexcitation, although important, is not the sole factor responsible for the 5-HT induced pressor response. Indeed, Inoue & Buñag (1989) were able to demonstrate that the pressor response caused by 5-HT was attenuated by pretreatment with a vasopressin $V_1$-receptor antagonist. More recently, it was demonstrated that the 5-HT pressor response could be abolished by pretreatment with combined $\alpha_1$-adrenoreceptor and $V_1$-receptor blockade. Neither antagonist alone abolished the response and it was concluded that vasopressin and autonomic mechanisms mediated the cardiovascular actions of central 5-HT in the rat (Pergola & Alper, 1991).

The site of action of the centrally mediated effects of 5-HT has been the subject of a recent review (Coote, 1990). It is difficult to locate the specific central site(s) involved in the cardiovascular effect following administration of 5-HT to the ventricles, since the drug is widely distributed throughout the brain using this procedure. However, certain studies in which 5-HT has been confined to more specific brain regions have suggested that forebrain structures are responsible for the 5-HT induced pressor response and hindbrain regions involved in the depressor response. In the anaesthetized rat the pressor response induced by administration of 5-HT is greater in the third than in the lateral ventricle and the pressor response is absent when 5-HT is administered to the fourth ventricle (Lambert et al., 1978). This would suggest that the 5-HT receptors causing the pressor effect in the rat are located close to the third ventricle. Moreover, Coote et al. (1987) demonstrated that administration of low doses of 5-HT (3 and 10 $\mu$g kg$^{-1}$) to the lateral ventricle of the anaesthetized cat caused an increase in blood pressure and heart rate whereas higher doses (30 and 100 $\mu$g kg$^{-1}$) caused falls in blood pressure, heart rate and renal sympathetic nerve activity. When the dose of 5-HT
was restricted to the forebrain of the animal following cannulation of the Aqueduct of Silvius (the aqueduct connecting the third and fourth ventricles) only increases in these variables were observed. This would suggest that forebrain structures are responsible for the pressor response in the cat. Hindbrain structures appear to be responsible for the reduction in blood pressure, heart rate and sympathetic nerve activity caused by lateral ventricular administration of 5-HT since this response was absent when the aqueduct of Silvius was cannulated preventing the penetration of 5-HT to the fourth ventricle and hindbrain. Furthermore, fourth ventricular administration of 5-HT caused a reduction in blood pressure, heart rate and sympathetic nerve activity in the anaesthetized cat (Coote et al., 1987; Shepheard et al., 1990a). The precise location of the receptors mediating the inhibitory effects of 5-HT is uncertain, although an action at the ventrolateral medulla has been suggested (see Coote, 1990). In previous experiments relatively high doses of 5-HT were administered to the lateral ventricle of the anaesthetized cat (Baum & Shropshire, 1975) and dog (Bhargava & Tangri, 1959; McCubbin et al., 1960; Dhawan et al., 1967). Therefore, it is possible that 5-HT following forebrain administration accessed the hindbrain of these animals, resulting in a depressor response and the apparent differences between these species and the rat could be a consequence of dose of 5-HT. The finding that the administration of high doses of 5-HT to the anaesthetized rat caused reductions in blood pressure further supports this opinion.

The microinjection of 5-HT into specific brain nuclei has enabled a more detailed understanding of the central sites mediating the excitatory and inhibitory effects of 5-HT. Several brain regions are thought to be involved in the cardiovascular response caused by 5-HT and these areas will be discussed below.
1.6.2 Evidence for a forebrain 5-HT pressor pathway

Smits & Struyker-Boudier (1976) demonstrated that local administration of 5-HT to a region of the hypothalamus termed the anterior hypothalamus/preoptic area caused a dose related increase in blood pressure associated with variable effects on heart rate in the urethane anaesthetized rat. The pressor response was abolished in rats pretreated (i.c.v.) with the mixed 5-HT₁ and 5-HT₂ receptor antagonist, methysergide (Smits & Struyker-Boudier, 1976) and following direct injection of the non selective 5-HT receptor antagonist metergoline into the anterior hypothalamus/preoptic area (Robinson, 1984). More recently, administration of 5-HT to the anterior hypothalamus/preoptic area or to the medial hypothalamus of the conscious rat was shown to produce a similar pressor response associated with either a marked bradycardia or tachycardia (Sukamoto et al., 1984).

Serotonergic nerve terminals have been found in the hypothalamus (Fuxe, 1965; Steinbusch, 1981) and the distribution of 5-HT within the hypothalamic nuclei has been investigated (Saavedra et al., 1974). The anterior hypothalamus/preoptic area accommodates numerous 5-HT containing terminals and high concentrations of 5-HT have been observed in this region. 5-HT₁ and 5-HT₂ receptors have been demonstrated in the hypothalamus (Pazos et al., 1985; Pazos & Palacios, 1985). The main serotonergic innervation to the hypothalamus originates from the dorsal and median raphé nuclei (Dalstrum & Fuxe, 1965; see Azmitia, 1978; Azmitia & Segal, 1978).

Electrical stimulation of the dorsal or median raphé nuclei has previously been shown to cause an increase in blood pressure accompanied with variable heart rate changes in the anaesthetized rat (Smits et al., 1978; Kuhn et al., 1980b; Robinson et al., 1985). Similarly, electrical and
chemical (DL-homocysteic acid) stimulation of the dorsal raphé nucleus of the anaesthetized cat caused an increase in blood pressure (Piper & Goadsby, 1985). More recently, chemical (DL-homocysteic acid) stimulation of the dorsal raphé nucleus of the anaesthetized rat produced a pressor response (Lovick, 1992) of similar magnitude to that elicited by electrical stimulation. This data suggests that electrical stimulation of the dorsal raphé nucleus was producing a response through activation of neuronal cell bodies and not axons of passage. In the anaesthetized rat, selective lesion of the 5-HT-containing neurones of the dorsal raphé nucleus using the neurotoxin 5,7-DHT attenuated the pressor response following electrical stimulation of this site. The pressor response elicited by stimulation of the median raphé was not affected by 5,7-DHT lesion of this nucleus (Robinson et al., 1985).

Moreover, administration (i.v.) of the 5-HT uptake inhibitor fluoxetine enhanced the pressor response to dorsal raphé stimulation without affecting the response to median raphé stimulation (Kuhn et al., 1980b). This suggests that the pressor response caused by stimulation of the dorsal raphé but not the median raphé was dependent, at least in part, on serotonergic neurones. Furthermore, the pressor response caused by stimulation of the dorsal raphé nucleus appeared to be mediated by an increase in sympathetic outflow since it was abolished by pretreatment with the noradrenergic neurone blocking agent bretylium (Kuhn et al., 1980b), intravenous phentolamine and transection of the spinal cord at the C1/C2 level (Piper & Goadsby, 1985).

A link between the dorsal raphé nucleus and the anterior hypothalamus/preoptic area was initially suggested by Smits et al. (1978). Administration of the non-selective 5-HT antagonists 2-bromolysergic acid diethylamide (BOL; Kuhn et al., 1980b) and metergoline (Robinson, 1984) into the anterior hypothalamus/preoptic area attenuated the pressor response caused following stimulation of the dorsal raphé nucleus.
Furthermore, lesion of the dorsal raphé but not the median raphé nucleus enhanced the pressor response to microinjection of 5-HT into the anterior hypothalamus/preoptic area, presumably due to the development of supersensitivity of 5-HT receptors located in the anterior hypothalamus/preoptic area following denervation or through the reduced uptake of 5-HT following the degeneration of the terminals leading to a decreased rate of termination of the neurotransmitter (see Robinson et al., 1985). The lesion of these nuclei did not cause a change in baseline cardiovascular variables per se. These observations indicated that activation of ascending 5-HT neurones from the dorsal raphé nucleus produced an increase in blood pressure which was dependent on the release of 5-HT and activation of 5-HT receptors located in the anterior hypothalamus/preoptic area. Furthermore, this pathway did not appear to be tonically active (Robinson et al., 1985).

An interaction between hypothalamic 5-HT and acetylcholine (ACh) has been demonstrated (see Robinson, 1984). In the anaesthetized rat, injection of the muscarinic antagonist atropine into the posterior hypothalamus abolished the pressor response caused either by the administration of 5-HT into the anterior hypothalamus/preoptic area or by electrical stimulation of the dorsal raphé nucleus. Depletion of ACh in the posterior hypothalamus following local or i.c.v. application of hemicholinium caused a similar blockade of these responses (Robinson, 1982). This data would suggest that stimulation of ascending fibres from the dorsal raphé nucleus caused the activation of 5-HT receptors located in the anterior hypothalamus/preoptic area which in turn led to the elevation of blood pressure by means of a cholinergic mechanism in the posterior hypothalamus.
The anterior hypothalamus/preoptic area may play an important role in the pressor response caused following i.c.v. administration of 5-HT (see Coote, 1990). Due to the close proximity of the anterior hypothalamus/preoptic area to the third ventricle, the neurones involved in the pressor response may be accessible following i.c.v. injection of 5-HT. In this respect, the response caused by 5-HT was greater following injection into the third ventricle compared to lateral ventricular administration suggesting that the site of action was close to the third ventricle (Lambert et al., 1978).

Furthermore, the response to 5-HT, whether administered i.c.v. or directly into the anterior hypothalamus/preoptic area was abolished following i.c.v. pretreatment with methysergide (Sukamoto et al., 1984; Inoue & Buñag, 1989; Smits & Struyker-Boudier, 1976). Interestingly, the pressor response caused by i.c.v. administration of 5-HT was attenuated following pretreatment (i.c.v.) with the cholinesterase inhibitor physostigmine, suggesting a cholinegic link in the response to 5-HT when administered into the lateral ventricle (Krstic & Djurkovic, 1987), a further similarity with the anterior hypothalamus/preoptic area pressor mechanism (see above).

Lastly, the rise in blood pressure caused by either i.c.v. administration 5-HT or following activation of the anterior hypothalamus/preoptic area were mediated through sympathoexcitation. This circumstantial evidence supports the idea that i.c.v. administered 5-HT elicits a pressor response following activation of 5-HT receptors located in the anterior hypothalamus/preoptic area. Therefore, it would appear that forebrain administration of 5-HT via the lateral cerebral ventricles is a convenient method of studying this effect.

The subtype of the 5-HT receptor(s) mediating the pressor response produced following i.c.v. or intrahypothalamic administration of 5-HT remains to be described. The 5-HT receptor antagonists previously used i.e. 2-bromolysergic acid diethylamide, methysergide and metergoline are non
selective (see Hoyer 1991) and in these studies the only agonist used was 5-HT.

1.6.3 Nucleus Tractus Solitarius

The nucleus tractus solitarius (NTS) is the site of termination of afferent fibres from baroreceptors and chemoreceptors and is a key structure in the integration of these reflexes (Miura & Reis, 1969; 1972; Jordan & Spyer; 1977 see Spyer, 1981). Stimulation of the NTS has been shown to cause marked cardiovascular changes and excitatory amino acid afferents and receptors are directly involved in the processing of baroreceptor information (e.g. Talman & Robertson, 1989; Le Galloudec et al., 1989; Leone & Gordon, 1989). A cardiovascular regulatory role for 5-HT at the level of the NTS has been suggested since it receives a dense central (Fuxe, 1965; Steinbusch, 1981; Schaffar et al., 1988) and peripheral (nodose ganglia; Nosjean et al., 1990) serotonergic innervation. Furthermore, several 5-HT receptor subtypes have been demonstrated to be present within the NTS; 5-HT$_{1A}$, 5-HT$_{1B}$ (Manaker & Verderame, 1990; Pazos & Palacios, 1985; Thor et al., 1992a), 5-HT$_{2}$ (Pazos et al., 1985) and 5-HT$_{3}$ (Pratt & Bowery, 1989; Pratt et al., 1990; Laporte et al., 1992). The 5-HT$_{3}$ receptor binding sites are thought to be presynaptic on vagal afferents as nodose ganglionectomy has been shown to reduce this binding (Pratt & Bowery, 1989). Local administration of 5-HT into the NTS has been shown to produce well defined cardiovascular responses and the receptor subtypes mediating these changes have been identified (Wolf et al., 1981; Laguzzi et al., 1984; Shvaloff & Laguzzi, 1986; Itoh & Buňag, 1991; Merahi et al., 1992a, 1992b). Low doses (pmol) of 5-HT have been demonstrated to cause a reduction in blood pressure and heart rate in the anaesthetized rat (Laguzzi et al., 1984; Shvaloff & Laguzzi, 1986; Merahi et al., 1992a). The depressor response was accompanied with falls in renal sympathetic nerve activity and the bradycardia was attenuated by prior treatment with
atropine methonitrate suggesting that the response was mediated by a reduction in sympathetic outflow and an increase in vagal drive to the heart (Itoh & Buñag, 1991). Reductions in blood pressure and heart rate were also observed following microinjection of the 5-HT$_2$/5-HT$_1$C receptor agonists DOB and DOM into the NTS. The inhibitory cardiovascular responses produced by 5-HT (low dose), DOB or DOM were abolished by prior microinjection of the 5-HT$_2$/5-HT$_1$C receptor antagonists ketanserin, ritanserin or pirenpirone suggesting that these effects were mediated through activation of 5-HT$_2$ and/or 5-HT$_1$C receptors (Shvaloff & Laguzzi, 1986; Merahi et al., 1992a). However, the bradycardia caused by activation of the baroreceptor reflex following intravenous phenylephrine was not affected by NTS administration of ketanserin suggesting that 5-HT$_2$/5-HT$_1$C receptors are not integral to the baroreceptor reflex arc (Merahi et al., 1992a). In addition, microinjection of subthreshold doses of 5-HT or DOB to the NTS have been shown to enhance the depressor and bradycardic response caused by NTS administration of N-methyl-D-aspartic acid (NMDA). The potentiation of the response to NMDA was antagonised by prior treatment with ketanserin, suggesting that 5-HT$_2$ receptors in the NTS may regulate the reflex control of blood pressure by modulating the glutamatergic transmission (Merahi et al., 1992a). In the anaesthetised cat, topical application of 5-HT to the entire NTS/obex region caused falls in blood pressure, heart rate and sympathetic nerve activity, however, this response could not be reproduced following microinjection of 5-HT within various regions of the NTS (Coote et al., 1987). It remains to be determined whether 5-HT has a cardiovascular regulatory role within the NTS of the cat and the differential effects of 5-HT in this species may reflect an action of 5-HT at a separate site (see Coote, 1990).

Higher (nmol) doses of 5-HT administered directly into the NTS (unilateral and bilateral) produced a dose related rise in blood pressure associated with
variable changes in heart rate (Wolf et al., 1981; Merahi et al., 1992b). An increase in sympathetic nerve activity would appear to mediate this effect since the 5-HT pressor response was abolished by prior treatment with prazosin (Merahi et al., 1992b). Similar increases in blood pressure were observed following microinjection of the 5-HT₃ receptor agonists phenylbiguanide and 2-methyl-5-HT. Furthermore, the bradycardia caused by activation of the baroreceptor reflex following intravenous phenylephrine was abolished following the administration of 5-HT and phenylbiguanide to the NTS. These effects of 5-HT and the 5-HT₃ receptor agonists were blocked by prior microinjection of the 5-HT₃ receptor antagonists zacopride and ondansetron, suggesting that these effects were mediated through activation of 5-HT₃ receptors (Merahi et al., 1992b). When comparing intact rats to rats with a unilateral nodose ganglionectomy, Merahi et al. (1992b) demonstrated that the cardiovascular response caused by administration (ipsilateral to the lesion) of 5-HT to the NTS was attenuated and concomitant autoradiographic studies revealed a reduction in the 5-HT₃ binding sites on the same side. This suggested that the 5-HT₃ receptors mediating the cardiovascular effects were presynaptic on vagal afferent fibres. Furthermore, since the GABAₐ receptor antagonist bicuculline abolished the pressor and baroreceptor inhibitory response of 5-HT, a 5-HT₃ receptor dependent activation of an inhibitory GABA neurone within the NTS may account for this cardiovascular response (Merahi et al., 1992b).

1.6.4 Nucleus ambiguus and the dorsal motor vagal nucleus
In the cat cardiac vagal motoneurones (CVMs) are mainly located in the nucleus ambiguus (see Hopkins, 1987) and immunohistochemical studies have demonstrated that 5-HT-immunoreactive boutons make synaptic contact with CVMs in this nucleus (Izzo et al., 1988). Furthermore, microinjection of 5-HT into the nucleus ambiguus of the anaesthetized cat produced bradycardia (Izzo et al., 1988). The nucleus ambiguus and the
dorsal motor vagal nucleus (DMVN) of the rat are innervated by 5-HT-immunoreactive fibres (Steinbusch, 1981) and both regions contain CVMs (Nosaka et al., 1982). Following the administration of 5-HT to the DMVN of the anaesthetized rat (pretreated with atenolol) a vagally mediated bradycardia was observed (Sporton et al., 1991). Autoradiographic studies have demonstrated the presence of several subtypes of 5-HT receptor in the nucleus ambiguus including 5-HT$_{1A}$ (Dashwood et al., 1988; Thor et al., 1992a; Pazos & Palacios, 1985), 5-HT$_{2}$ (Pazos et al., 1985) and 5-HT$_{3}$ (see Pratt et al., 1990). Microinjection studies have shown that the 5-HT$_{1A}$ receptor agonists 8-OH-DPAT and flesinoxan administered to the nucleus ambiguus of the anaesthetized cat (Izzo et al., 1988) and the DMVN of the anaesthetized rat (Sporton et al., 1991) produced reductions in heart rate. In the latter study agonists at 5-HT$_{2}$ and 5-HT$_{3}$ receptors did not affect heart rate. These studies suggest that activation of 5-HT$_{1A}$ receptors located in the nucleus ambiguus and the DMVN cause an increase in vagal drive to the heart. An action at these receptors may explain the ability of 5-HT$_{1A}$ agonists administered i.v. to cause an increase in vagal drive to the heart in the cat (Ramage and Fozard, 1987; Ramage et al., 1988) and rat (Gradin et al., 1985; Fozard et al., 1987; Cherqui et al., 1988).

Furthermore, this hypothesis may also account for the bradycardia caused following i.c.v. administration of 5-HT to the conscious rat which has been shown to be partly due to an increase in vagal drive (Dalton, 1986).

1.6.5 The ventrolateral medulla

It is now accepted that structures in the ventrolateral medulla play an important role in cardiovascular regulation (for a recent review see Guyenet, 1990). Administration of pentobarbital or glycine to the ventrolateral surface of the medulla caused reductions in blood pressure (Feldberg & Guertzenstein, 1972; Gurtzenstein & Silver, 1974). Electrical stimulation of the rostral ventrolateral medulla (RVLM) caused a pressor response and an
increase in sympathetic activity (e.g. Dampney et al., 1982; Howe et al., 1983; Ross et al., 1984b) as did microinjection of glutamate into this region (e.g. Dampney et al., 1982; Ross et al., 1984b; Willette et al., 1987; Bachelard et al., 1990). Conversely, microinjection of inhibitory amino acids into the RVLM produced falls in blood pressure (Ross et al., 1984b; Hayes & Weaver; 1990). Neurones in the RVLM have been shown to project to the intermediolateral cell column (IML) of the spinal cord, the main site of origin of sympathetic preganglionic neurones (Ross et al., 1984a; see Loewy & Neil, 1981). Findings from functional, neuroanatomical and electrophysiological studies (for reviews see Guyenet, 1990; Calaresu & Yardley, 1988) have led to the hypothesis that the RVLM is essential for the maintenance of tonic sympathetic nerve activity and blood pressure. Further studies have shown that the cells in the RVLM control different components of sympathetic outflow (Lovick, 1987; Stein et al., 1989; Hayes & Weaver, 1990). Immunocytochemical studies have shown that rostral ventrolateral medulla is rich in 5-HT-immunoreactive terminals (Steinbusch, 1981) and 5-HT\textsubscript{1A} binding sites have been demonstrated in this region (Thor et al., 1990; 1992b). Bilateral administration of 5-HT into the RVLM has been shown to cause a reduction in blood pressure and heart rate associated with an increase in vascular conductance in the femoral bed (Lovick, 1989a, 1989b). This action of 5-HT appeared to involve the activation of 5-HT\textsubscript{1A} receptors (see below).

1.6.6 Medullary Raphé Nuclei
The presence of a dense network of serotonergic fibres in the IML was initially demonstrated by Dalstrom & Fuxe (1964) and this innervation was shown to originate from the medullary raphé nuclei, the raphé pallidus (B1), obscurus (B2) and magnus (B3) (Loewy, 1981; Loewy & McKellar, 1981; Loewy & Neil, 1981). Electrical stimulation of these raphé nuclei has been shown to cause both depressor and pressor responses with concomitant
increases and decreases in sympathetic nerve activity, respectively (e.g. Adiar et al., 1977; Coote & Macleod; 1974; McCall, 1984; see Coote, 1988). Iontophoretic application of 5-HT in the region of preganglionic sympathetic neurones has been shown to excite these neurones (de Groat & Ryall, 1967; McCall, 1983; Lewis & Coote, 1990). Evidence of this type has led to the suggestion that there is a serotonergically mediated sympathoexcitatory response to stimulation of the medullary raphé (see McCall, 1990). However, the involvement of 5-HT in a sympathoinhibitory response following the stimulation of the raphé nuclei has also been proposed (Gilbey et al., 1981; see Coote, 1988). Furthermore, intrathecal administration of 5-HT caused both an increase and a decrease in renal postganglionic sympathetic nerve activity (Yusof & Coote, 1988). In this study 5-HT\textsubscript{1A} receptors mediated sympathoexcitatory effects and 5-HT\textsubscript{2}/5-HT\textsubscript{1C} receptors mediated the sympathoinhibitory response.

1.7 Central 5-HT receptors in blood pressure regulation
The complex response observed following central administration of 5-HT may result from the activation of different receptor subtypes for 5-HT. Evidence in the literature does suggest that activation of 5-HT\textsubscript{1A} and 5-HT\textsubscript{2}/5-HT\textsubscript{1C} receptors can differentially affect blood pressure causing hypotension and hypertension, respectively. 5-HT\textsubscript{3} receptors have also been shown to affect cardiovascular function, however, this has been discussed in 1.6.3 and this section will concentrate on the actions of 5-HT\textsubscript{1A} and 5-HT\textsubscript{2}/5-HT\textsubscript{1C} receptors.

1.7.1 Central 5-HT\textsubscript{1A} receptors and cardiovascular regulation
The characterization of selective agonists for 5-HT\textsubscript{1A} receptors such as 8-OH-DPAT (Middlemiss & Fozard, 1983; Fozard et al., 1987), flesinoxan (Wouters et al., 1988a, 1988b), urapidil and 5-methyl-urapidil (Gross et al., 1987), ipsapirone (Dompert et al., 1985) and DP-5-CT (Mir et al., 1987)
have allowed the investigation of the cardiovascular effects of 5-HT$_{1A}$ receptors.

Administration of 8-OH-DPAT and flesinoxan have been shown to cause a reduction in blood pressure in rats (Fozard et al., 1987; Gradin et al., 1985; Dabire et al., 1987), rabbits (Hof & Fozard., 1989; Shepheard et al., 1990b), cats (Ramage & Fozard, 1987; Ramage et al., 1988) and dogs (Di Francesco et al., 1988; Laubie et al., 1989). The depressor response was accompanied with bradycardia in all species tested. Furthermore, these changes in blood pressure and heart rate occurred in conscious and anaesthetized preparations and in normotensive and hypertensive animals (e.g. rat; Dreteler et al., 1990; Gradin et al., 1985). The reduction in blood pressure caused by 8-OH-DPAT and flesinoxan in the cat, rat and rabbit has been shown to be caused mainly by a increase in total peripheral conductance, however, a reduction in cardiac output also contributed in the cat and rabbit (Wouters et al., 1988; Dreteler et al., 1989; 1991b; Hof & Fozard, 1989).

These cardiovascular effects of 8-OH-DPAT and flesinoxan were not mediated through 5-HT$_2$ or 5-HT$_3$ receptors since antagonists for these subtypes did not block the response (Fozard et al., 1987; Dabire et al., 1987; Dreteler et al., 1990). The cardiovascular responses to 8-OH-DPAT and flesinoxan were inhibited by the putative 5-HT$_{1A}$ receptor antagonists methiothepin (non-selective), metergoline (non-selective), buspirone (partial agonist), 8-MeO-CIEPAT and spiroxatrine (Fozard et al., 1987; Dabire et al., 1987; Dreteler et al., 1990; Doods et al., 1988). Furthermore, spiperone (a 5-HT$_{1A}$ and 5-HT$_2$ receptor antagonist) reversed the cardiovascular effects of 8-OH-DPAT in anaesthetized cats (McCall et al., 1987). The β-adrenoreceptor antagonists pindolol and cyanopindolol which are potent antagonists at 5-HT$_{1A}$ receptors blocked the hypotension and
bradycardia caused by 8-OH-DPAT, flesinoxan and DP-5-CT (Fozard et al., 1987; Doods et al., 1988; Wouters et al., 1988a; Dreteler et al., 1990). These studies demonstrate that the decrease in blood pressure and heart rate is mediated by 5-HT$_{1A}$ receptors.

The ability of 8-OH-DPAT and flesinoxan to cause a reduction in blood pressure associated with bradycardia rather than reflex tachycardia pointed to a centrally mediated cardiovascular effect. Moreover, the hypotension and bradycardia caused by 8-OH-DPAT was present in sino-aortic denervated animals, ruling out an action on baroreceptor afferent fibres or modification of baroreceptor sensitivity (Petty et al., 1988; King & McCall, 1991). A central action for 8-OH-DPAT and flesinoxan was further supported by the finding that the cardiovascular effects of these compounds were absent in pithed rats even when baseline blood pressure was raised with infusions of angiotensin II or vasopressin (Gradin et al., 1985; Fozard et al., 1987). An enhanced hypotensive potency was observed for 8-OH-DPAT, DP-5-CT and flesinoxan following administration into the vertebral artery or intracisternal injection (flesinoxan) compared to i.v. administration in the anaesthetized cat, again supporting a central effect (Doods et al., 1988; Wouters et al., 1988b). Direct evidence for a centrally mediated effect came from experiments in which pre and postganglionic sympathetic nerve activity was monitored. Intravenous administration of 8-OH-DPAT, flesinoxan and ipsaperone in anaesthetized cats caused hypotension which was associated with a moderate reduction in thoracic preganglionic sympathetic nerve activity (Ramage & Fozard, 1987; Ramage et al., 1988). In these studies there was a lack of correlation of the thoracic sympathoinhibitory action and the hypotensive action of 8-OH-DPAT. This was suggested to be due to a differential effect on sympathetic outflow since it was demonstrated that 8-OH-DPAT and flesinoxan produced a non-uniform reduction in renal, splanchnic and
cardiac sympathetic nerve activity when these variables were measured simultaneously in anaesthetized cats. In this preparation renal nerve activity was most sensitive to the sympathoinhibitory actions of the 5-HT$_{1A}$ receptor agonists and cardiac nerve activity was least affected (Ramage & Wilkinson, 1989; see Ramage, 1990). This patterning of sympathetic outflow was also observed following administration of 8-OH-DPAT to the fourth ventricle (Shepheard et al., 1989). Furthermore, the increase in total peripheral conductance caused by flesinoxan appeared to result from an organ-specific vasodilatation since renal conductance was seen to increase whilst femoral conductance was unaltered. Again, a differential sympathoinhibition to vascular beds was suggested (Ramage et al., 1988).

This patterning of sympathetic outflow may be species dependent since cardiac nerve activity was more sensitive to the inhibitory actions of 8-OH-DPAT than renal nerve activity in the anaesthetized rabbit (Shepheard et al., 1990b). These studies clearly showed that 5-HT$_{1A}$ receptor agonists caused a centrally mediated hypotension associated with differential sympathoinhibition.

Interestingly, in anaesthetized and conscious rats intracisternal administration of 8-OH-DPAT was no more effective in producing hypotension than i.v. administration (Gradin et al., 1985; Mir & Fozard, 1987; Dreteler et al., 1990) and lateral or third ventricular injection of 8-OH-DPAT was less effective (Martin & Liz, 1985; Mir & Fozard, 1987) than i.v. administration. However, i.c. administration of 8-MeO-CIEPAT blocks the cardiovascular response to i.v. 8-OH-DPAT supporting the view that the response was centrally mediated (see Fozard et al., 1987). This would suggest that the hypotensive action of 8-OH-DPAT was at a site in the hindbrain (see Mir & Fozard, 1987).
The bradycardia caused by i.v. 8-OH-DPAT or flesinoxan in anaesthetized cats was reversed by atropine methonitrate and was abolished following vagotomy indicating that the 5-HT\textsubscript{1A} agonists caused a centrally mediated increase in vagal tone (Ramage & Fozard, 1987; Ramage \textit{et al.}, 1988). The bradycardia elicited by 8-OH-DPAT or flesinoxan in conscious normotensive and hypertensive rats was also caused by an increase in vagal drive (Gradin \textit{et al.}, 1985; Dreteler \textit{et al.}, 1990; Cherqui \textit{et al.}, 1988). This may be due to activation of 5-HT\textsubscript{1A} receptors located in the nucleus ambiguus and the DMVN (see 1.6.4 and Ramage, 1990).

It is now accepted that activation of 5-HT\textsubscript{1A} receptors produces a centrally mediated fall in blood pressure associated with sympathoinhibition and an increase in vagal drive (see Ramage, 1990; see Dabire, 1991). The central site(s) involved in the actions of the 5-HT\textsubscript{1A} receptor agonists have been the subject of a number of studies. The observed increase in hypotensive potency of 5-HT\textsubscript{1A} agonists after administration via the vertebral artery compared to i.v. application (Doods \textit{et al.}, 1988; Wouters \textit{et al.}, 1988b; Laubie \textit{et al.}, 1989) suggested that hindbrain regions were important in the inhibitory actions of these compounds. Topical application of 8-OH-DPAT or urapidil to the ventral surface of the medulla caused a depressor response and bradycardia in the anaesthetized cat which was antagonised by spiperone or spiroxatrine (Gillis \textit{et al.}, 1989; Mandal \textit{et al.}, 1989; King & Holtman, 1990). Similarly, topical application of 8-OH-DPAT to the ventral surface of the medulla of anaesthetized rats also reduced blood pressure and heart rate (Helke \textit{et al.}, 1993). These studies confirm the importance of the ventrolateral medulla in the hypotensive effects of 8-OH-DPAT.

Several lines of evidence suggest that the rostral ventrolateral medulla (RVLM), a region shown to maintain tonic sympathetic activity and blood
pressure (see above), is an important site for the inhibitory action of 5-HT$_{1A}$ agonists. Moreover, 5-HT$_{1A}$ binding sites have been demonstrated in this region (Thor et al., 1990, 1992b). Bilateral microinjection of 5-HT and the 5-HT$_{1A}$ receptor agonists, 8-OH-DPAT and 5-CT into the RVLM has been shown to cause a reduction in blood pressure and heart rate associated with an increase in vascular conductance in the femoral bed of the anaesthetized rat (Lovick, 1989a, 1989b; Helke et al., 1993). In anaesthetized rats i.v. 8-OH-DPAT caused hypotension, a reduction in lumbar sympathetic nerve activity and slowed the discharge rate of sympathoexcitatory neurones in the RVLM. Bilateral microinjection of 8-OH-DPAT into the RVLM also produced a reduction in blood pressure and lumbar sympathetic nerve activity (Nosjean & Guyenet, 1991). Furthermore, iontophoretic application of 5-HT and agonists at 5-HT$_{1A}$ receptors inhibited ongoing activity of neurones in the RVLM (Wang & Lovick, 1992b). Taken together, these studies suggest that activation of 5-HT$_{1A}$ receptors located in the RVLM caused direct inhibition of neurones that send excitatory projections to the spinal sympathetic outflow leading to a reduction in blood pressure in rats (see Wang & Lovick, 1992b). In anaesthetized dogs microinjection of 8-OH-DPAT to the rostral ventrolateral medulla (corresponding to the ventrolateral pressor area) caused a reduction blood pressure, heart rate and renal sympathetic nerve activity and this response was prevented or reversed by microinjection of methiothepin (Laubie et al., 1989). Similar findings were reported in the anaesthetized cat (Mandal et al., 1991), again, suggesting that the cardiovascular response of 8-OH-DPAT was due to activation of 5-HT$_{1A}$ receptors in the RVLM. In the anaesthetized cat, i.v. administration of 8-OH-DPAT reduced blood pressure, heart rate and cardiac sympathetic nerve activity. The fall in nerve activity exactly paralleled the inhibition of neuronal firing of sympathoexcitatory neurones in the RVLM (Clement & McCall, 1990a). However, in this study, iontophoretic application of 5-HT and 8-OH-DPAT onto sympathoexcitatory neurones in
the RVLM failed to affect the firing rate of these neurones suggesting that 8-OH-DPAT may act on central sympathetic neurones which lie antecedent to the ventrolateral sympathoexcitatory neurones in the cat. These results were inconsistent with those of Wang & Lovick (1992b) and may reflect a difference between the cat and rat. Recent studies have implicated the sympathoexcitatory neurones in the lateral tegmental field (LTF) in the sympatholytic effect of 8-OH-DPAT. 8-OH-DPAT administered directly into the LTF caused a reduction in blood pressure and heart rate associated with a reduction in cardiac and renal sympathetic nerve activity. A fall in the firing rate of LTF sympathoexcitatory neurones was also reported (Clement & McCall, 1992a; Vayssettes-Courchay et al., 1993). Intravenous administration of 8-OH-DPAT caused a reduction in the firing rate of sympathoexcitatory neurones in the LTF in conjunction with a decrease in renal nerve activity and these effects were reversed with spiperone (Clement & McCall, 1992a; Vayssettes-Courchay et al., 1993). Furthermore, chemical lesion of the LTF produced by microinjection of kainic acid prevented the cardiovascular effects of i.v. 8-OH-DPAT (Clement & McCall, 1992b; Vayssettes-Courchay et al., 1993). This data suggests that the LTF is an important site for the hypotensive effects of 8-OH-DPAT in cats.

The involvement of presynaptic 5-HT$_{1A}$ receptors in the cardiovascular effects of 5-HT$_{1A}$ receptor agonists has been suggested in a number of studies. Serotonergic neurones located in midline medullary raphé nuclei project to sympathetic preganglionic neurones in the intermediolateral cell column and tonically excite sympathetic preganglionic neurones (see 1.6.6 and McCall, 1990). McCall and co-workers (1989) suggested that the sympatholytic action of 8-OH-DPAT may result from the inhibition of the tonic sympathoexcitatory drive from the medullary raphé 5-HT neurones through a mechanism involving a presynaptic 5-HT$_{1A}$ autoreceptor.
lontophoretic application of 8-OH-DPAT caused a reduction in the tonic discharge of identified medullary 5-HT neurones (McCall & Clement, 1989). In anaesthetized cats comparisons of the inhibitory actions of i.v. 8-OH-DPAT on medullary 5-HT neuronal activity and cardiac or renal sympathetic nerve activity were made. 8-OH-DPAT completely suppressed the firing of medullary 5-HT neurones and this was correlated to the inhibition of renal but not cardiac sympathetic nerve activity (McCall et al., 1989; Ramage et al., 1992). These studies suggest that medullary 5-HT neurones supply tonic excitatory drive to sympathetic preganglionic neurones which innervate postganglionic renal sympathetic neurones and that 8-OH-DPAT can inhibit sympathetic activity through a presynaptic inhibitory effect on medullary 5-HT neurones (Ramage et al., 1992).

Microinjection of the 5-HT$_{1A}$ receptor agonists 8-OH-DPAT and 5-methyl-urapidil into the raphé pallidus and magnus (B$_1$/B$_3$ cell groups) caused a reduction in blood pressure and heart rate in the anaesthetized rat and these effects were prevented by pretreatment with the 5-HT$_{1A}$ receptor antagonists spiroxatrine and spiperone (Valenta & Singer, 1990). These studies suggest that the medullary raphé nuclei are involved in the hypotensive effect of 5-HT$_{1A}$ receptor agonists and that these compounds may act at presynaptic 5-HT$_{1A}$ autoreceptors, thereby inhibiting a tonic sympathoexcitatory drive. However, it does not appear that this is the major site of action for 8-OH-DPAT since destruction of serotonergic neurones (with 5,7-DHT; Helke et al., 1993) or depletion of 5-HT stores (with PCPA; Fozard et al., 1987) did not affect the hypotensive effects of 8-OH-DPAT administered i.v. or to the ventral surface of the medulla, respectively. Furthermore, high i.v. doses of 8-OH-DPAT were required to totally suppress cardiac and renal nerve activity in anaesthetized cats, whereas the firing rate of raphé neurones was abolished at much lower doses (Ramage et al., 1992; Clement & McCall, 1989). These studies
suggested that postsynaptically located 5-HT_{1A} receptors are more important in producing the cardiovascular effects of 8-OH-DPAT.

Microinjection of 8-OH-DPAT, flesinoxan and 5-CT into the dorsal raphé nucleus has been shown to cause falls in blood pressure and heart rate in the conscious rat. The hypotension and bradycardia were attenuated by prior treatment with atropine methonitrate suggesting a vagal component to the response. The cardiovascular effects were prevented by pretreatment with (-) pindolol implicating 5-HT_{1A} receptors in this response. The dorsal raphé nucleus contains a high density of somatodendritic 5-HT_{1A} autoreceptors and activation of these receptors was suggested as the mechanism of the hypotensive response (Connor & Higgins, 1990).

The studies reviewed above suggest that more than one central site may be involved in the hypotensive effect of systemically administered 5-HT_{1A} receptor agonists and there may be differences between species. Furthermore, the similarity between the response caused following 5-HT_{1A} receptor activation and the hypotensive, bradycardic and sympathoinhibitory response caused by i.c.v. administration of 5-HT (see above) suggests that this action of 5-HT was mediated following activation of hindbrain 5-HT_{1A} receptors.

1.7.2 Central 5-HT\textsubscript{2}/5-HT\textsubscript{1C} receptors and cardiovascular regulation

It is now accepted that activation of central 5-HT\textsubscript{2}/5-HT\textsubscript{1C} receptors in anaesthetized cats causes hypertension and sympathoexcitation. Intravenous administration of the 5-HT\textsubscript{2}/5-HT\textsubscript{1C} receptor agonist DOI (Shannon et al., 1984; Glennon et al., 1986; Van Wijngaarden et al., 1990) has been shown to cause a rise in blood pressure accompanied with a marked increase in splanchnic and cardiac sympathetic nerve activity and these effects were antagonised by the selective 5-HT\textsubscript{2}/5-HT\textsubscript{1C} receptor
antagonists LY 53857 and ketanserin (McCall et al., 1987; McCall & Harris, 1988; Clement & McCall, 1990b). Further examination of the effect of i.v. DOI revealed that activation of peripheral 5-HT\(_2\) receptors located on vascular and bronchial smooth muscle caused vasoconstriction and bronchoconstriction. The bronchoconstriction was demonstrated to severely compromise respiration leading to an over-estimation of the sympathoexcitatory effects of DOI (Ramage et al., 1993). However, in cats pretreated with the peripherally acting 5-HT\(_2\)/5-HT\(_1\)C receptor antagonist BW501C67, DOI caused a pressor response associated with femoral arterial constriction and an increase in sympathetic nerve activity (Ramage et al., 1993) confirming that there was a central sympathoexcitatory component to the response of DOI. Fourth ventricular administration of DOI in anaesthetized cats pretreated with BW501C67 (to prevent any peripheral response) was also shown to cause an increase in blood pressure and constriction of the femoral vasculature. Sympathoexcitation was inferred in this study (Shepheard et al., 1991). This evidence further supported a central sympathoexcitatory role for 5-HT\(_2\)/5-HT\(_1\)C receptors and suggested a hindbrain location for these receptors. In this respect, several studies have suggested that the hypertensive and sympathoexcitatory effects of 5-HT\(_2\)/5-HT\(_1\)C receptors are located in the hindbrain. King & Holtman (1989) demonstrated that DOI applied to the intermediate area (Schlaefke's area) of the ventral surface of the medulla caused an increase in blood pressure which was prevented by prior treatment with ketanserin. These results were confirmed in a study in which quipazine (a 5-HT\(_2\) and 5-HT\(_3\) receptor agonist) administered to the same area caused a increase in blood pressure, heart rate and splanchnic sympathetic nerve activity. These effects were prevented or reversed by topical application of the 5-HT\(_2\)/5-HT\(_1\)C receptor antagonists LY 53857 and cyproheptadine, to the rostral ventrolateral medulla (Vayssettes-Courchay et al., 1991). Microinjection of quipazine (Vayssettes-Courchay et al., 1992) or DOI
(Mandal et al., 1990, 1991) directly into the subretrofacial nucleus located in the rostral ventrolateral medulla of anaesthetized cats caused an increase in blood pressure associated with tachycardia and sympathoexcitation and these effects were antagonised by microinjection of LY 53857 and BW501C67 (Vayssettes-Courchay et al., 1992), confirming that these effects were mediated by 5-HT\(_2\) and/or 5-HT\(_{1C}\) receptors. These studies show that activation of 5-HT\(_2\) and/or 5-HT\(_{1C}\) receptors located in the ventrolateral medulla cause sympathoexcitation. Electrophysiological findings from experiments in cats and rats supports this conclusion. In anaesthetized cats iontophoretically applied DOI was not able to increase the firing rate of sympathoexcitatory neurones in the rostral ventrolateral medulla; i.v. administration did increase the firing rate of these neurones and it was suggested that DOI was acting on neurones antecedent to the neurones of the rostral ventrolateral medulla or at distal dendrites of these neurones (Clement & McCall, 1990b). However, the 5-HT\(_2\)/5-HT\(_{1C}\) receptor agonist \(\alpha\)-methyl-5-HT caused excitation of neurones in the rostral ventrolateral medulla of anaesthetized rats which was prevented by ketanserin (Wang & Lovick, 1992a).

The medullary areas mediating sympathoexcitatory actions of the 5-HT\(_2\)/5-HT\(_{1C}\) receptor agonists do not appear to receive tonic serotonergic input as selective 5-HT\(_2\)/5-HT\(_{1C}\) receptor antagonists such as LY 53857 or cinanserin do not reduce blood pressure or inhibit sympathetic nerve discharge (Ramage, 1985; 1988b; McCall & Harris, 1987).

Other 5-HT\(_2\)/5-HT\(_{1C}\) receptor antagonists such as ketanserin, methysergide, methiothepin, metergoline and cyproheptadine have been shown to cause a reduction in blood pressure and sympathetic nerve activity (McCall & Schuette, 1984: McCall & Harris, 1987; Antonaccio & Taylor, 1977; McCall & Humphrey, 1982; Ramage, 1985; 1988b). However, these
agents are not selective for 5-HT receptors and have activity at other receptor systems (see Ramage, 1988b). In this respect, the sympathoinhibitory action of ketanserin has been suggested to be caused by activation of central $\alpha_1$-adrenoreceptors (McCall & Schuette, 1984; McCall & Harris, 1987; Ramage, 1985). Methysergide, metergoline and methiothepin have affinity for 5-HT$_1$ receptor subtypes (see Hoyer, 1991). Therefore, it is difficult to attribute the cardiovascular changes caused by the classical 5-HT$_2$ receptor antagonists to antagonism of 5-HT$_2$ and/or 5-HT$_{1C}$ receptors (Ramage, 1988b; Dabire, 1991).

Recently, sympathoinhibitory actions of 5-HT$_2$ and/or 5-HT$_{1C}$ receptor activation have been reported. Yusof & Coote (1988) demonstrated that intrathecal administration of $\alpha$-methyl-5-HT inhibited renal sympathetic nerve activity and this action was prevented by ketanserin in anaesthetized rats. Reductions in blood pressure and heart rate were also observed following microinjection of 5-HT and the selective 5-HT$_2$/5-HT$_{1C}$ receptor agonists DOB and DOM into the nucleus tractus solitarius (NTS) in rats and these inhibitory cardiovascular responses were abolished by prior microinjection of the 5-HT$_2$/5-HT$_{1C}$ receptor antagonists ketanserin, ritanserin and pirenpirone suggesting that these effects were mediated through activation of 5-HT$_2$ and/or 5-HT$_{1C}$ receptors (Shvaloff & Laguzzi, 1986; Merahi et al., 1992a; see 1.6.3). Furthermore, microinjection of quipazine into the NTS of the anaesthetized cat caused a reduction in blood pressure, heart rate and sympathetic nerve activity and this was blocked by LY 53857 and BW501C67 suggesting that a inhibitory 5-HT$_2$ and/or 5-HT$_{1C}$ mechanism occurred in cats (Vayssettes-Courchay et al., 1992). In that study an inhibitory action of 5-HT$_2$ and/or 5-HT$_{1C}$ receptors was also demonstrated in the caudal ventrolateral medulla.
Thus, activation of central 5-HT$_2$ and/or 5-HT$_{1C}$ receptors causes sympathoexcitation in cats. There is little evidence for a similar cardiovascular action of 5-HT$_2$ and/or 5-HT$_{1C}$ receptors in rats. This may represent a species difference between the cat and rat.
1.8 Aims of the thesis

Central administration of 5-HT can cause both increases and decreases in blood pressure and sympathetic outflow. These effects are complex and activation of different 5-HT receptor subtypes can cause opposing cardiovascular effects. The site of central administration of 5-HT can also influence the cardiovascular response observed.

5-HT receptors located in the hindbrain of several species have been shown to cause cardiovascular changes. In this respect, activation of 5-HT\textsubscript{1A} receptors have been shown to cause reductions in blood pressure and sympathetic nerve activity in rats and cats whereas activation of 5-HT\textsubscript{2} and/or 5-HT\textsubscript{1C} receptors (in cats) produce hypertension and sympathoexcitation.

5-HT receptors have also been demonstrated within regions of the forebrain involved in cardiovascular control. The dorsal and median raphé nuclei have been shown to provide ascending serotonergic innervation to many areas in the forebrain including regions implicated in the control of blood pressure. Stimulation of the dorsal raphé nucleus has been shown to cause an increase in blood pressure through activation of 5-HT receptors located in the anterior hypothalamus/preoptic area. Similarly, administration of 5-HT into forebrain areas via the lateral cerebral ventricle causes an increase in blood pressure in rats and cats. However, the 5-HT receptor subtypes mediating these effects of 5-HT and the physiological basis for the pressor changes are presently unknown.

The objective of the present study is to investigate the role of 5-HT receptors in cardiovascular regulation in rats and cats. The aim of the studies described in this thesis is to investigate further the pressor response caused by forebrain administration of 5-HT via the lateral cerebral ventricle.
(i.c.v.) in rats and cats. The specific aims of this study are to determine the
type of 5-HT receptors mediating the cardiovascular effects of 5-HT and to
determine the physiological basis of the cardiovascular changes.

Since anaesthesia has been shown to affect the cardiovascular response
caused by i.c.v. administration of 5-HT, initial experiments will compare the
effects of 5-HT in conscious rats and rats anaesthetized with different
anaesthetics. The objective of these studies is to determine an anaesthetic
regimen which best models the conscious state. In an anaesthetized rat
model the effects of 5-HT and selective agonists and antagonists for the
various 5-HT receptor subtypes will be administered i.c.v. to determine the
5-HT receptors mediating the cardiovascular actions of 5-HT. Sympathetic
nerve activity will be monitored in these experiments to determine whether
changes in sympathetic tone are involved in the cardiovascular response of
5-HT. In a second study, the regional haemodynamic changes caused
following of activation central 5-HT receptors in conscious rats will be
described. This will provide an understanding of the blood flow changes
responsible for the 5-HT induced pressor response. Lastly, the effect of
forebrain administration of 5-HT on cardiovascular variables and
sympathetic outflow will be investigated in anaesthetized cats. Selective
agonists and antagonists will be used to determine the nature of the 5-HT
receptor subtype(s) mediating the cardiovascular effects of 5-HT.
CHAPTER 2

Characterisation of the 5-HT receptor subtype(s) involved in the cardiovascular effects of centrally administered 5-HT in rats

2.1 Introduction

Intracerebroventricular (i.c.v.) injections of 5-HT in anaesthetized rats causes a rise in blood pressure and variable effects on heart rate (Lambert et al., 1975, 1978; Krstic & Djurkovic, 1976, 1980). In conscious rats i.c.v. administration of 5-HT also causes a pressor effect but in these animals consistently produces a bradycardia (Sukamoto et al., 1984; Dalton, 1986). More recently, Inoue & Buñag (1989) were able to demonstrate that i.c.v. 5-HT induces a pressor response associated with a consistent bradycardia and sympathoinhibition in anaesthetized rats.

The precise nature of the 5-HT receptor subtype(s) involved in the centrally mediated pressor response to 5-HT is unknown, although the effect is antagonised by the non-selective 5-HT receptor antagonists methysergide or bromolysergic acid diethylamide (Lambert et al., 1978; Sukamoto et al., 1984; Inoue & Buñag, 1989). Furthermore, in conscious rats Dalton (1986) demonstrated that the bradycardia caused by i.c.v. 5-HT is attenuated by methysergide and the non-selective 5-HT$_2$/5-HT$_1$C receptor antagonist cyproheptidine. These studies support the notion that 5-HT receptors mediate the cardiovascular effects of 5-HT but the non-selective nature of the antagonists used provides little insight into the nature of the receptor types involved. Moreover, no information is presently available on the cardiovascular effects of more selective 5-HT receptor agonists.
The central and peripheral mechanisms responsible for the pressor response caused by i.c.v. administration of 5-HT in the rat are unclear. It has been reported that the rise in blood pressure produced by 5-HT is attenuated by cervical transection of the spinal cord, adrenalectomy, adrenergic blocking agents and α-adrenoceptor antagonists (Krstic & Djurkovic, 1980), suggesting that there is a sympathoexcitatory component to the response of 5-HT. However, it has been demonstrated that the rise in blood pressure is associated with a reduction in splanchnic sympathetic nerve activity (Inoue & Buñag, 1989). Furthermore, hexamethonium, at doses shown to block autonomic ganglia, enhances the 5-HT induced rise in blood pressure in the anaesthetized rat (Lambert et al., 1978; Krstic & Djurkovic, 1980). Similar results were found in the conscious rat pretreated with chlorisondamine (Pergola & Alper, 1991). Taken together these data suggest that sympathoexcitation, although important, is not the sole factor responsible for the 5-HT-induced pressor response. Indeed, Inoue & Buñag (1989) were able to demonstrate that the pressor response caused by 5-HT is attenuated by pretreatment with a vasopressin V₁-receptor antagonist, suggesting a role for vasopressin in the 5-HT-induced pressor response.

It is evident from these observations that further evaluation of the relative roles for the autonomic and neurohumoral systems in the response to central administration of 5-HT is required. In this chapter the mechanism and nature of the receptors involved in cardiovascular effects of forebrain administration 5-HT via the lateral cerebral ventricle has been further investigated. Kuhn et al. (1980a) suggested that the cardiovascular effects of 5-HT may be related to the central dose administered and whether the animal model was anaesthetized or conscious. Furthermore, the type of anaesthetic used has also been shown to affect the response to i.c.v. 5-HT (Sukamoto et al., 1984). For these reasons, in preliminary experiments, the dose-response relationship for i.c.v. 5-HT was examined in conscious rats.
and a comparison between the effects of 5-HT in conscious and anaesthetized rats was made. Subsequently, the effects of 5-HT on blood pressure, heart rate and sympathetic nerve activity in rats anaesthetized with α-chloralose were investigated using more selective agonists and antagonists for the different 5-HT receptor subtypes. In addition respiratory variables were monitored.
2.2 Methods

2.2.1 Conscious rat experiments

Male normotensive Sprague-Dawley rats (250-300g) were used. Animals were housed individually and maintained under a 12:12 h light-dark cycle (lights on at 6 a.m.) with free access to food and water. Surgery was performed in 2 stages and after each surgical stage wounds were closed and dusted with Acramide. The animals were also given an injection of ampicillin (Penbritin, 7 mg kg\(^{-1}\), i.m.).

Chronic cannulation of the lateral ventricle

Rats were anaesthetized with a short acting barbiturate (sodium methohexitone, 60 mg kg\(^{-1}\), i.p.) and placed in a stereotaxic head holder. A midline incision was made in the scalp and connective tissue was removed. A hole was drilled in the skull and a guide cannula (22 gauge) was stereotaxically placed in the right lateral ventricle. The co-ordinates used from bregma were 4 mm ventral, 1.5 mm lateral and 1 mm posterior. The cannula was secured to the skull with screws and dental cement and the scalp sutured. Drug and vehicle solutions were administered through an i.c.v. injection cannula (28 gauge) attached by a length of polythene tubing to a 25 \(\mu\)l syringe (Hamilton). At the end of the experiment, the cannula placement was confirmed by the administration of 50 ng angiotensin II i.c.v.; animals with an intact cannula showed a dipsogenic response shortly after injection. Cannula position was also confirmed histologically following administration of 5 \(\mu\)l of 2% pontamine sky blue dye (v/v).

Chronic implantation of vascular catheters

At least one week after the implantation of the i.c.v. cannula and 1-2 days before experimentation, animals were anaesthetized using sodium methohexitone (40 mg kg\(^{-1}\), i.p.). Two intra-venous catheters were implanted in the right jugular vein for drug administration and an
intra-arterial catheter was placed in the distal abdominal aorta, via the caudal artery (for blood pressure and heart rate recordings). The vascular catheters were tunnelled subcutaneously and exteriorized at the back of the neck.

Experimental protocols
Experiments were performed with the animals in their home cages and they were given free access to food and water. Each animal wore a specially designed harness which was attached to a counterbalanced spring. The vascular catheters led out of the cage through the spring and the arterial catheter connected to a pressure transducer (Bell & Howell, type 4-442). Arterial blood pressure was displayed on a chart recorded (Gould ES1000) and heart rate derived electronically from the blood pressure signal (Gould Biotach Amplifier). Each animal received 3 separate doses of 5-HT and a single volume of 5 μl of saline. The doses of 5-HT were administered according to a Latin Square design over 3 days and a single injection of 5-HT was given per day; saline was given before the low dose of 5-HT. Injections of saline and 5-HT were made when the animals were still and when cardiovascular variables were stable. The response produced by vehicle and drug were recorded for at least 30 min.

2.2.2 Anaesthetized rat experiments
Experiments were performed on male normotensive Sprague-Dawley rats (250-350g). Anaesthesia was induced with either :
1) urethane (1500 mg kg⁻¹, i.p.). Supplementary doses of urethane (200 mg kg⁻¹, i.v.) were given as required.
2) halothane and α-chloralose. Anaesthesia was induced with halothane (2.5 % in oxygen) and maintained with α-chloralose (80 mg kg⁻¹, i.v.). Supplementary doses of α-chloralose (10-20 mg kg⁻¹, i.v.) were given as
required. Depth of anaesthesia was assessed by the stability of cardiovascular variables and corneal and toe pinch reflexes.

The left carotid artery was cannulated for the measurement of blood pressure. Blood pressure was measured using a pressure transducer (Gould Statham P23XL) and heart rate was derived electronically from the blood pressure signal (Gould Biotach Amplifier). Blood pressure and heart rate were displayed on a chart recorder (Gould 2600 series). The left jugular vein was cannulated for drug administration and a tracheal cannula was implanted. Body temperature was monitored by a rectal probe and maintained at 36-38°C with a homeothermic blanket system (Harvard). Rats were either allowed to breathe spontaneously or were artificially ventilated.

**Artificially respired rat**

All animals in this group were anaesthetized using halothane and α-chloralose (see above). The animals were artificially ventilated (rate 50 min⁻¹, stroke volume 8 ml kg⁻¹) with oxygen-enriched room air by use of a positive pressure pump (Harvard Rodent Ventilator 683) and neuromuscular blockade was produced with decamethonium (3 mg kg⁻¹ i.v.). Blood samples were taken from a T-piece on the carotid arterial cannula and blood gases and pH were monitored with a Corning pH/blood gas analyser. Blood gases were maintained between 90-130 mmHg PO₂, 40-50 mmHg PCO₂ and pH 7.3-7.4. Adjustments of the respiratory pump volume were made as necessary to maintain blood gas and pH balance. Once ventilated, the animals were infused (6 ml kg⁻¹ h⁻¹) via the jugular vein with a solution comprising 10 ml plasma substitute (gelofusine), 10 ml distilled water, 0.04g glucose, 0.168g sodium bicarbonate and 30 mg decamethonium. This was to prevent the development of non-respiratory acidosis and to maintain blood volume and neuromuscular blockade.
Cannulation of the lateral ventricle

The rats were placed in a stereotaxic head holder and a stainless steel guide cannula (22 gauge) was implanted unilaterally into the right lateral cerebral ventricle. The co-ordinates used were 4 mm ventral, 1.5 mm lateral and 1 mm posterior from bregma. Drug and vehicle solutions were administered in a volume of 5μl via an i.c.v. injection cannula (28 gauge) attached by a length of polythene tubing to a 100 μl syringe (Hamilton 710 series). At the end of each experiment, the cannula placement was confirmed by the administration of 5 μl of 2% pontamine sky blue dye. Only, experiments in which dye was seen in the ventricular system were included in the mean result.

Whole nerve recordings

Simultaneous whole nerve extracellular recordings were taken from the renal sympathetic (postganglionic) nerve and the phrenic nerve in artificially resired, α-chloralose anaesthetized rats.

The right phrenic nerve was exposed by deflecting the scapula forwards and dissecting the nerve clear of overlying muscle and connective tissue. The nerve was cut leaving a long central process which was placed on a bipolar silver hook electrode. Phrenic nerve activity was amplified (Digitimer NL104), filtered (Digitimer NL125; frequency band 200-2000 Hz) and quantified by counting the number of action potentials above the noise level over 5s using a spike processor (Digitimer D130). The noise level was verified at the end of the experiment after the administration of pentobarbitone sodium (20 mg per animal). To maintain phrenic nerve activity, a measure of central inspiratory drive, the blood Pco₂ values in these animals were maintained at 40-50 mmHg. This usually locked the rate of phrenic nerve firing to the rate of the animals chest movements caused by the respiration pump. Therefore, changes in phrenic nerve
activity can be interpreted as the result of changes in the size of each inspiratory burst.

The right kidney was exposed by a retroperitoneal approach and was deflected laterally to reveal the right renal artery and renal nerve. The nerve was cleared of connective tissue, crushed close to the kidney and the long central process positioned on a bipolar silver hook electrode. The renal nerve activity was amplified (Digitimer NL104), filtered (Digitimer NL125; frequency band 100-500 Hz) and quantified by integrating the signal above background noise over 5s with a solid state integrator (Medical Electronics work shop, RFHSM). The validity of the integrator threshold setting was verified at the end of the experiment by measuring noise following the abolition of nerve activity after administration of pentobarbitone sodium (20 mg per animal). In a single set of experiments simultaneous recordings of renal, splanchnic and adrenal nerve activities were made. The splanchnic and adrenal nerves were accessible following the retroperitoneal approach used to expose the renal nerve. Recordings of nerve activity were made in a similar manner as described for renal nerve activity except that the splanchnic and adrenal nerves were not crushed. The adrenal nerve was cleared of connective tissue and recordings were made close to the adrenal gland.

At the beginning of each experiment the baroreceptor reflex response was tested by observing whether renal nerve activity and heart rate were decreased by a rise in blood pressure caused by noradrenaline (25 ng per animal, i.v.) and were increased by a reduction in blood pressure caused by sodium nitroprusside (0.6 μg per animal, i.v.). Only preparations with an intact baroreceptor reflex were used. Renal nerve activity was considered to be postganglionic if the activity was abolished by hexamethonium bromide (6 mg kg⁻¹, i.v.) given at the end of each experiment.
Experimental protocols

Two anaesthetized rat models were used. Initial experiments were performed in rats anaesthetized either with urethane alone or with halothane and α-chloralose (see above). Blood pressure and heart rate were measured and the rats were allowed to breathe spontaneously. This initial set of experiments allowed a comparison of the effect of i.c.v. administration of 5-HT in urethane anaesthetized animals (the anaesthetic regimen used in previous studies; see Lambert et al., 1978) and α-chloralose anaesthetized animals (an anaesthetic known to spare the baroreceptor reflex; see Barringer & Buñag, 1990). In a second model, animals were anaesthetized with α-chloralose and artificially ventilated following neuromuscular blockade; sympathetic nerve activity and phrenic nerve activity were recorded. Pharmacological analysis of the response to 5-HT i.c.v. was performed using this model.

Arterial blood pressure, heart rate, the integrator output of renal nerve activity and phrenic nerve activity counts were displayed on a chart recorder (Gould 2600 series). Blood pressure and unfiltered nerve activity from renal and phrenic nerves were recorded on electromagnetic tape using a Racal Store 4 tape recorder.

The preparation was allowed to stabilise for 30 min before the administration of saline (5 μl i.c.v.). After a 5 min control period a single dose of test compound or saline control was given i.c.v. and the response was followed for at least 30 min. In antagonist studies, antagonists administered i.c.v. were given 10 min before the injection of test drug. However, both LY 53857 and spiroxatrine were administered in two doses of 150 nmol kg\(^{-1}\) alone or combined, 5 min apart and the test drug administered 5 min after the last dose of these antagonists. Vehicle for
spiroxatrine (0.01N HCl) was administered in 2 volumes of 5 μl 5 min apart and the test agonist was given 5 min after the last dose of vehicle. When antagonists were given i.v. the test drug was administered 5-10 min later. However, for methiothepin the test drug was administered following a stabilisation period of 20 min. These pretreatment times were chosen to allow stabilisation of any changes in the variables being recorded caused by administration of the antagonists. In each rat the cardiovascular response of a single dose of the test drug was recorded.

2.2.3 Analysis of results

Baseline values were taken 1 minute before the addition of drug or vehicle. All results are expressed as changes from baseline values. Nerve activity was measured as the average of the integrated values over 1 minute in arbitrary units and was expressed as the percentage change from baseline. Changes in mean arterial pressure, heart rate, renal and phrenic nerve activity caused by the test drug were compared with time-matched vehicle controls using two-way analysis of variance and were subsequently analysed using the least significant difference test (Sokal & Rohlf, 1969). Biphasic responses in some variables were observed following i.c.v. administration of 5-HT and these phases were analysed separately. Thus, the maximum change for each phase of the 5-HT response was measured and compared to the maximum change in vehicle controls during the same period using Student’s $t$ test for unpaired data for anaesthetized animals and Student’s $t$ test for paired data for conscious animals. Changes in variables caused by antagonist or vehicle pretreatments were compared to the pre-dose baseline using Student’s $t$ test for paired data. All values are expressed as the mean ± s.e.mean; differences in the mean were taken as significant when $P < 0.05$. 
2.2.4 Drugs and solutions

The drugs used were arginine vasopressin (Parke-Davis, Pontypool, Gwent, U.K.); atenolol (ICI Pharmaceuticals Ltd, Macclesfield, U.K.); atropine methonitrate (Sigma Chemical Co., Poole, Dorset, U.K.); BW501C67 (2-anilino-N-(2-(3-chlorophenoxy)propyl) acetamide HCl);
5-carboxamidotryptamine maleate (5-CT);
N,N-di-n-propyl-5-carboxamidotryptamine maleate (DP-5-CT; these were gifts from Wellcome Research Laboratories, Beckenham, Kent, U.K.);
α-chloralose (Sigma Chemical Co., Poole, Dorset, U.K.); cinanserin HCl (Squibb Inc., Princeton, U.S.A.); 1-(2,5-di-methoxy-4-iodophenyl)-2-aminopropane (DOI; Research Biochemicals Inc., Semat, St. Albans, U.K.); decamethonium iodide (Koch-Light, Haverhill, Suffolk, U.K.);
Gelofusine (Consolidated Chem., Wrexham, Clwyd, U.K.);
8-hydroxy-2-(di-N-propylamino)tetralin HBr (8-OH-DPAT; Research Biochemicals Inc., Semat, St. Albans, U.K.); 5-hydroxytryptamine creatinine sulphate, 5-HT (BDH, Poole, Dorset, U.K.); halothane (ICI Pharmaceuticals Ltd, Macclesfield, U.K.); hexamethonium bromide (Koch-Light, Haverhill, Suffolk, U.K.); idazoxan HCl (Sigma Chemical Co., Poole, Dorset, U.K.); isoprenaline sulphate BP. (Wellcome, London, U.K.);
6-methyl-1-(1-methylethyl)ergoline-8-carboxylic acid
2-hydroxy-1-methyl-propylester-2-butenedioate (LY53857; Eli Lilly, Indianapolis, U.S.A.); [ß-Mercapto-ß,ß-cyclopentamethylenepropionyl]¹, O-Me-Tyr²,Arg⁸]-Vasopressin, (d(CH₂)₅Tyr(Me)AVP; Sigma Chemical Co., Poole, Dorset, U.K.); methiothepin mesylate (Research Biochemicals Inc., Semat, St. Albans, U.K.); noradrenaline acid tartrate (Winthrop, Guildford, Surrey, U.K.); ondansetron (a gift from Glaxo Group Research, Ware, Hertfordshire, U.K.); sodium nitroprusside (Sigma Chemical Co., Poole, Dorset, U.K.); sodium pentobarbitone (Sigma Chemical Co., Poole, Dorset, U.K.); spiroxatrine (a gift from Janssen, Wantage, Oxon, U.K.): sumatriptan
Wellcome Laboratories, Beckenham, Kent, U.K.); and urethane (Sigma Chemical Co., Poole, Dorset, U.K.).

Drugs given i.c.v. were dissolved in 0.9% w/v saline except for spiroxatrine and the combination of spiroxatrine and LY 53857 which were dissolved in 0.01 N hydrochloric acid (HCl). Solutions were administered in a dose volume of 5 µl over a 20 s period. All drugs given i.v. were dissolved in saline. In some experiments an infusion (6 ml kg⁻¹ h⁻¹) of vasopressin (2 mU ml⁻¹) was given i.v. to cause an increase in blood pressure.
2.3 Results

2.3.1 Conscious rats

**Effect of i.c.v. administration of saline.**

Saline i.c.v. (5 μl; n = 6) had little effect on blood pressure or heart rate and these variables remained stable for the duration of the experiment (Figure 1). There were no changes in behaviour associated with administration of saline. Baseline values for blood pressure and heart rate are given in Table 2.1.

**Effect of i.c.v. administration of 5-HT.**

Baseline values of blood pressure and heart rate for this group of experiments are given in Table 2.1. 5-HT 4 nmol kg⁻¹ caused an immediate, significant increase in blood pressure of 16 ± 1 mmHg (maximum; 1-4 min) and heart rate of 70 ± 18 beats min⁻¹ (maximum; 10-30 min). These variables remained elevated for 30 min; Figure 1 shows the time course of effect and maximum changes are illustrated in Figure 2. 5-HT (40 and 120 nmol kg⁻¹) caused an immediate increase in blood pressure (maxima 19 ± 3 and 19 ± 3 mmHg, respectively) which remained elevated for 5-10 min. This was associated with an initial, significant fall in heart rate (nadir 62 ± 12 and 43 ± 9 beats min⁻¹). The initial bradycardia (phase 1; 1-5 min) was followed by a tachycardia (maxima 57 ± 17 and 68 ± 24 beats min⁻¹; phase 2; 10-30 min). The time course of these effects are shown in Figure 1. As biphasic responses were observed for heart rate, each phase of response was analysed (Student’s paired t test) separately and the maximum changes between 1-5 min and 10-30 min were compared to maximum changes in saline controls (Figure 2). The rise in blood pressure caused by 5-HT was independent of dose (Figure 2). However, heart rate changes were dose related, the low dose (4 nmol kg⁻¹) of 5-HT caused monophasic tachycardia whereas higher doses (40 and 120 nmol kg⁻¹)
Figure 1  Conscious rats: a comparison of the changes from baseline values over time (min) caused by i.c.v. saline (□; n = 6), 5-HT 4 nmol kg\(^{-1}\) (▼; n = 6), 5-HT 40 nmol kg\(^{-1}\) (■; n = 6) and 5-HT 120 nmol kg\(^{-1}\) (●; n = 6) in mean arterial blood pressure (MAP) and heart rate (HR). Each point represents the mean value and the vertical lines show s.e.mean.
Figure 2 Conscious rats: histograms showing the maximum changes in mean arterial blood pressure (MAP) and heart rate (HR) during the first phase (1-5 min) and the second phase (10-30 min) of the response to i.c.v. 5-HT (4 nmol kg⁻¹, □ ; 40 nmol kg⁻¹, □□ ; 120 nmol kg⁻¹, □□□ ). Each bar represents the mean value and the vertical lines show s.e.mean. * P < 0.05 and ** P < 0.01 compared (Student’s paired t test) to i.c.v. saline over the same period (not shown).
changes in behaviour were observed following the administration of 5-HT
but these changes were not quantified. 5-HT 4 nmol kg\(^{-1}\) caused an arousal
response (6/6 animals); animals started to explore their cages. 5-HT (40
and 120 nmol kg\(^{-1}\)) caused an initial arousal response which lasted for 3-4
min. This was followed by flat body posture with hindlimb abduction (6/6
animals) that was maintained for approximately 20 min.

2.3.2 Anaesthetized spontaneously breathing rats
The effects of 5-HT i.c.v. were examined in urethane and \(\alpha\)-chloralose
anaesthetized rats. This was to allow a comparison between the
anaesthetic used in previous studies, urethane (see introduction) with
\(\alpha\)-chloralose. The dose of 5-HT used for comparison was 40 nmol kg\(^{-1}\) as
this dose had been previously shown to cause all aspects of the 5-HT
response (see 2.3.1).

Effect of i.c.v. administration of saline in urethane and \(\alpha\)-chloralose
anaesthetized rats.
Saline administered i.c.v. (5 \(\mu\)l) to urethane (\(n = 4\)) or \(\alpha\)-chloralose (\(n = 5\))
anaesthetized rats caused little effect on blood pressure or heart rate and
these variables remained stable for the duration of the experiment (Figures 3
and 4). Baseline values for blood pressure and heart rate are given in Table
2.2.

Effect of i.c.v. administration of 5-HT in urethane and \(\alpha\)-chloralose
anaesthetized rats.
In urethane anaesthetized rats, i.c.v. administration of 5-HT 40 nmol kg\(^{-1}\)
produced a rise in arterial blood pressure of 25 ± 7 mmHg (maximum
Figure 3 Urethane anaesthetized rats: a comparison of the changes from baseline values over time (min) caused by i.c.v. saline (□; n = 4) and 5-HT 40 nmol kg⁻¹ (■; n = 4) in mean arterial blood pressure (MAP) and heart rate (HR). Each point represents the mean value and the vertical lines show s.e.mean. * P < 0.05 and ** P < 0.01 compared (ANOVA) to i.c.v. saline.
Figure 4  α-chloralose anaesthetized rats: (a) a comparison of the changes from baseline values over time (min) caused by i.c.v. saline (□; n = 6) and 5-HT 40 nmol kg\(^{-1}\) (■; n = 5) in mean arterial blood pressure (MAP) and heart rate (HR). Each point represents the mean value and the vertical lines show s.e.mean. (b) Histograms showing the maximum changes in mean arterial blood pressure (MAP) and heart rate (HR) during the first phase (1-5 min) and the second phase (10-30 min) of the response to i.c.v. saline (□) and 5-HT 40 nmol kg\(^{-1}\) (■). Each bar represents the mean value and the vertical lines show s.e.mean. * P < 0.05 and ** P < 0.01 compared (Student’s unpaired t test) to i.c.v. saline over the same period.
change, 2-10 min, n = 4) and an increase in heart rate of 24 ± 11 beats min⁻¹ (maximum change, 1-10 min, n = 4). The onset of these changes was immediate and blood pressure and heart rate remained elevated for 10-15 min. The time course of these changes is shown in Figure 3. In α-chloralose anaesthetized rats, 5-HT 40 nmol kg⁻¹ i.c.v. caused an increase in arterial blood pressure which reached a maximum of 20 ± 3 mmHg between 2-10 min (n = 5). The onset of the pressor response was immediate and the duration was between 25-30 min. The pressor response was associated with biphasic changes in heart rate (Figure 4a). An initial bradycardia (nadir 38 ± 6 beats min⁻¹, 2-4 min) was followed by tachycardia (maximum rise 34 ± 15, 15-30 min). As biphasic responses were observed for heart rate, each phase of response was analysed separately and the maximum changes between 1-5 min (phase 1) and 10-30 min (phase 2) were compared (Student's unpaired t test) to maximum changes in saline controls over the same period (Figure 4b).

Initial (1-5 min) changes in blood pressure and heart rate caused by 5-HT (40 nmol kg⁻¹ i.c.v.) in conscious and anaesthetized rats are compared in Figure 5. A similar profile of response was observed in conscious and α-chloralose anaesthetized rats; a pressor response was associated with initial bradycardia. In urethane anaesthetized rats, 5-HT caused an increase in blood pressure, however, this was associated with initial tachycardia. As the response to 5-HT (40 nmol kg⁻¹ i.c.v.) in α-chloralose anaesthetized animals was similar to that observed in conscious animals, this anaesthetic regimen was used in all subsequent experiments.

Urethane is an antagonist at α₂-adrenoceptors (see 2.1) and this property may affect the response to 5-HT i.c.v. Therefore, in three animals anaesthetized with α-chloralose, idazoxan (100 μg kg⁻¹) was administered i.v. 10 min before the injection of 5-HT (40 nmol kg⁻¹ i.c.v.). Idazoxan
Table 2.1. Baseline values for MAP and HR in conscious rats. Values are the mean ± s.e.mean.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>n</th>
<th>MAP (mmHg)</th>
<th>HR (beats min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline control</td>
<td>6</td>
<td>108 ± 3</td>
<td>357 ± 8</td>
</tr>
<tr>
<td>5-HT 4 nmol kg⁻¹</td>
<td>6</td>
<td>102 ± 1</td>
<td>348 ± 11</td>
</tr>
<tr>
<td>5-HT 40 nmol kg⁻¹</td>
<td>6</td>
<td>109 ± 2</td>
<td>364 ± 13</td>
</tr>
<tr>
<td>5-HT 120 nmol kg⁻¹</td>
<td>6</td>
<td>105 ± 2</td>
<td>349 ± 7</td>
</tr>
</tbody>
</table>

Table 2.2. Baseline values for MAP and HR in anaesthetized rats. Values are the mean ± s.e.mean.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>n</th>
<th>MAP (mmHg)</th>
<th>HR (beats min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urethane Anaesthesia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline control</td>
<td>4</td>
<td>93 ± 5</td>
<td>405 ± 17</td>
</tr>
<tr>
<td>5-HT 40 nmol kg⁻¹</td>
<td>4</td>
<td>86 ± 6</td>
<td>444 ± 27</td>
</tr>
<tr>
<td>Halothane/α-chloralose Anaesthesia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline control</td>
<td>5</td>
<td>117 ± 7</td>
<td>371 ± 16</td>
</tr>
<tr>
<td>5-HT 40 nmol kg⁻¹</td>
<td>5</td>
<td>121 ± 7</td>
<td>384 ± 9</td>
</tr>
</tbody>
</table>

Table 2.3. Baseline values and changes in MAP and HR following the administration of atropine methonitrate (n = 5) and atenolol (n = 4) in α-chloralose anaesthetized rats. Values are the mean ± s.e.mean. Significant changes (Student's paired t test) in baseline values caused by the pretreatments are shown as * P < 0.05.

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Atropine</th>
<th>Atenolol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose</td>
<td>0.5 mg kg⁻¹</td>
<td>1 mg kg⁻¹</td>
</tr>
<tr>
<td>Injection route</td>
<td>i.v.</td>
<td>i.v.</td>
</tr>
<tr>
<td>Pretreatment time</td>
<td>10 min</td>
<td>10 min</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Basal</th>
<th>Change</th>
<th>Basal</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mmHg)</td>
<td>91 ± 3</td>
<td>6 ± 3</td>
<td>110 ± 8</td>
<td>2 ± 3</td>
</tr>
<tr>
<td>HR (beats min⁻¹)</td>
<td>366 ± 16</td>
<td>39 ± 8*</td>
<td>391 ± 13</td>
<td>-35 ± 11*</td>
</tr>
</tbody>
</table>
pretreatment caused no significant change in baseline blood pressure and
heart rate values which were 124 ± 18 mmHg and 376 ± 28 beats min⁻¹,
respectively. In the presence of idazoxan, 5-HT (40 nmol kg⁻¹ i.c.v.)
produced an immediate rise in blood pressure (maximum rise 16 ± 6
mmHg, 1-5 min) with little change in heart rate (-1 ± 3 beats min⁻¹).

Effects of pretreatment with either atropine methonitrate or atenolol on the
response to 5-HT in α-chloralose anaesthetised rats.

Preliminary studies in separate animals showed that:-
a) Pretreatment with atropine methonitrate (0.5 mg kg⁻¹, i.v., 10 min)
abolished the bradycardia induced by right vagus nerve stimulation
(stimulation parameters :- frequency 16 Hz , supramaximal voltage 10 v ,
duration 5 sec, pulse width 0.4 msec, 3 minute interval). The duration of
block was in excess of 1 h (n = 2; data not shown).
b) Pretreatment with atenolol (1 mg kg⁻¹, i.v., 10 min) abolished the
tachycardia produced by the β-adrenoceptor agonist, isoprenaline (10-100
ng kg⁻¹, i.v.). The duration of block was in excess of 1 h (n = 2; data not
shown).

Administration of either atropine methonitrate (0.5 mg kg⁻¹, i.v.; n = 5) or
atenolol (1 mg kg⁻¹, i.v.; n = 4) had no effect on basal blood pressure.
However, atropine methonitrate caused a significant increase in heart rate
whereas atenolol pretreatment caused a significant reduction in heart rate
(Table 2.3). Neither atropine methonitrate nor atenolol affected the
magnitude of the blood pressure rise caused by 5-HT (40 nmol kg⁻¹ i.c.v.;
Figure 6). However, the initial bradycardia caused by 5-HT was
significantly attenuated by both atropine methonitrate and atenolol (Figure
6). The tachycardic phase of the 5-HT response was significantly
attenuated by pretreatment atropine methonitrate (Figure 6).
Figure 5 Histograms comparing the maximum changes from baseline values in mean arterial blood pressure (MAP) and heart rate (HR) in the first phase (1-5 min) of the response to i.c.v. 5-HT 40 nmol kg⁻¹ in conscious rats (□; n=6), α-chloralose anaesthetized rats (◼; n=5) and urethane anaesthetized rats (▲; n=4). Each bar represents the mean value and the vertical lines show s.e.mean.
Figure 6 α-chloralose anaesthetized rats: a comparison of the changes from baseline values over time (min) caused by 5-HT (40 nmol kg\(^{-1}\), i.c.v.) in non-pretreated control animals ( ■ ; \( n = 5\)) and animals pretreated with atropine methonitrate ( ▽ ; 0.5 mg kg\(^{-1}\), i.v.; \( n = 5\)) or atenolol ( ○ ; 1 mg kg\(^{-1}\), i.v.; \( n = 4\)) in mean arterial blood pressure (MAP) and heart rate (HR). Each point represents the mean value and the vertical lines show s.e.mean. * \( P < 0.05\) and ** \( P < 0.01\) compared (ANOVA) to i.c.v. 5-HT in the absence of antagonist.
2.3.3 Anaesthetized artificially respired rats

In this group of experiments sympathetic nerve activity and phrenic nerve activity as well as blood pressure and heart rate were recorded in $\alpha$-chloralose anaesthetized rats which were artificially respired following neuromuscular blockade with decamethonium.

Effect of i.c.v. administration of saline.

Saline i.c.v. (5 $\mu$l; n = 6) had little effect on blood pressure, heart rate, renal or phrenic nerve activity and these variables remained stable for the duration of the experiment (see Figure 7a). Baseline values for blood pressure and heart rate are given in Table 2.4.

Effect of i.c.v. administration of 5-HT

5-HT [40 (n = 6); 120 (n = 8) nmol kg$^{-1}$] caused immediate, dose-related increases in arterial blood pressure (Figure 7) which reached maxima, between 1 and 5 min after injection, of 6 ± 1 and 19 ± 2 mmHg respectively. The rise in blood pressure remained elevated for 5 min after the low dose and 20 min after the high dose. In addition, 5-HT (40 and 120 nmol kg$^{-1}$) caused initial significant falls in heart rate of 17 ± 5 and 16 ± 2 beats min$^{-1}$ and in renal nerve activity of 23 ± 8 % and 41 ± 8 %, respectively. The initial decrease in these variables (phase 1) was followed by an increase (phase 2). Maximum increases in heart rate and renal nerve activity produced by 5-HT (40 and 120 nmol kg$^{-1}$) were 19 ± 5 and 63 ± 14 beats min$^{-1}$ and 47 ± 15 and 137 ± 32 %, respectively and occurred between 10-20 min. These biphasic changes in heart rate and renal nerve activity were temporally matched (Figure 7a). 5-HT (120 nmol kg$^{-1}$) caused an initial (1-5 min) significant reduction in phrenic nerve activity of 22 ± 5 %. In 3 animals this was followed by a secondary rise in phrenic nerve activity (227 ± 84 %, 10-20 min). However, in the remaining animals phrenic nerve activity returned to baseline levels after 5
<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>n</th>
<th>MAP (mmHg)</th>
<th>HR (beats min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 μl</td>
<td>6</td>
<td>122 ± 6</td>
<td>417 ± 13</td>
</tr>
<tr>
<td>5-HT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40 nmol kg⁻¹</td>
<td>6</td>
<td>112 ± 6</td>
<td>417 ± 31</td>
</tr>
<tr>
<td>120 nmol kg⁻¹</td>
<td>8</td>
<td>112 ± 4</td>
<td>417 ± 18</td>
</tr>
<tr>
<td>DOI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 nmol kg⁻¹</td>
<td>4</td>
<td>112 ± 9</td>
<td>406 ± 18</td>
</tr>
<tr>
<td>40 nmol kg⁻¹</td>
<td>4</td>
<td>126 ± 5</td>
<td>424 ± 21</td>
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<td>120 nmol kg⁻¹</td>
<td>6</td>
<td>124 ± 5</td>
<td>430 ± 8</td>
</tr>
<tr>
<td>120 nmol kg⁻¹, i.v.</td>
<td>3</td>
<td>117 ± 4</td>
<td>429 ± 22</td>
</tr>
<tr>
<td>5-CT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 nmol kg⁻¹</td>
<td>5</td>
<td>115 ± 7</td>
<td>417 ± 15</td>
</tr>
<tr>
<td>DP-5-CT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.3 nmol kg⁻¹</td>
<td>4</td>
<td>118 ± 5</td>
<td>427 ± 11</td>
</tr>
<tr>
<td>3 nmol kg⁻¹</td>
<td>7</td>
<td>114 ± 4</td>
<td>442 ± 9</td>
</tr>
<tr>
<td>8-OH-DPAT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 nmol kg⁻¹</td>
<td>6</td>
<td>96 ± 4</td>
<td>391 ± 11</td>
</tr>
<tr>
<td>40 nmol kg⁻¹</td>
<td>8</td>
<td>122 ± 5</td>
<td>428 ± 10</td>
</tr>
<tr>
<td>120 nmol kg⁻¹</td>
<td>8</td>
<td>108 ± 2</td>
<td>411 ± 16</td>
</tr>
<tr>
<td>sumatriptan</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 nmol kg⁻¹</td>
<td>6</td>
<td>114 ± 4</td>
<td>392 ± 12</td>
</tr>
</tbody>
</table>

Table 2.4. Baseline values for MAP and HR in α-chloralose anaesthetized rats which were artificially resired following neuromuscular blockade. All compounds were administered i.c.v. unless stated. Values are the mean ± s.e.mean.
Figure 7 Artificially respired α-chloralose anaesthetized rats: (a) a comparison of the changes from baseline values over time (min) caused by i.c.v. saline (●; n = 6), 5-HT 40 nmol kg⁻¹ (Δ; n = 6) and 5-HT 120 nmol kg⁻¹ (□; n = 8) in mean arterial blood pressure (MAP), heart rate (HR), renal nerve activity (RNA) and phrenic nerve activity (PNA). Each point represents the mean value and the vertical lines show s.e.mean. (b) Histograms showing the maximum changes in these variables during the first phase (1-5 min) and the second phase (10-30 min) of the response to i.c.v. 5-HT (40 nmol kg⁻¹, [ ] ; 120 nmol kg⁻¹, [ ]). Each point represents the mean value and the vertical bars show s.e.mean. * P < 0.05 and ** P < 0.01 compared (Student’s unpaired t test) to i.c.v. saline over the same periods.
min. Baseline values for blood pressure and heart rate for 5-HT 40 and 120 nmol kg\(^{-1}\) are shown in Table 2.4. As biphasic responses were observed for heart rate and renal nerve activity, each phase of response was analysed (Student's paired t test) separately and the maximum changes between 1-5 min (phase 1) and 10-30 (phase 2) min were compared to maximum changes in saline controls (Figure 7b). A representative tracing of the response to 5-HT 120 nmol kg\(^{-1}\) is shown in Figure 8.

The effect of pretreatment (i.c.v) with either cinanserin or LY 53857 on the response to 5-HT

The effect of central 5-HT\(_2/5-HT_{1C}\) receptor blockade on the response to 5-HT i.c.v. was examined and representative tracings of the effects of cinanserin and LY 53857 on this response are shown in Figure 8 and 9. Cinanserin (300 nmol kg\(^{-1}\), i.c.v.; n = 6) or LY 53857 (300 nmol kg\(^{-1}\), given in 2 i.c.v. doses of 150 nmol kg\(^{-1}\) 5 min apart; n = 6) had no significant (Student's paired t test) effect on baseline values for blood pressure, heart rate, renal nerve activity or phrenic nerve activity (Table 2.5). Baseline values for blood pressure and heart rate for these groups are shown in Table 2.5. Neither drug prevented the rise in blood pressure caused by 5-HT (120 nmol kg\(^{-1}\)) but LY 53857 reduced the duration of the pressor response (Figure 10). In the presence of LY 53857 or cinanserin the initial bradycardia and inhibition of renal nerve activity caused by 5-HT were prevented and immediate monophasic increases in these variables were observed (Figure 10). The tachycardia and renal sympatoexcitation were maximal after 5 min and were maintained for 30 min. The increases in these variables were similar in magnitude to the second phase tachycardia and renal sympatoexcitation caused by 5-HT in the absence of antagonist. The 5-HT induced changes in phrenic nerve activity were not significantly altered by cinanserin and LY 53857 (Figure 10).
<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Dose</th>
<th>Injection route</th>
<th>Pretreatment time</th>
<th>MAP (mmHg)</th>
<th>HR (beats min⁻¹)</th>
<th>RNA (%)</th>
<th>PNA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cinanserin</td>
<td>300 nmol kg⁻¹</td>
<td>i.c.v.</td>
<td>Basal: 102 ± 4</td>
<td>398 ± 10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>LY 53857</td>
<td>300 nmol kg⁻¹</td>
<td>i.c.v.</td>
<td>Basal: 102 ± 5</td>
<td>391 ± 13</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>BW501C67</td>
<td>100 μg kg⁻¹</td>
<td>i.v.</td>
<td>Basal: 115 ± 11</td>
<td>420 ± 22</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 2.5. Baseline values and changes in MAP, HR and renal (RNA) and phrenic (PNA) nerve activities following the administration of cinanserin (n = 6), LY 53857 (given as a split dose of 2 × 150 nmol kg⁻¹ at 0 and 5 min; n = 6) and BW501C67 (n = 4) in α-chloralose anaesthetized, artificially respired rats. Values are the mean ± s.e.mean. There were no significant changes (Student’s paired t test) in baseline values following the administration of cinanserin, LY 53857 or BW501C67.
Figure 8 Traces showing the effects of i.c.v 5-HT (120 nmol kg⁻¹) in the presence of (a) saline (5μl, i.c.v.) and (b) cinanserin (300 nmol kg⁻¹, i.c.v.) and the effects of (c) DP-5-CT (3 nmol kg⁻¹, i.c.v.) on arterial blood pressure (BP), heart rate (HR), renal nerve activity (RNA) and phrenic nerve activity (PNA) in artificially respired α-chloralose anaesthetized rats.
Figure 9 Traces showing the effects of i.c.v 5-HT (120 nmol kg⁻¹) in the presence of (a) saline, (b) LY 53857 (300 nmol kg⁻¹, i.c.v.) and (c) dC₂H₅Tyr(Me)AVP (10 μg kg⁻¹, i.v.) on arterial blood pressure (BP), heart rate (HR), renal nerve activity (RNA) and phrenic nerve activity (PNA) in artificially respired α-chloralose anaesthetized rats.
Figure 10 Artificially respired α-chloralose anaesthetized rats: a comparison of the changes from post-pretreatment values over time (min) caused by 5-HT (120 nmol kg\(^{-1}\), i.c.v.) in the presence of saline (□ ; 5 μl i.c.v.; n = 8), cinanserin (● ; 300 nmol kg\(^{-1}\), i.c.v.; n = 6) or LY 53857 (○ ; 300 nmol kg\(^{-1}\), i.c.v.; n = 6) in mean arterial blood pressure (MAP), heart rate (HR), renal nerve activity (RNA) and phrenic nerve activity (PNA). Each point represents the mean value and the vertical lines show s.e.mean. * P < 0.05 and ** P < 0.01 compared (ANOVA) to 5-HT in the presence of saline.
Effect of i.v. pretreatment with BW501C67 on the response to i.c.v. 5-HT

The effect of peripheral 5-HT$_2$/5-HT$_1C$ receptor blockade on the response to 5-HT i.c.v. was examined. Preliminary studies in separate animals showed that a 5 min pretreatment with BW501C67 100 µg kg$^{-1}$ i.v. abolished the pressor response and the bronchoconstriction produced by 5-HT 1-100 µg kg$^{-1}$ i.v. in vagotomized anaesthetized rats treated with atenolol (1 mg kg$^{-1}$, i.v.). Pretreatment with BW501C67 (100 µg kg$^{-1}$ i.v.; n = 4) had no significant (Student's paired t test) effect on blood pressure, heart rate, renal nerve activity or phrenic nerve activity (Table 2.5). 5-HT (120 nmol kg$^{-1}$) administered i.c.v. in animals pretreated with BW501C67 caused similar effects to those observed in non-pretreated animals; there was an immediate increase in blood pressure and biphasic changes in heart rate and renal nerve activity. However, the duration of the bradycardia and the renal sympathoinhibition was significantly prolonged (Figure 11). Baseline values for blood pressure and heart rate are given in Table 2.5. The profile of response produced by 5-HT i.c.v. in the presence of BW501C67 was dissimilar to that observed in animals pretreated i.c.v. with the 5-HT$_2$/5-HT$_1C$ receptor antagonists cinanserin and LY 53857 (compare Figures 10 and 11)

The effect of combined pretreatment (i.c.v.) with LY 53857 and spiroxatrine on the response to 5-HT

The effect of 5-HT$_{1A}$ receptor blockade on the pressor response, tachycardia and renal sympathoinhibition caused by 5-HT in the presence of central 5-HT$_2$/5-HT$_1C$ receptor blockade was investigated. Combined pretreatment with LY 53857 (300 nmol kg$^{-1}$) and spiroxatrine (300 nmol kg$^{-1}$; n = 4) (administered in a split dose of 150 nmol kg$^{-1}$ i.c.v. 5 min apart) did not significantly change baseline values (see Table 2.6). However, the effect of 5-HT (120 nmol kg$^{-1}$ i.c.v.) on all variables was significantly (ANOVA) reduced in animals pretreated with this combination
Figure 11  Artificially respired $\alpha$-chloralose anaesthetized rats: a comparison of the changes from baseline or post-pretreatment values over time (min) caused by 5-HT (120 nmol kg$^{-1}$, i.c.v.) in the absence (□; n = 8) and presence of BW501C67 0.1 mg kg$^{-1}$ i.v. (■; n = 4) in mean arterial blood pressure (MAP), heart rate (HR), renal nerve activity (RNA) and phrenic nerve activity (PNA). Each point represents the mean value and the vertical lines show s.e.mean. * P < 0.05 and ** P < 0.01 compared (ANOVA) to 5-HT in non-pretreated animals.
Figure 12  Artificially respired α-chloralose anaesthetized rats: a comparison of the changes from post-pretreatment values over time (min) caused by 5-HT (120 nmol kg\(^{-1}\), i.c.v.) in the presence of LY 53857 (300 nmol kg\(^{-1}\), i.c.v.; ○; \(n = 6\)) with the combined pretreatment of LY 53857 (300 nmol kg\(^{-1}\), i.c.v.) and spiroxatrine (300 nmol kg\(^{-1}\), i.c.v.; ▲; \(n = 4\)) in mean arterial blood pressure (MAP), heart rate (HR), renal nerve activity (RNA) and phrenic nerve activity (PNA). Each point represents the mean value and the vertical lines show s.e.mean. * \(P < 0.05\) and ** \(P < 0.01\) compared (ANOVA) to 5-HT in the presence of LY 53857.
Table 2.6. Baseline values and changes in MAP, HR and renal (RNA) and phrenic (PNA) nerve activities following the combined administration of LY 53857 and spiroxatrine (given as a split dose of 2 x 150 nmol kg⁻¹ at 0 and 5 min; n = 4) and administration of d(CH₂)₅Tyr(Me)AVP (n = 5) and ondansetron (n = 5) in α-chloralose anaesthetized, artificially resired. Values are the mean ± s.e.mean. There were no significant changes (Student's paired t test) in baseline values following administration of LY 53857 and spiroxatrine, d(CH₂)₅Tyr(Me)AVP or ondansetron.
compared to animals pretreated with LY 53857 (300 nmol kg\(^{-1}\) i.c.v.) alone (see Figure 12). The combination of LY 53857 and spiroxatrine was dissolved in 0.01N HCl (vehicle for spiroxatrine) whereas previously LY 53857 had been dissolved in saline. Therefore, in 2 separate experiments to control for the effect of acidified vehicle on the effect of LY 53857, animals were pretreated with LY 53857 dissolved in 0.01N HCl. In these experiments the response to 5-HT (120 nmol kg\(^{-1}\) i.c.v.; data not shown) was similar to that observed previously in animals pretreated with LY 53857 alone dissolved in saline.

The effect of pretreatment (i.v) with d(CH\(_2\)_5Tyr(Me)AVP on the response to 5-HT

In six experiments, the effect of vasopressin V\(_1\)-receptor antagonism on the response to 5-HT i.c.v. was examined and a representative tracing is depicted in Figure 9. d(CH\(_2\)_5Tyr(Me)AVP (10 \(\mu\)g kg\(^{-1}\) i.v.) had no significant effect on blood pressure, heart rate, renal or phrenic nerve activity. Baseline values and changes caused by a 5 min pretreatment of d(CH\(_2\)_5Tyr(Me)AVP are given in Table 2.6. Pretreatment with d(CH\(_2\)_5Tyr(Me)AVP did not prevent the rise in blood pressure caused by 5-HT (120 nmol kg\(^{-1}\) i.c.v.) but the duration of the pressor response was significantly (ANOVA) attenuated, see Figure 13. The 5-HT-induced bradycardia and renal sympathoinhibition were reversed to an immediate tachycardia and sympathoexcitation in the presence of d(CH\(_2\)_5Tyr(Me)AVP (Figure 13). Changes in phrenic nerve activity caused by 5-HT were unaffected. The profile of response produced by 5-HT i.c.v. in the presence of d(CH\(_2\)_5Tyr(Me)AVP was similar to that observed in animals pretreated with the 5-HT\(_2\)/5-HT\(_1\)C receptor antagonists cinanserin and LY 53857 (see Figures 8 and 9).
Figure 13 Artificially respired α-chloralose anaesthetized rats: a comparison of the changes from baseline or post-pretreatment values over time (min) caused by 5-HT (120 nmol kg\(^{-1}\), i.c.v.) in the absence (□; n = 8) and presence of dC\(_2\)H\(_5\)Tyr(Me)AVP 10 μg kg\(^{-1}\) i.v. (▲; n = 6) in mean arterial blood pressure (MAP), heart rate (HR), renal nerve activity (RNA) and phrenic nerve activity (PNA). Each point represents the mean value and the vertical lines show s.e.mean. * P < 0.05 and ** P < 0.01 compared (ANOVA) to 5-HT in non-pretreated animals.
In three separate experiments d(CH$_2$)$_5$Tyr(Me)AVP (10 µg kg$^{-1}$ i.v.; 5 min pretreatment) was shown to abolish the pressor response produced by an infusion of vasopressin (2 mU kg$^{-1}$ min$^{-1}$). Vasopressin caused an increase in blood pressure of 22 ± 6 mmHg (basal 100 ± 4 mmHg) after 5 min of infusion; this was associated with a bradycardia of 46 ± 6 beats min$^{-1}$ (basal 390 ± 11 beats min$^{-1}$) and a reduction in renal nerve activity of 69 ± 10%. After 30 min the vasopressin challenge was repeated in the presence of d(CH$_2$)$_5$Tyr(Me)AVP (10 µg kg$^{-1}$ i.v.; 5 min pretreatment) and the response was significantly (Student's paired $t$ test) attenuated (blood pressure change 0 ± 1 mmHg after 5 min, $P < 0.05$; heart rate change 2 ± 1 beats min$^{-1}$, $P < 0.01$; renal nerve activity change 0 ± 4%, $P < 0.01$)

**Effect of pretreatment (i.v.) of ondansetron (GR 38032) on the response to 5-HT**

The effect of 5-HT$_3$ receptor blockade on the response to 5-HT i.c.v. was examined. Pretreatment with ondansetron (1 mg kg$^{-1}$ i.v.; $n = 5$) had no significant (Student's paired $t$ test) effect on blood pressure, heart rate, renal nerve activity or phrenic nerve activity (Table 2.6). 5-HT (120 nmol kg$^{-1}$) administered i.c.v. in animals pretreated with ondansetron caused similar effects to those observed in non-pretreated animals; there was an immediate increase in blood pressure associated with initial reductions in heart rate and renal nerve activity. The initial decrease in these variables was followed by an increase (Figure 14). Phrenic nerve activity was not significantly changed. Baseline values for blood pressure and heart rate are given in Table 2.6.
Figure 14 Artificially respired α-chloralose anaesthetized rats: a comparison of the changes from baseline or post-pretreatment values over time (min) caused by 5-HT (120 nmol kg⁻¹, i.c.v.) in the absence (□; n = 8) and presence of odansetron 1 mg kg⁻¹ i.v. (▲; n = 5) in mean arterial blood pressure (MAP), heart rate (HR), renal nerve activity (RNA) and phrenic nerve activity (PNA). Each point represents the mean value and the vertical lines show s.e.mean. * P < 0.05 and ** P < 0.01 compared (ANOVA) to 5-HT in non-pretreated animals.
Effect of i.c.v. and i.v. administration of DOI

The effect of central and peripheral administration of the 5-HT$_2$/5-HT$_1$C receptor agonist, DOI (see introduction) was investigated. 12 nmol kg$^{-1}$ DOI (i.c.v.) had no effect on blood pressure, heart rate, renal and phrenic nerve activity (data not shown; n = 4). DOI (40 nmol kg$^{-1}$, n = 4; 120 nmol kg$^{-1}$, n = 6; i.c.v.) produced maximum increases in blood pressure of 7 ± 1 and 10 ± 2 mmHg, respectively and decreases in heart rate and renal nerve activity of 18 ± 6 and 19 ± 9 beats min$^{-1}$ and 31 ± 9 and 53 ± 7 %, respectively 5 min after injection. There was no change in phrenic nerve activity (Figure 15). Baseline values for blood pressure and heart rate are given in Table 2.4.

DOI 120 nmol kg$^{-1}$ (n = 3) given i.v. produced a rise in blood pressure of 22 ± 2 mmHg which was maintained for 20 min. Bradycardia and renal sympathoinhibition were observed and reached maxima of 21 ± 3 beats min$^{-1}$ and 54 ± 6 % respectively 1 min following injection. The bradycardia was not maintained and had returned to baseline by 10 min. However, the renal sympathoinhibition was maintained for 20 min. Phrenic nerve activity was not measured in these animals. Baseline values for blood pressure and heart rate are shown in Table 2.4. Pretreatment with BW501C67 (0.1 mg kg$^{-1}$ i.v.; n = 2) abolished the rise in blood pressure caused by i.v. DOI (data not illustrated). DOI (120 nmol kg$^{-1}$) produced a similar response independent of peripheral or central administration.

Effect of pretreatment (i.v.) with BW501C67 on the response to i.c.v. DOI

The effect of antagonism of peripheral 5-HT$_2$/5-HT$_1$C receptors on the response to DOI i.c.v. was examined. Pretreatment with BW501C67 (0.1 mg kg$^{-1}$ i.v.), which had no effect per se (Table 2.7), significantly (ANOVA) attenuated the rise in blood pressure, bradycardia and renal sympathoinhibition caused by DOI (120 nmol kg$^{-1}$; n = 4; Figure 16).
Figure 15 Artificially respired α-chloralose anaesthetized rats: a comparison of the changes over time (min) from baseline values caused by i.c.v. saline (●; n=6), DOI 40 nmol kg⁻¹ (□; n=4) and DOI 120 nmol kg⁻¹ (○; n=6) in mean arterial blood pressure (MAP), heart rate (HR), renal nerve activity (RNA) and phrenic nerve activity (PNA). Each point represents the mean value and the vertical lines show s.e.mean. * P < 0.05 and ** P < 0.01 compared (ANOVA) to i.c.v. saline.
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Table 2.7. Baseline values and changes in MAP, HR and renal (RNA) and phrenic (PNA) nerve activities following administration of BW501C67 (n = 4) and d(CH₂)₅Tyr(Me)AVP (n = 3) in α-chloralose anaesthetized, artificially respired rats. Values are the mean ± s.e.mean. There were no significant changes (Student's paired t test) in baseline values following administration of BW501C67 or d(CH₂)₅Tyr(Me)AVP.
Figure 16  Artifically respired α-chloralose anaesthetized rats: a comparison of the changes over time (min) from baseline or post-pretreatment values caused by i.c.v. DOI 120 nmol kg⁻¹ in the absence (○; n=6) and presence of BW501C67 0.1 mg kg⁻¹ i.v. (●; n=4) in mean arterial blood pressure (MAP), heart rate (HR), renal nerve activity (RNA) and phrenic nerve activity (PNA). Each point represents the mean value and the vertical lines show s.e.mean. * P < 0.05 and ** P < 0.01 compared (ANOVA) to DOI in non-pretreated animals.
Figure 17 Artificially respired α-chloralose anaesthetized rats: a comparison of the changes over time (min) from baseline or post-pretreatment values caused by i.c.v. DOI 120 nmol kg⁻¹ in the absence (○; n = 6) and presence of dC₂H₅Tyr(Me)AVP 10 μg kg⁻¹ i.v. (●; n = 3) in mean arterial blood pressure (MAP), heart rate (HR), renal nerve activity (RNA) and phrenic nerve activity (PNA). Each point represents the mean value and the vertical lines show s.e.mean. * P < 0.05 and ** P < 0.01 compared (ANOVA) to DOI in non-pretreated animals.
Baseline values for blood pressure and heart rate in animals pretreated with BW501C67 are given in Table 2.7. Therefore, the response produced by i.c.v. administration of DOI was attenuated following peripheral 5-HT$_2$/5-HT$_1$C receptor blockade.

Effect of i.v. pretreatment with d(CH$_2$)$_5$Tyr(Me)AVP on the response to i.c.v. DOI
To determine the effect of vasopressin V$_1$-receptor blockade on the response caused by central administration of DOI, three animals were pretreated with d(CH$_2$)$_5$Tyr(Me)AVP (10 μg kg$^{-1}$ i.v.) 5 min before the addition of DOI (120 nmol kg$^{-1}$ i.c.v.). d(CH$_2$)$_5$Tyr(Me)AVP caused no significant (Student's paired t test) changes in baseline values (see Table 2.7). In the presence of d(CH$_2$)$_5$Tyr(Me)AVP, DOI caused an immediate increase in blood pressure associated with a bradycardia and a reduction in renal nerve activity. Phrenic nerve activity was not changed. The initial (1-5 min) rise in blood pressure was significantly (ANOVA) greater in animals pretreated with d(CH$_2$)$_5$Tyr(Me)AVP compared to non-pretreated animals (see Figure 17), the bradycardia and renal sympathoinhibition were of a similar magnitude in pretreated and non-pretreated animals. Therefore, antagonism of vasopressin V$_1$-receptors did not prevent the response produced by i.c.v. administration of DOI.

Effect of i.c.v. administration of 5-HT$_1$ receptor agonists
In this group of experiments the effect of selective agonists for the various subtypes of the 5-HT$_1$ receptor category were examined in anaesthetized, artificially respired rats.

Effect of i.c.v. administration of 5-CT
5-CT (3 nmol kg$^{-1}$ i.c.v.; n = 5) caused an immediate, significant rise in blood pressure of 17 ± 5 mmHg after 2 min. This rise in blood pressure
Figure 18 Artificially respired α-chloralose anaesthetized rats: a comparison of the changes over time (min) from baseline values caused by i.c.v. saline (○; n = 6) and 5-CT (■; 3 nmol kg⁻¹; n = 5) in mean blood pressure (MAP), heart rate (HR), renal nerve activity (RNA) and phrenic nerve activity (PNA). Each point represents the mean value and the vertical lines show s.e.mean. * P < 0.05 and ** P < 0.01 compared to i.c.v. saline.
was associated with significant increases in heart rate of $48 \pm 7$ beats min$^{-1}$ and in renal nerve activity of $77 \pm 35\%$ (Figure 18). The rise in heart rate and renal nerve activity were temporally matched and maintained for 20 min. However, blood pressure remained elevated for only 2 min (Figure 18) and in three animals, between 3 and 5 min after injection, the rise in blood pressure was followed by a substantial fall of $21 \pm 3$ mmHg below baseline. 5-CT caused no significant changes in phrenic nerve activity. Baseline values for blood pressure and heart rate are shown in Table 2.4.

**Effect of i.c.v. and i.v. administration of 8-OH-DPAT**

8-OH-DPAT (3 nmol kg$^{-1}$; $n=6$) caused significant increases in blood pressure, heart rate and renal nerve activity (Figure 19). The onset of these changes was immediate and was maximal at 10 minutes, reaching $7 \pm 3$ mmHg, $49 \pm 11$ beats min$^{-1}$ and $57 \pm 17\%$, respectively. Phrenic nerve activity was also significantly increased ($75 \pm 24\%$, 15 min), however the onset of this response was delayed, see Figure 19. Baseline values for blood pressure and heart rate are given in Table 2.4. Higher doses, 40 (n=8) and 120 (n=8) nmol kg$^{-1}$, of 8-OH-DPAT produced small reductions in blood pressure of $5 \pm 2$ and $3 \pm 1$ mmHg, respectively. These blood pressure changes were associated with dose-related tachycardia of $21 \pm 5$ and $38 \pm 14$ beats min$^{-1}$, respectively, after 10 min. 8-OH-DPAT (40 and 120 nmol kg$^{-1}$) produced variable changes in renal nerve activity which did not reach significance (ANOVA). Phrenic nerve activity was significantly increased 10 min after the administration of 8-OH-DPAT 40 nmol kg$^{-1}$ (Figure 20). Baseline values for blood pressure and heart rate are shown in Table 2.4.

In some animals 8-OH-DPAT 40 $\mu$g kg$^{-1}$ (120 nmol kg$^{-1}$; $n=8$) was administered i.v. 1 h after the i.c.v. dose. 8-OH-DPAT caused reductions
Figure 19  Artificially respired α-chloralose anaesthetized rats: a comparison of the changes over time (min) from baseline values caused by i.c.v. saline (○; n=6) and 8-OH-DPAT (●; 3 nmol kg⁻¹; n=6) in mean blood pressure (MAP), heart rate (HR), renal nerve activity (RNA) and phrenic nerve activity (PNA). Each point represents the mean value and the vertical lines show s.e.mean. * P < 0.05 and ** P < 0.01 compared to i.c.v. saline.
Figure 20  Artificially respired α-chloralose anaesthetized rats: a comparison of the changes over time (min) from baseline values caused by i.c.v. saline (○; n=6), 8-OH-DPAT 40 nmol kg⁻¹ (□; n=8) and 8-OH-DPAT 120 nmol kg⁻¹ (■; n=8) in mean blood pressure (MAP), heart rate (HR), renal nerve activity (RNA) and phrenic nerve activity (PNA). Each point represents the mean value and the vertical lines show s.e.mean. * P < 0.05 and ** P < 0.01 compared to i.c.v. saline.
in blood pressure, heart rate and renal nerve activity of 9 ± 1 mmHg (basal 113 ± 5 mmHg), 25 ± 4 beats min⁻¹ (basal 452 ± 8 beats min⁻¹) and 47 ± 8 %, respectively. Phrenic nerve activity was not quantified. Therefore, the response observed following the administration of 8-OH-DPAT was dose-related and dependent on the route of administration.

**Effect of i.c.v. administration of DP-5-CT**

DP-5-CT (0.3 nmol kg⁻¹; n = 4) caused immediate, significant (ANOVA) increases in heart rate and renal nerve activity (reaching a maximum by 10 min of 30 ± 9 beats min⁻¹ and 28 ± 7 %, respectively) without causing a change in blood pressure (Figure 21). A higher dose of DP-5-CT (3 nmol kg⁻¹; n = 8) caused significant (ANOVA) increases in blood pressure, heart rate and renal nerve activity reaching a maximum by 5 min of 9 ± 3 mmHg, 39 ± 5 beats min⁻¹ and 83 ± 15 %, respectively. These changes were maintained for at least 30 min. There was no significant change in phrenic nerve activity with DP-5-CT (0.3, 3 nmol kg⁻¹ i.c.v.). A representative trace of the response of DP-5-CT (3 nmol kg⁻¹) is depicted in Figure 8 and a time course of response is shown in Figure 21. Baseline values for blood pressure and heart rate for these animals are given in Table 2.4. The administration of DP-5-CT (3 nmol kg⁻¹ i.c.v.) was repeated in three animals in which renal, splanchnic and adrenal sympathetic nerve activities were recorded. Blood pressure, heart rate and phrenic nerve activity were also recorded in these animals. Initially, the response to i.c.v. administration of saline (5 μl; n = 3) was recorded in these animals to provide vehicle control experiments. Saline caused little change in any of the variables being recorded (Figure 22; basal blood pressure and heart rate were 105 ± 1 mmHg and 399 ± 22 beats min⁻¹). At least 30 min after the administration of saline, DP-5-CT was administered i.c.v. DP-5-CT (3 nmol kg⁻¹; n = 3) caused immediate, significant (ANOVA; compared to i.c.v. saline)
Figure 21 Artificially respired \( \alpha \)-chloralose anaesthetized rats: a comparison of the changes over time (min) from baseline values caused by i.c.v. saline ( \( \bigcirc \); \( n = 6 \)), DP-5-CT 0.3 nmol kg\(^{-1} \) ( \( \blacktriangle \); \( n = 4 \)) and DP-5-CT 3 nmol kg\(^{-1} \) ( \( \blacktriangledown \); \( n = 8 \)) in mean blood pressure (MAP), heart rate (HR), renal nerve activity (RNA) and phrenic nerve activity (PNA). Each point represents the mean value and the vertical lines show s.e.mean. * \( P < 0.05 \) and ** \( P < 0.01 \) compared to i.c.v. saline.
Figure 22 Artificially respired α-chloralose anaesthetized rats: a comparison of the changes over time (min) from baseline values caused by i.c.v. saline (○; n=3) and DP-5-CT 3 nmol kg⁻¹ (▼; n=3) in mean blood pressure (MAP), heart rate (HR), renal nerve activity (RNA), splanchnic nerve activity (SNA), adrenal nerve activity (ANA) and phrenic nerve activity (PNA). Each point represents the mean value and the vertical lines show s.e.mean. * P < 0.05 and ** P < 0.01 compared (ANOVA) to i.c.v. saline.
increases in blood pressure and heart rate which reached $11 \pm 6$ mmHg (basal $101 \pm 3$ mmHg) and $95 \pm 26$ beats min$^{-1}$ (basal $376 \pm 19$ beats min$^{-1}$), respectively after 5 min. These changes were associated with significant increases in renal, splanchnic and adrenal nerve activities which were $110 \pm 34 \%$, $86 \pm 8 \%$ and $148 \pm 54 \%$, respectively after 5 min. There was also an increase in phrenic nerve activity in these animals (see Figure 22).

The effect of pretreatment with either methiothepin or spiroxatrine on the response to DP-5-CT

The effect of methiothepin and spiroxatrine on the cardiovascular response caused by DP-5-CT was investigated. Methiothepin (1 mg kg$^{-1}$ i.v.; $n = 4$) caused a significant (Student's paired $t$ test) reduction in blood pressure of $32 \pm 2$ mmHg 5 min following injection, blood pressure was still reduced but stable after 20 min. This was associated with an initial increase in heart rate and renal nerve activity, reaching a maximum by 5 min of $51 \pm 20$ beats min$^{-1}$ and $64 \pm 14 \%$, respectively. These variables returned to near baseline levels after 20 min (see Table 2.8). Baseline values for blood pressure and heart rate are given in Table 2.8. Following a 20 min pretreatment with methiothepin the pressor response, tachycardia and renal sympathoexcitation produced by DP-5-CT (3 nmol kg$^{-1}$) administered i.c.v. were significantly attenuated (Figure 23).

Spiroxatrine was dissolved in 0.01N HCl and the resultant solution had a pH of 2.5-3. Therefore, six vehicle control experiments were performed in which 0.01N HCl was administered i.c.v. in 2 volumes of 5 $\mu$l, 5 min apart and DP-5-CT (3 nmol kg$^{-1}$ i.c.v.) was given 5 min after the last volume of vehicle. In parallel experiments, spiroxatrine (300 nmol kg$^{-1}$; $n = 6$) was administered i.c.v. in 2 doses of 150 nmol kg$^{-1}$, 5 min apart and DP-5-CT (3 nmol kg$^{-1}$ i.c.v.) was given 5 min after the last dose of spiroxatrine.
Chapter 2

Figure 23 Artificially respired α-chloralose anaesthetized rats: histograms comparing changes from post-pretreatment values 5 min after i.c.v. administration of DP-5-CT (3 nmol kg⁻¹) in the presence of saline (□ ; 5 μl i.c.v.; n=8) and methiothepin (■ ; 1 mg kg⁻¹ i.v.; n=4) in mean arterial blood pressure (MAP), heart rate (HR), renal nerve activity (RNA) and phrenic nerve activity (PNA). Each bar represents the mean value and the vertical lines show s.e.mean. * P < 0.05 and ** P < 0.01 compared to DP-5-CT in the presence of saline.
Figure 24 Artificially respired α-chloralose anaesthetized rats: a comparison of the changes from post-pretreatment values over time (min) caused by DP-5-CT (3 nmol kg⁻¹, i.c.v.) in the presence of 0.01 N HCl (vehicle for spiroxatrine ; ▼; 10 μl, i.c.v.; n = 6) and spiroxatrine (▼; 300 nmol kg⁻¹, i.c.v.; n = 6) in mean arterial blood pressure (MAP), heart rate (HR), renal nerve activity (RNA) and phrenic nerve activity (PNA). Each point represents the mean value and the vertical lines show s.e.mean. * P < 0.05 and ** P < 0.01 comparing (ANOVA) the effects of DP-5-CT in animals pretreated with 0.01 N HCl and spiroxatrine. * P < 0.05 and ** P < 0.01 compared to DP-5-CT in non-pretreated animals (not shown).
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Table 2.8. Baseline values and changes in MAP, HR and renal (RNA) and phrenic (PNA) nerve activities following the administration of methiothepin \((n = 4)\), spiroxatrine \(\text{given as a split dose of 2 x 150 nmol kg}^{-1}\) at 0 and 5 min; \(n = 6\) and 0.01 N HCl \(\text{given in 2 volumes of 5} \mu l\) at 0 and 5 min; \(n = 6\) in \(\alpha\)-chloralose anaesthetized, artificially respired rats. Values are the mean ± s.e.mean. Significant changes (Student’s paired \(t\) test) in baseline values are shown as * \(P < 0.05\), ** \(P < 0.01\).
Vehicle pretreatment did not affect baseline blood pressure values (shown in Table 2.8) but caused a significant (Student's paired t test) rise in heart rate of $19 \pm 4$ beats min$^{-1}$ and in renal nerve activity of $27 \pm 10\%$, 10 min after the first injection (Table 2.8). Spiroxatrine pretreatment did not alter baseline values per se (see Table 2.8).

In the presence of 0.01N HCl (vehicle for spiroxatrine) DP-5-CT caused an immediate increase in blood pressure of similar magnitude and duration to the response of DP-5-CT in non-pretreated animals (see Figure 24). The pressor response was associated with tachycardia and an increase in renal and phrenic nerve activity. The tachycardia was significantly (ANOVA) greater in vehicle-pretreated animals compared to non-pretreated animals (see Figure 24).

The effect of DP-5-CT (3 nmol kg$^{-1}$ i.c.v.) on all variables was significantly attenuated in spiroxatrine (300 nmol kg$^{-1}$ i.c.v.) pretreated animals compared (ANOVA) to vehicle pretreated animals, see Figure 24.

**Effect of i.c.v. administration of sumatriptan**

The $5\text{-HT}_1$ receptor agonist sumatriptan (GR43175; 10 nmol kg$^{-1}$ i.c.v.; $n = 6$) caused a small, significant (ANOVA) reduction in blood pressure of $6 \pm 2$ mmHg 5 min after administration and this was maintained for 20 min. The depressor response was associated with increases in heart rate, renal nerve activity and phrenic nerve activity (reaching $28 \pm 8$ beats min$^{-1}$, $26 \pm 5\%$ and $70 \pm 29\%$, respectively after 5 min) which were maintained for 10-15 min (see Figure 25). Baseline values for blood pressure and heart rate are shown in Table 2.4.
The effect of pretreatment with spiroxatrine on the response to sumatriptan

The effect of 5-HT\textsubscript{1A} receptor antagonism on the response to i.c.v. administration of sumatriptan was examined. Pretreatment with spiroxatrine (300 nmol kg\textsuperscript{-1} i.c.v.; n = 4) did not significantly change baseline values (see Table 2.9). In the presence of spiroxatrine the reduction in blood pressure and the increase in heart rate caused by sumatriptan were significantly (ANOVA) attenuated (Figure 25).

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Table 2.9. Baseline values and changes in MAP, HR and renal (RNA) and phrenic (PNA) nerve activities following the administration of spiroxatrine (given as a split dose of 2 x 150 nmol kg\textsuperscript{-1} at 0 and 5 min; n = 4) in α-chloralose anaesthetized, artificially respired rats. Values are the mean ± s.e.mean. There were no significant changes (Student’s paired t test) in baseline values following administration of spiroxatrine.
Figure 25 Artificially respired α-chloralose anaesthetized rats: a comparison of the changes over time (min) from baseline or post-pretreatment values caused by i.c.v. saline (○; 5 µl; n=6) and sumatriptan 10 nmol kg⁻¹ in the absence (□; n=6) and presence of spiroxatrine (▲; 300 nmol kg⁻¹, i.c.v.; n=4) in mean blood pressure (MAP), heart rate (HR), renal nerve activity (RNA) and phrenic nerve activity (PNA). Each point represents the mean value and the vertical lines show s.e.mean. * P < 0.05 and ** P < 0.01 compared (ANOVA) to i.c.v. saline. τ P < 0.05 and ττ P < 0.01 compared (ANOVA) to i.c.v. sumatriptan in non-pretreated animals.
2.4 Discussion

5-HT administered via the lateral ventricle in conscious rats caused a pressor response which was not dependent on the dose of 5-HT used. The pressor response was of similar magnitude and duration to that previously reported for 5-HT (Sukamoto et al., 1984; Dalton, 1986; Pergola & Alper, 1991; Dedeoglu & Fisher, 1991a). However, the heart rate changes associated with this increase in blood pressure were related to dose; a low dose of 5-HT (4 nmol kg\(^{-1}\)) caused an increase in heart rate whereas higher doses of 5-HT (40 and 120 nmol kg\(^{-1}\)) initially caused bradycardia which was followed by tachycardia. These findings are in agreement with the findings of a previous study (Dedeoglu & Fisher, 1991a). Similarly, the bradycardia produced by higher doses of 5-HT in the present study is comparable to that observed in previous studies (Sukamoto et al., 1984; Dalton, 1986; Pergola & Alper, 1991).

Lateral ventricular administration of 5-HT has also been shown to cause a pressor response in the urethane anaesthetized rat (Lambert et al., 1975; 1978; Krstic & Djurkovic, 1976; 1980; Sukamoto et al., 1984). However, in these studies variable changes in heart rate were observed. More recently, Inoue & Buñag (1989) demonstrated that i.c.v. 5-HT induced a pressor response associated with a consistent bradycardia in the urethane anaesthetized rat. In the present study, i.c.v. injection of 5-HT caused a pressor response associated with an immediate tachycardia in urethane anaesthetized rats. A similar pressor response was observed in rats anaesthetized with \(\alpha\)-chloralose; however, this was associated with an initial bradycardia which was followed by a tachycardia. These results clearly demonstrate that the type of anaesthetic used affects the 5-HT-induced heart rate changes and that the response to i.c.v. 5-HT produced in \(\alpha\)-chloralose anaesthetized rats is similar to the response seen in conscious animals (see Figure 5 for a comparison). The present results
also show that urethane can affect the 5-HT-induced heart rate response without having a marked influence on the pressor response. This is in agreement with the findings of a previous study (Sukamoto et al., 1984).

Urethane has been shown to antagonise cardiovascular responses mediated following activation of $\alpha_2$-adrenoceptors (Armstrong et al., 1982). Therefore, the differential effect on heart rate seen in urethane anaesthetized rats compared to $\alpha$-chloralose anaesthetized rats or conscious rats may be a consequence of $\alpha_2$-adrenoceptor blockade. This was tested in $\alpha$-chloralose anaesthetized rats pretreated with the $\alpha_2$-adrenoceptor antagonist, idazoxan at a dose that is known to be selective for $\alpha_2$-adrenoceptors (Doxey et al. 1983). Under these conditions no change in heart rate was observed following i.c.v. administration of 5-HT. Although this experiment does not conclusively indicate that $\alpha_2$-adrenoceptor antagonism accounts for the differences seen between urethane and $\alpha$-chloralose anaesthesia, it does highlight the problem of using urethane anaesthesia in cardiovascular studies. $\alpha_2$-Adrenoceptor antagonists are known to inhibit the baroreceptor reflex (Huchet et al., 1981, 1983). Urethane has also been shown to attenuate baroreceptor mediated responses (Barringer & Buñag, 1990). Therefore, the absence of a 5-HT-induced bradycardia in urethane anaesthetized rats may reflect an inhibition of this reflex. In this respect, baroreceptor reflex mediated responses are known to be well preserved in animals anaesthetized with $\alpha$-chloralose (Barringer & Buñag, 1990). The use of urethane may explain the variable heart rate effects observed in previous studies (Lambert et al., 1975; 1978; Krstic & Djurkovic, 1976, 1980; Sukamoto et al., 1984). However, the ability of Inoue & Buñag (1989) to detect a bradycardia following injection of 5-HT is inconsistent with the present study but may be explained by the reduced dose of urethane used in their study. All further experiments in the present study in which anaesthesia was required
utilised α-chloralose anaesthesia. This would appear to be justified since the response to 5-HT in α-chloralose anaesthetized rats was similar to that seen in conscious rats (Figure 5).

The present study demonstrated that the cardiovascular effects caused by lateral ventricular administration of 5-HT are dependent on the dose of 5-HT given and in anaesthetized animals, the anaesthetic used. These results confirm an earlier proposal of Kuhn et al. (1980a). However, the physiological basis for the cardiovascular changes observed in rats and the 5-HT receptor subtype(s) mediating these changes remained unknown. To allow the investigation of these aspects of the response, 5-HT was administered i.c.v. in α-chloralose anaesthetized rats in which sympathetic nerve activity was recorded and respiration was controlled and monitored. The nature of the 5-HT receptors mediating these effects of 5-HT was investigated using selective agonists and antagonists for the different 5-HT receptor subtypes.

In α-chloralose anaesthetized rats treated with a neuromuscular blocking agent, i.c.v administration of 5-HT caused a dose-related increase in blood pressure. This was associated with an initial reduction in heart rate and renal sympathetic nerve activity. The changes in heart rate and renal nerve activity were biphasic; the initial falls in these variables were followed by tachycardia and renal sympathoexcitation. Interestingly the changes in heart rate mirrored the changes in sympathetic nerve activity. Therefore in anaesthetized rats, 5-HT administered i.c.v. caused a pressor response associated with both sympathoinhibition and sympathoexcitation.

Lateral ventricular administration of low doses (0.3-3 nmol kg\(^{-1}\)) of DP-5-CT, 5-CT and 8-OH-DPAT caused immediate renal sympahtoexcitation, tachycardia and a rise in blood pressure. In further
experiments in which several sympathetic outflows were monitored, DP-5-CT caused a marked increase in splanchnic, renal and adrenal nerve activities, suggesting that this agonist produced a generalised sympathoexcitation. Furthermore, 5-HT administered i.c.v. in animals pretreated with the selective 5-HT2/5-HT1c receptor antagonists cinanserin or LY 53857 (Rubin et al., 1964; Cohen et al., 1983a, 1985; see Hoyer 1991) also caused an immediate increase in renal sympathetic nerve activity, heart rate and blood pressure. In the presence of the 5-HT2/5-HT1c receptor antagonists the initial bradycardia and renal sympathoinhibition caused by i.c.v. 5-HT were prevented and sympathoexcitatory effects of 5-HT were unmasked.

DP-5-CT is a highly selective agonist for 5-HT1A receptors (Mir et al., 1987; Doods et al., 1988; see Schoeffter & Hoyer, 1988) and the sympathoexcitation, tachycardia and rise in blood pressure caused by i.c.v administration of DP-5-CT were blocked in animals pretreated (i.v.) with methiothepin, a non-selective antagonist that has been shown to block 5-HT1A receptors (Fozard et al., 1987; Schoeffter & Hoyer, 1988). However, methiothepin caused a reduction in blood pressure, presumably a consequence of its ability to antagonise α1-adrenoceptors and thus it is difficult to ascribe the attenuation of the response to DP-5-CT to a selective action at 5-HT1A receptors. To overcome this difficulty rats were pretreated with spiroxatrine, a selective antagonist for 5-HT1A receptors (Nelson & Taylor, 1986; see Hoyer 1991) and a low dose of this antagonist was administered i.c.v. to minimise the reported systemic cardiovascular effects (Dreteler et al., 1990). In the presence of spiroxatrine the sympathoexcitation, tachycardia and pressor response caused by DP-5-CT were abolished. Therefore it is concluded that the sympathoexcitation caused by DP-5-CT is due to the activation of central 5-HT1A receptors. Furthermore, 8-OH-DPAT, a selective 5-HT1A receptor agonists (Middlemiss
& Fozard, 1983; Fozard et al., 1987) and 5-CT, a non-selective 5-HT\textsubscript{1} receptor agonist with agonist activity at 5-HT\textsubscript{1A} receptors (Schoeffter & Hoyer, 1988; Van Wijngaarden et al., 1990; see Hoyer, 1991) also caused renal sympathoexcitation, tachycardia and a rise in blood pressure supporting the view that this sympathoexcitation is mediated following 5-HT\textsubscript{1A} receptor activation. The observation that the rise in blood pressure, sympathoexcitation and tachycardia caused by 5-HT in the presence of the 5-HT\textsubscript{2}/5-HT\textsubscript{1C} antagonist LY 53857 could be blocked by the addition of spiroxatrine, suggests that the sympathoexcitation caused by 5-HT is also due to activation of 5-HT\textsubscript{1A} receptors.

The above data indicating that activation of 5-HT\textsubscript{1A} receptors, reached by i.c.v. administration, causes sympathoexcitation contrasts with the findings from previous studies using 8-OH-DPAT and other non-structurally related 5-HT\textsubscript{1A} agonists. These agonist given i.v. have been demonstrated to cause a centrally mediated decrease in blood pressure and sympathoinhibition in rats, cats, rabbits and dogs (see introduction 1.7.1). However, in the rat there is some evidence that activation of 5-HT\textsubscript{1A} receptors can also cause a pressor response and/or sympathoexcitation. In conscious spontaneously hypertensive rats i.v. 8-OH-DPAT caused an initial tachycardia and rise in blood pressure which was then followed by a bradycardia and hypotension (Fozard et al., 1987). 8-OH-DPAT (i.v.) in conscious and anaesthetized rats mediates the release of adrenaline by central sympathoexcitation of the adrenal glands (Chaouloff & Jeanrenaud, 1987; Chaouloff et al., 1990a, 1990b; Bagdy et al., 1989a, 1989b; Bouhelal & Mir, 1990, 1992). Microinjection of 5-HT\textsubscript{1A} agonists into the raphé obscurus causes a pressor response (Dreteler et al., 1991a). Furthermore, i.c.v. administration of low doses of 8-OH-DPAT in conscious rats also causes a pressor response which is attenuated by methiothepin (Dedeoglu & Fisher, 1991a). These combined data, at the least in the rat,
demonstrate that activation of 5-HT$_{1A}$ receptors can cause sympathoexcitation as well as sympathoinhibition.

In the present study higher doses of 8-OH-DPAT (40 and 120 nmol kg$^{-1}$) administered i.c.v. caused falls in blood pressure associated with tachycardia and variable changes in renal sympathetic nerve activity. Falls in blood pressure were also observed with higher doses of 8-OH-DPAT in conscious rats (Dedeoglu & Fisher, 1991a). A possible explanation for this depressor response is that the sympathoexcitation is masked by the sympathoinhibitory action of 8-OH-DPAT. A high dose of 8-OH-DPAT when given i.c.v. may diffuse to the mid and hind brain regions where activation of 5-HT$_{1A}$ receptors is known to cause a fall in blood pressure and/or sympathoinhibition, in areas such as the dorsal raphe (Connor & Higgins, 1990), raphe magnus and pallidus (Valenta & Singer, 1990) and the rostral ventrolateral medulla (Lovick, 1989a; Nosjean & Guynet, 1991) (see introduction 1.7.1). In the present study, this explanation is supported by the finding that i.v. administration of the hypotensive i.c.v. dose of 8-OH-DPAT (120 nmol kg$^{-1}$), caused a fall in blood pressure associated with bradycardia and sympathoinhibition. However, the reduction in blood pressure and concomitant tachycardia caused by 8-OH-DPAT could also be explained by a centrally mediated release of adrenaline from the adrenal medulla (Chaouloff et al., 1990a, 1990b; Bagdy et al., 1989a, 1989b; Bouhelal & Mir, 1990, 1992). Activation of peripheral $\beta_2$- and $\beta_1$-adrenoceptors could cause peripheral vasodilatation and direct tachycardia, respectively. The contribution of adrenaline release in the response to high i.c.v. doses of 8-OH-DPAT is presently unknown. This could be tested by examining the effect of a selective $\beta_2$-adrenoceptor antagonist (e.g. ICI 118551) on the depressor response caused by 8-OH-DPAT. If adrenaline release was involved, the depressor response caused by i.c.v. 8-OH-DPAT may be reversed. Additionally, the plasma
concentrations of adrenaline could be monitored before and after i.c.v. administration of 8-OH-DPAT.

In the present experiments the blood pressure rise caused by 5-CT was not maintained yet both the tachycardia and renal sympathoexcitation caused by this 5-HT₁ receptor agonist were maintained. In three animals falls in blood pressure were observed 3-5 min after i.c.v. administration. Systemic administration of this compound has previously been shown to cause falls in blood pressure mediated by vasodilatation following activation of '5-HT₁-like' receptors in conscious rats (Dalton et al., 1986) and anaesthetized cats (Connor et al., 1986). The ability of 5-CT to cause a fall in blood pressure in pithed rats (Dabire et al., 1988) confirmed the vasodilator action of this agonist and demonstrated that this was due to a peripheral action on vascular smooth muscle. In this respect, 5-CT has been shown to cause direct relaxation of isolated vasculature (Feniuk et al., 1984; Trevethick et al., 1986; Martin et al., 1987). Thus, in the present study 5-CT following i.c.v. administration may have caused this hypotensive effect through activation of peripheral 5-HT₁-like receptors mediating vasodilatation, although this remains to be established. 5-CT is a non-selective 5-HT₁ receptor agonist and has high affinity for 5-HT₁A, 5-HT₁B, 5-HT₁D receptors and is a potent agonist at 5-HT₁-like receptors (see Hoyer, 1991). Therefore, it is possible that 5-CT was activating at central 5-HT₁B receptors to cause the reduction in blood pressure in these rats. Sumatriptan (GR43175), a novel 5-HT₁-like agonist, has been shown to be selective for 5-HT₁-like receptors mediating contraction of isolated vascular tissue (Humphrey et al., 1988; Connor et al., 1989) and has affinity for 5-HT₁D, 5-HT₁B and 5-HT₁A receptors (Schoeffter & Hoyer, 1989b). In an attempt to activate 5-HT₁B receptors without the complication of peripheral vasodilatation, sumatriptan was administered i.c.v. Sumatriptan given to the lateral ventricle caused a slight fall in blood
pressure which was associated with a modest increase in heart rate and renal sympathetic nerve activity; however, these effects were antagonised by the selective 5-HT$_{1A}$ receptor antagonist spiroxatrine. As spiroxatrine has little affinity for 5-HT$_{1B}$ or 5-HT$_{1D}$ (see Hoyer, 1991), it is concluded that sumatriptan caused these effects following the activation of 5-HT$_{1A}$ receptors. The ability of this agonist to cause a fall in blood pressure through activation of 5-HT$_{1A}$ receptors was unexpected since selective agonists for this receptor subtype (e.g. DP-5-CT) caused a pressor response. The reason for this difference is unknown but may reflect a weak agonist action at this receptor by sumatriptan. However, it is possible that 5-CT and sumatriptan cause a depressor response through similar mechanisms proposed for higher doses of 8-OH-DPAT (see above), although this requires further investigation. Sumatriptan has previously been shown to reduce blood pressure and sympathetic outflow in anaesthetized cats (Shepheard et al., 1989). The present study demonstrates that sumatriptan can also cause sympathoexcitation following activation of central 5-HT$_{1A}$ receptors.

A major finding of the present study is that 5-HT can cause sympathoexcitation through activation of central 5-HT$_{1A}$ receptors. Previous studies have also suggested that 5-HT administered i.c.v. caused sympathoexcitation. This was based on the observation that the rise in blood pressure produced by 5-HT was attenuated by cervical transection of the spinal cord, adrenalectomy, adrenergic blocking agents and $\alpha$-adrenoceptor antagonists (Krstic & Djurkovic, 1980). However, in the present study 5-HT i.c.v. caused a rise in blood pressure which was associated with an initial reduction in renal sympathetic nerve activity. Similarly, Inoue & Buñag (1989) demonstrated that 5-HT caused a rise in blood pressure associated with a reduction in splanchnic sympathetic nerve activity. Furthermore, hexamethonium, at doses shown to block autonomic
ganglia, enhanced the 5-HT induced rise in blood pressure in the anaesthetized rat (Lambert et al., 1978; Krstic & Djurkovic, 1980); similar results were found in the conscious rat pretreated with chlorisondamine (Pergola & Alper, 1991). Taken together these data indicate that another pressor mechanism is involved in the response to central administration of 5-HT. Several lines of evidence now support a pressor role for vasopressin in this response. 5-HT has previously been shown to regulate a number of neuroendocrine responses including the release of vasopressin in conscious rats (Steardo & Lovino, 1986; see Van de Kar, 1991). Furthermore, in anaesthetized rats the pressor response to i.c.v. 5-HT is blocked by pretreatment with the vasopressin V₁-receptor antagonist d(CH₂)₅Tyr(Me)AVP, although the associated bradycardia is only attenuated in the first 5 min (Inoue & Buñag, 1989). In the present study, pretreatment with the selective V₁-receptor antagonist d(CH₂)₅Tyr(Me)AVP (Kruszynski et al., 1980; Manning & Sawyer, 1986), at a dose which was shown to abolish the pressor response to injected vasopressin (present study; Buñag & Miyajima, 1984), only attenuated the duration of the rise in blood pressure caused by i.c.v. 5-HT. This difference may be explained by the different anaesthetics used; in the present study α-chloralose was used while in that of Inoue & Buñag (1989) urethane was used. Interestingly, in the present study the bradycardia and sympathoinhibition caused by 5-HT were prevented in the presence of d(CH₂)₅Tyr(Me)AVP and tachycardia and sympathoexcititation were immediately observed after administration of 5-HT (see trace Figure 9). A similar observation has been made in conscious rats (Pergola & Alper, 1991) and in that study the pressor response to i.c.v. 5-HT was completely blocked by combined α₁-adrenoceptor and vasopressin V₁-receptor blockade. The finding that i.c.v. administration of 5-HT produced a rapid (maximum response within 5 min) and brief (< 15 min duration) increase in plasma vasopressin concentrations further supports the involvement of vasopressin in the pressor response (Pergola et
al., 1993). Taken together these data indicate that i.c.v. 5-HT in conscious and anaesthetized rats causes the release of vasopressin.

Certain evidence now suggests that the initial bradycardia and sympathoinhibition caused by i.c.v. 5-HT were reflexly mediated following baroreceptor activation as a consequence of the peripherally mediated increase in blood pressure caused by the release of vasopressin. In the present study in α-chloralose anaesthetized rats, the initial bradycardia caused by i.c.v. 5-HT was attenuated in animals pretreated with atropine or atenolol, indicating that this bradycardia was mediated by an increase in vagal drive to the heart and a reduction in sympathetic tone, respectively. Furthermore, when pressure was raised to levels similar to those reached following i.c.v. injection of 5-HT with an infusion of vasopressin, marked falls in heart rate and renal nerve activity resulted, indicating that baroreceptor mediated responses could be elicited in this preparation. In conscious rats in which chronic sinoaortic deafferentation had been performed (Pergola & Alper, 1992) i.c.v. 5-HT, although causing a pressor response, produced a marked tachycardia. Taken together these data support the view that the peripherally mediated pressor response caused by the release of vasopressin induces a baroreceptor mediated sympathoinhibition and bradycardia which masks the ability of 5-HT to cause sympathoexcitation and tachycardia through activation of 5-HT$_{1A}$ receptors. However, in the present study the bradycardia caused by infusing vasopressin was greater than that caused by i.c.v. 5-HT in spite of the similar increase in pressure. This suggests that the bradycardia caused by i.c.v. 5-HT was blunted by the sympathoexcitatory action of this amine. Alternatively, 5-HT may have altered the sensitivity of the baroreceptor reflex. Such an action has previously been reported for 5-HT when administered into the NTS. Microinjection of 5-HT to the NTS caused a sympathetically mediated pressor response and inhibited the baroreceptor
reflex through an action at 5-HT₃ receptors (Merahi et al., 1992b; see general introduction). The involvement of 5-HT₃ receptors in the cardiovascular response caused by i.c.v. 5-HT was investigated in rats pretreated with the selective centrally acting 5-HT₃ receptor antagonist ondansetron (GR38032F; Butler et al., 1988). Ondansetron did not modify the response to i.c.v. administration of 5-HT and it is concluded that 5-HT₃ receptors were not involved in the cardiovascular action of i.c.v. 5-HT. This does not preclude an action of i.c.v. administered 5-HT on baroreceptor sensitivity and this point remains to be determined.

It is of interest that in the present study and that of Dedeoglu & Fisher (1991a), a low dose of 5-HT (4 nmol kg⁻¹) given i.c.v. caused a pressor response associated with a tachycardia in conscious rats, whereas a higher dose (120 nmol kg⁻¹) produced a pressor response and a biphasic effect on heart rate; bradycardia followed by tachycardia. Presumably the low dose of 5-HT produced only the sympathoexcitatory component of the 5-HT response. Therefore the pattern of response caused by i.c.v. administration of 5-HT is dependent on the dose of 5-HT given. Previous studies in anaesthetized rats (Krstic & Djurkovic, 1981) and conscious normotensive and hypertensive rats (Dalton, 1986) have shown that 5-HT given i.c.v. can cause a small secondary depressor response (< 20 mmHg). It is interesting that in this study as well as that of Dedeoglu & Fisher (1991a), 5-HT administered i.c.v. did not cause a fall in blood pressure. These conflicting results may be explained by the dissimilar doses of 5-HT used in these studies; in this respect falls in blood pressure were only observed following administration of high doses of 5-HT (240-1600 nmol kg⁻¹).

In the present experiments the initial inhibitory actions of 5-HT on heart rate and sympathetic nerve activity were prevented in animals which were pretreated (i.c.v.) with the 5-HT₂/5-HT₁C receptor antagonists cinanserin
and LY 53857. Under these conditions only the sympathoexcitatory effects of 5-HT, mediated through activation of 5-HT$_{1A}$ receptors, were observed. This data indicates that the bradycardia and renal sympathoinhibition caused by 5-HT were mediated through 5-HT$_2$ and/or 5-HT$_{1C}$ receptors. The reversal of bradycardia to tachycardia has previously been reported in conscious rats pretreated with the 5-HT$_2$/5-HT$_{1C}$ receptor antagonist cyproheptadine (Dalton, 1986). Furthermore, when peripheral vasopressin V$_1$-receptors were blocked with d(CH$_2$)$_5$Tyr(Me)AVP the initial inhibitory effects of 5-HT were reversed to tachycardia and renal sympathoexcitation. As the vasopressin V$_1$-receptor antagonist modified the response to i.c.v. 5-HT in a similar manner to the 5-HT$_2$/5-HT$_{1C}$ receptor antagonists (for comparison see Figure 9), it is concluded that the release of vasopressin by i.c.v. administration of 5-HT is mediated through 5-HT$_2$ or 5-HT$_{1C}$ receptors. This conclusion is supported by previous studies in conscious rats in which the 5-HT receptors mediating the release of vasopressin were characterized and demonstrated to be 5-HT$_2$ and/or 5-HT$_{1C}$ receptors (Brownfield et al., 1988; Bagdy et al., 1992; Pergola et al., 1993; see Van de Kar, 1991).

The selective 5-HT$_2$/5-HT$_{1C}$ receptor agonist DOI (Shannon et al., 1984; Glennon et al., 1986, 1988c; Van Wijngaarden et al., 1990) given i.c.v. caused a dose-related rise in blood pressure associated with bradycardia and renal sympathoinhibition. Intravenous administration of DOI produced a response similar in magnitude and duration to that observed following i.c.v. administration of this compound. The effects of DOI were attenuated by the peripherally acting 5-HT$_2$/5-HT$_{1C}$ receptor antagonist BW501C67 (Mawson & Whittington 1970; Fuller et al., 1986) and it is concluded that the pressor response produced by DOI was due to activation of peripheral 5-HT$_2$ receptors. DOI given i.v. to conscious rats has previously been shown to cause a pressor response associated with a reduction in renal
blood flow and bradycardia (Alper, 1990). The pressor response, bradycardia and renal constriction caused by DOI were antagonised by the peripherally acting 5-HT$_2$/5-HT$_{1c}$ receptor antagonist xylamidine and pretreatment with chlorisondamine enhanced the pressor response and abolished the bradycardia caused by this agonist (Alper, 1990). Moreover, DOI caused a dose-dependent increase in blood pressure in pithed rats which was antagonised by LY 53857 (Dabire et al., 1989). The findings from these studies supports the view that the cardiovascular effects of DOI are peripherally mediated and that the bradycardia is a consequence of activation of the baroreceptor reflex. Such a mechanism would explain the bradycardia and renal sympathoinhibition in the present study. Alper (1990) has previously reported that the increase blood pressure produced by DOI is due to a direct vasoconstriction mediated by 5-HT$_2$ receptors and indirectly through the production of angiotensin II resulting from enhanced renin release due to the decreased renal perfusion pressure. Therefore, in the present study, it is likely that DOI administered i.c.v. gained access to the systemic circulation and caused a pressor response through direct vasoconstriction mediated by 5-HT$_2$ receptors and indirectly through activation of the renin-angiotensin system, leading to a baroreflex mediated bradycardia and sympathoinhibition. The rise in blood pressure and the initial bradycardia and renal sympathoinhibition caused by i.c.v. 5-HT as a consequence of the release of vasopressin (see above) was essentially unaffected by i.v. BW501C67, at a dose shown to block peripheral 5-HT$_2$ receptors and confirms that the actions of 5-HT are centrally mediated. Pretreatment with the vasopressin V$_1$-receptor antagonist d(CH$_2$)$_5$Tyr(Me)AVP did not antagonise and may have enhanced the cardiovascular actions of DOI administered i.c.v. Thus it is concluded that the pressor response elicited by DOI was not through activation of central 5-HT$_2$ or 5-HT$_{1c}$ receptors to release vasopressin. This is an unexpected finding and the reason for the lack of vasopressin release with this
5-HT₂/5-HT₁C receptor agonist is unknown and requires further study. In support of these findings, DOI given i.v. has previously been shown not to cause the release of vasopressin (Bagdy et al., 1992; see Van de Kar, 1991). However, in a recent study Dedeoglu & Fisher (1991b) reported that DOI (100 nmol kg⁻¹) caused a pressor response which was slightly attenuated by vasopressin V₁-receptor blockade, although this finding requires confirmation. DOI is a low efficacy agonist and can block the pressor effect produced by 5-HT in pithed rats (Dabire et al., 1989). Therefore, it is possible that DOI did not have efficacy at the 5-HT₂/5-HT₁C receptor responsible for the release of vasopressin. This could be tested by determining whether the 5-HT-induced release of vasopressin was susceptible to antagonism by DOI in the presence of BW501C67 (to block the peripheral actions of DOI). An attempt was made to investigate the release of vasopressin with the putative 5-HT₂ and 5-HT₁C receptor agonist α-methyl-5-HT. However in two animals, i.c.v. administration of α-methyl-5-HT caused a pressor response associated with biphasic changes in heart rate and renal nerve activity in a similar manner to that of 5-HT (data not shown). This is consistent with an action at both 5-HT₂ and/or 5-HT₁C and 5-HT₁A receptors in this model. The non-selective nature of this agonist has previously been reported (Ismaiel et al., 1990; Leff & Martin, 1988). Quipazine has been shown to cause an increase in blood pressure caused in part through the release of vasopressin (Zink et al., 1990), although this compound is also non-selective for 5-HT receptors. The more specific characterization of the receptor mediating the release of vasopressin awaits the availability of more selective compounds for either 5-HT₂ or 5-HT₁C receptors.

Interestingly, in the anaesthetized cat, activation of central 5-HT₂ or 5-HT₁C receptors with either DOI or the combined 5-HT₂/5-HT₃ receptor agonist quipazine has been shown to cause sympathoexcitation (McCall & Harris,
1988; Vayssettes-Courchay et al., 1991; Shepheard et al., 1991; Ramage et al., 1993; see Chapter 3). In the present study and in previous studies (Alper, 1990; Vayssettes-Courchay et al., 1990) there is no evidence for a centrally mediated increase in sympathetic tone in the rat. Therefore, it appears that the cat and rat are different in this respect.

In the present experiments phrenic nerve activity was monitored to give an indication that the preparation had central respiratory drive, a factor that is reduced by general anaesthesia. Lambert et al., (1978) have suggested that the pressor effect was a consequence of hypoxia or hypercapnia resulting from a depression of respiration induced by 5-HT. However, when respiration was controlled (as in the present study), 5-HT caused a pressor response and an initial decrease in phrenic nerve activity. This indicates that the decrease in respiration observed by Lambert et al (1978) was not involved in cardiovascular effects of 5-HT. The initial decrease of inspiratory drive in the present experiments may be a consequence of the 5-HT induced pressor response since respiratory drive has been shown to be modulated by the baroreceptor reflex (see Daly, 1986). In previous studies activation of 5-HT pathways has been demonstrated to increase central respiratory drive (Holtman et al., 1986a, 1986b; Dreteler et al., 1991a). Furthermore, central administration of 8-OH-DPAT has been shown to increase respiratory rate (Gillis et al., 1989) and phrenic nerve activity (Sporton et al., 1991), a measure of central inspiratory drive. The present results demonstrate that 8-OH-DPAT, DP-5-CT and sumatriptan can cause an increase in central inspiratory drive and this variable tended to increase following i.c.v. injection of 5-HT. Spiroxatrine abolished the increase in phrenic nerve activity caused by DP-5-CT and sumatriptan. These findings are consistent with previous studies (see above) and suggest that 5-HT$_{1A}$ receptors can cause an increase in respiratory drive. The
reason for the variable response caused by 5-HT on inspiratory drive is unknown.

It is difficult to locate the specific central site(s) involved in the cardiovascular effect following administration of 5-HT to the ventricles, since the drug is widely distributed throughout the brain using this procedure. However, certain studies in which 5-HT has been confined to more specific brain regions have suggested that forebrain structures are responsible for the 5-HT induced pressor response (see general introduction). The precise site/sites in the brain where 5-HT is acting to cause these cardiovascular effects remain to be determined but the rapid onset of response would suggest a brain area close to the lateral or 3rd ventricles. Previous microinjection studies have shown that 5-HT injected into the anterior hypothalamus/preoptic area caused a rise in blood pressure through an increase in sympathetic outflow (Smits & Struyker-Boudier, 1976; Sukamoto et al., 1984). This response was antagonised by metergoline, a non-selective antagonist with affinity at 5-HT_{1A} receptors (Robinson, 1984). Therefore the sympathoexcitatory effects of 5-HT observed in the present study may be mediated by 5-HT_{1A} receptors located in the anterior hypothalamus/preoptic area. In this way, 5-HT administered i.c.v. may diffuse to this structure, which is close to the third ventricle, to cause sympathoexcitation. In doing so 5-HT may mimic the effects of endogenously released 5-HT from ascending serotonergic fibres originating in the dorsal raphé nucleus. Electrical and chemical stimulation of the dorsal raphé nucleus has previously been shown to cause a pressor response through an action at the anterior hypothalamus/preoptic area (Smits et al., 1978; Kuhn et al., 1980b; Robinson et al., 1985; Lovick, 1992). The evidence for this forebrain pressor pathway has been reviewed in the general introduction and the experiments to verify an action of i.c.v. 5-HT at these sites will be discussed in Chapter 5.
The central site of the 5-HT$_2$/5-HT$_1C$ receptors involved in the release of vasopressin are presently unknown. However, a direct or indirect action of 5-HT at the paraventricular nucleus or the supraoptic nucleus, sites which are important for the production and release of vasopressin, is suggested. The role of 5-HT in the regulation of vasopressin release and the brain loci involved in this response are discussed in Chapter 5.

In conclusion, the present study demonstrates that i.c.v. administration of 5-HT causes sympathoexcitation by activation of 5-HT$_1A$ receptors and the release of vasopressin through activation of 5-HT$_2$ or 5-HT$_1C$ receptors. An interaction between these mechanisms is responsible for the profile of the response to 5-HT given i.c.v. The pressor response caused by 5-HT is caused by both the direct sympathoexcitation and the vasoconstrictor actions of vasopressin and the biphasic changes in heart rate and sympathetic nerve activity are the product of baroreceptor mediated inhibition and direct sympathoexcitation.
CHAPTER 3

Regional haemodynamic effects of centrally administered 5-HT and DP-5-CT in conscious Long-Evans and Brattleboro rats

3.1 Introduction

Administration of 5-HT to the lateral ventricle of conscious and anaesthetized rats has been shown to cause an increase in blood pressure (Krstic & Djurkovic, 1976, 1980; Lambert et al., 1975, 1978; Sukamato et al., 1984; Dalton, 1986; Inoue & Buñag, 1989; Pergola & Alper, 1991; Dedeoglu & Fisher, 1991a; Anderson et al., 1992). Recently, this pressor response was investigated and shown to be mediated by an increase in sympathetic outflow following activation of 5-HT$_{1A}$ receptors and the release of vasopressin through activation of 5-HT$_2$ and/or 5-HT$_{1C}$ receptors (Anderson et al., 1992; see Chapter 2). Although the mechanism of the pressor response caused by 5-HT has previously been described, the haemodynamic changes associated with this increase in blood pressure are at present unknown. In the previous study central administration of the selective 5-HT$_{1A}$ receptor agonist DP-5-CT was shown to produce a pressor response, tachycardia and an increase in sympathetic outflow in splanchnic, renal and adrenal sympathetic nerves (see Chapter 2). Again, the regional haemodynamic changes associated with this generalised sympathoexcitation caused by activation of central 5-HT$_{1A}$ receptors remains to be described.

Therefore, the regional haemodynamic changes caused by central administration of 5-HT and DP-5-CT were investigated in freely moving conscious rats with chronically implanted miniature Doppler flow probes. To provide further evidence for the involvement of vasopressin in the response to i.c.v. 5-HT and to investigate the cardiovascular actions of
5-HT in the absence of this hormone, the effects of 5-HT were compared in Long-Evans rats and vasopressin deficient Brattleboro rats. Brattleboro rats are genetically derived from the Long-Evans strain and are homozygous for diabetes insipidus. These animals have functioning receptors for vasopressin (e.g. Gardiner et al., 1989) but produce a mutant vasopressin precursor which is not processed correctly in vivo (Ivell et al., 1986).

Since the cardiovascular response caused by i.c.v. 5-HT is related to dose; low doses of 5-HT cause a pressor response and tachycardia whereas high doses of 5-HT cause a pressor response associated with biphasic heart rate changes (see Chapter 2; Dedeoglu & Fisher, 1991a), the regional haemodynamic changes were monitored over a similar dose range for 5-HT as used previously.

5-HT and selective 5-HT$_{1A}$ receptor agonists have been shown to cause a behavioural syndrome (Hjorth et al., 1982: Tricklebank et al., 1984, 1985), therefore behavioural changes were also monitored in this study.
Chapter 3

3.2 Methods

3.2.1 Conscious chronically instrumented rat

Experiments were performed on male normotensive Long-Evans and Brattleboro rats (300-400g). Animals were housed individually and maintained under a 12:12 h light-dark cycle (lights on at 6 a.m.) with free access to food and water. Surgery was performed in 3 stages and after each surgical stage wounds were closed and dusted with Acramide. The rats were also given an injection of ampicillin (Penbritin, 7 mg kg\(^{-1}\) i.m.).

Chronic cannulation of the lateral ventricle

A guide cannula was placed in the right lateral ventricle using the method described in 2.2.1.

Chronic implantation of Doppler flow probes

At least one week after the i.c.v. cannula was implanted the animals were anaesthetized (sodium methohexitone 40 mg kg\(^{-1}\), i.p., supplemented as required) and laparotomized. The superior mesenteric and left renal arteries were separated from surrounding connective tissue. The abdominal aorta below the level of the ileocaecal artery was prepared similarly. Pulsed Doppler flow probes were sutured around the vessels and their leads tunnelled subcutaneously to exit at the back of the neck. The cuffs of the Doppler flow probes were manufactured 'in house'; a silastic cuff (internal diameter of 1.1-1.6 mm) was built on a DBF-120A-XS crystal plus wire subassembly (Crystal Biotech, Holliston, U.S.A.; see Gardiner et al., 1990; Haywood et al., 1981). Viscera were replaced in the abdominal cavity and irrigated with sterile saline. The incision was closed with sterile cat gut and the skin was sewn with suture (Ethicon). The pulsed Doppler probe leads were soldered to a connector (Microtech Inc, Boothwyn, U.S.A.) which was clamped into a harness worn by the rat. Once animals had regained consciousness, they were given free access to food and water.
Chronic implantation of vascular catheters

At least two weeks after the implantation of the pulsed Doppler probes and 1-2 days before the start of the experiment an intra-arterial catheter and two intra-venous catheters were implanted in a similar manner as described in 2.2.1.

3.2.2 Experimental design

Only healthy animals with acceptable blood pressure and pulsed Doppler signals were used. Experiments were performed with the animals in their home cages and they were given free access to food and water. Each animal wore a specially designed harness which was attached to a counterbalanced spring. The vascular catheters were led out of the cage through the spring and the arterial catheter was connected to a pressure transducer (Bell & Howell, type 4-442). Blood pressure was recorded and heart rate was derived electronically from the blood pressure signal (Gould Biotach Amplifier). Each animal was connected to a pulsed Doppler monitoring system (VF-1 mainframe fitted with a 20-MHz pulsed Doppler flow meter [HVPD-20], Crystal Biotech, Holliston, U.S.A.) and only animals with 3 acceptable phasic Doppler shift signals (signal:noise, > 20:1) were used. Doppler probe-to-mainframe connections were made remote from the animal and the probe leads from the animal were led out of the cage supported by the spring. This system allowed the animals to move freely in their cage. Simultaneous recordings of phasic and mean (electronically derived from the phasic signal) arterial pressure, heart rate and Doppler shift signals were made on a chart recorder (Gould ES1000). Phasic and mean renal, mesenteric and hindquarters (abdominal aorta) Doppler shift signals were measured. Phasic Doppler shift signals from chronically implanted pulsed Doppler flow probes have previously been shown to give a good index of volume flow (see Gardiner & Bennett, 1988).
3.2.3 Experimental protocol

Each animal received 3 separate doses of test compound and a single volume of saline. The doses of test compound were arranged using a Latin Square design and were given over 3 days. A single i.c.v. injection of test compound was administered per day; saline was given before the low dose of test compound. All injections were made when the animals were still and when cardiovascular variables were stable. The responses produced by vehicle and test compound were recorded for at least 30 min. This protocol was attempted initially in animals without Doppler flow probes to assess feasibility and to allow comparisons between the effects of 5-HT i.c.v. in Long-Evans rats (the genetic control for the Brattleboro rat strain) and Sprague-Dawley rats (used in previous studies).

In some experiments Long-Evans rats were pretreated with d(CH$_2$)$_5$Tyr(Me)-AVP (bolus 10 $\mu$g kg$^{-1}$ i.v.; infusion 10 $\mu$g kg$^{-1}$ h$^{-1}$) 10 min before the administration of 5-HT i.c.v. In other experiments Brattleboro rats were pretreated with ICI 118551 (bolus 0.2 mg kg$^{-1}$ i.v.; infusion 0.1 mg kg$^{-1}$ h$^{-1}$) 1 h before the administration of 5-HT i.c.v. These doses and pretreatment times for d(CH$_2$)$_5$Tyr(Me)-AVP and ICI 118551 have been shown to selectively block vasopressin $V_1$-receptors (Gardiner et al., 1989) and $\beta_2$-adrenoceptors (Gardiner & Bennett, 1988), respectively.

3.2.4 Analysis of results

Baseline values were taken 1 minute before the addition of drug or vehicle.
All results are expressed as changes from baseline values. The mean Doppler shift was divided by mean arterial pressure to provide estimates of vascular conductance and this was expressed as the percentage change from baseline. As the vascular conductance change gives an indication of vasoconstriction or vasodilatation of a particular vascular bed this variable is
described in the results section and the changes in mean Doppler shift are
given in the appendix. Changes in mean arterial pressure, heart rate, renal,
mesenteric and hindquarters vascular conductances caused by the test drug
were compared with time-matched vehicle controls using two-way analysis
of variance and were subsequently analysed using the least significant
difference test (Sokal & Rohlf, 1969). Changes in variables caused by
antagonist or vehicle pretreatments were compared to the pre-dose baseline
using Student's $t$ test for paired data. All values are expressed as the mean
± s.e.mean. Differences in the mean were taken as significant when $P <
0.05.$

3.2.5 Drugs and solutions
The drugs used were acramide (Dales Pharmaceuticals, U.K.);
N,N-di-n-propyl-5-carboxamidotryptamine maleate, DP-5-CT (a gift from
Wellcome Research Laboratories Beckenham, Kent, U.K.);
5-hydroxytryptamine creatinine sulphate, 5-HT (BDH, Poole, Dorset, U.K.);
erthro-(±)-1-[7-methylindan-4-yloxy]-3-isopropyl-aminobutan-2-ol,
ICI 118551 (ICI Pharmaceuticals Ltd, Macclesfield, U.K.); [β-Mercapto-β,β-
cyclopentamethylenepropionyl$^{1}$,O-Me-Tyr$^{2}$,Arg$^{8}$]-Vasopressin,
d(CH$_{2}$)$_{5}$Tyr(Me)AVP (Sigma Chemical Co., Poole, Dorset, U.K.); ampicillin
(Beecham, U.K.); sodium methohexitone (Eli Lilly, U.K.).
Drugs given i.c.v. were dissolved in 0.9% w/v saline and were administered
in a dose volume of 5 $\mu$l over a 20 s period. All drugs given i.v. were
dissolved in saline (ICI 118551 was gently warmed) and given in a volume
of 100 $\mu$l. Infusions of drugs were given at a rate of 300 $\mu$g h$^{-1}$. 
3.3 Results

3.3.1 Conscious Long-Evans rats: effect of 5-HT on blood pressure and heart rate

In preliminary experiments, blood pressure and heart rate were measured in conscious Long-Evans rats to allow a comparison between the effect of i.c.v. administration of 5-HT in this strain with the Sprague-Dawley rat strain used in previous studies. Saline i.c.v. (5 μl; n = 6) had little effect on blood pressure or heart rate and these variables remained stable for the duration of the experiment (data not shown). 5-HT (4, 40, 120 nmol kg⁻¹; n = 6) administered i.c.v. caused a rise in arterial blood pressure. This was associated with dose related heart rate changes; the low dose (4 nmol kg⁻¹) of 5-HT caused tachycardia whereas higher doses (40 and 120 nmol kg⁻¹) caused bradycardia followed by tachycardia. Baseline values for blood pressure and heart rate are shown in Table 3.1. As biphasic responses were observed for heart rate, each phase of response was analysed (Student’s paired t test) separately and the maximum changes between 1-5 min and 10-30 min were compared to time-matched changes in saline controls. Maximum changes for blood pressure and heart rate for each dose of 5-HT are depicted in Figure 26. The changes caused by 5-HT (4, 40, 120 nmol kg⁻¹ i.c.v.) in the Long-Evans rat strain were similar in magnitude and duration to those observed in the Sprague-Dawley strain (compare Figures 2 and 26).

3.3.2 Regional haemodynamic changes caused by i.c.v. 5-HT in conscious Long-Evans and Brattleboro rats

Effect of i.c.v. administration of saline

Saline (5 μl i.c.v.) administered to Long-Evans (n = 8) and Brattleboro (n = 8) rats caused little change in blood pressure, heart rate and renal, mesenteric
<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>n</th>
<th>MAP (mmHg)</th>
<th>HR (beats min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline 5 µl</td>
<td>6</td>
<td>105 ± 2</td>
<td>348 ± 14</td>
</tr>
<tr>
<td>4 nmol kg⁻¹</td>
<td>6</td>
<td>104 ± 2</td>
<td>345 ± 13</td>
</tr>
<tr>
<td>40 nmol kg⁻¹</td>
<td>6</td>
<td>104 ± 2</td>
<td>352 ± 15</td>
</tr>
<tr>
<td>120 nmol kg⁻¹</td>
<td>6</td>
<td>102 ± 2</td>
<td>335 ± 14</td>
</tr>
</tbody>
</table>

Table 3.1. Baseline values for MAP and HR in conscious Long-Evans rats. All compounds were administered i.c.v. Values are the mean ± s.e.mean.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>n</th>
<th>MAP (mmHg)</th>
<th>HR (beats min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Administration of saline and 5-HT in Long-Evans rats</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline 5 µl</td>
<td>8</td>
<td>103 ± 1</td>
<td>348 ± 9</td>
</tr>
<tr>
<td>4 nmol kg⁻¹</td>
<td>8</td>
<td>101 ± 1</td>
<td>354 ± 14</td>
</tr>
<tr>
<td>40 nmol kg⁻¹</td>
<td>11</td>
<td>101 ± 1</td>
<td>345 ± 7</td>
</tr>
<tr>
<td>120 nmol kg⁻¹</td>
<td>8</td>
<td>102 ± 2</td>
<td>353 ± 11</td>
</tr>
<tr>
<td>Administration of saline and 5-HT in Brattleboro rats</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline 5 µl</td>
<td>8</td>
<td>112 ± 4</td>
<td>309 ± 5</td>
</tr>
<tr>
<td>4 nmol kg⁻¹</td>
<td>8</td>
<td>110 ± 3</td>
<td>324 ± 7</td>
</tr>
<tr>
<td>40 nmol kg⁻¹</td>
<td>8</td>
<td>113 ± 4</td>
<td>323 ± 7</td>
</tr>
<tr>
<td>120 nmol kg⁻¹</td>
<td>8</td>
<td>110 ± 4</td>
<td>321 ± 9</td>
</tr>
<tr>
<td>Administration of saline and DP-5-CT in Long-Evans rats</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline 5 µl</td>
<td>4</td>
<td>104 ± 4</td>
<td>334 ± 7</td>
</tr>
<tr>
<td>3 nmol kg⁻¹</td>
<td>4</td>
<td>103 ± 5</td>
<td>351 ± 13</td>
</tr>
<tr>
<td>30 nmol kg⁻¹</td>
<td>4</td>
<td>101 ± 7</td>
<td>330 ± 17</td>
</tr>
<tr>
<td>100 nmol kg⁻¹</td>
<td>4</td>
<td>101 ± 4</td>
<td>337 ± 14</td>
</tr>
</tbody>
</table>

Table 3.2. Baseline values for MAP and HR in conscious Long-Evans and Brattleboro rats. All compounds were administered i.c.v. Values are the mean ± s.e.mean.
Figure 26 Conscious Long-Evans rats: histograms showing the maximum changes in mean arterial blood pressure (MAP) and heart rate (HR) during the first phase (1-5 min) and the second phase (10-30 min) of the response to i.c.v. 5-HT (4 nmol kg\(^{-1}\), \[\square\]; 40 nmol kg\(^{-1}\), \[\square\square\]; 120 nmol kg\(^{-1}\), \[\square\square\square\]). Each bar represents the mean value and the vertical lines show s.e.mean. * P < 0.05 and ** P < 0.01 compared (Student’s paired \(t\) test) to i.c.v. saline over the same periods (not shown).
and hindquarters vascular conductances. These variables remained stable for the duration of the experiment (see Figures 27 and 32). There were no changes in behaviour associated with administration of saline in Long-Evans or Brattleboro rats. Baseline values for blood pressure and heart rate are given in Table 3.2.

Effect of i.c.v. administration of 5-HT in Long-Evans rats

5-HT 4 nmol kg\(^{-1}\) i.c.v. \((n=8)\) caused an immediate significant increase in arterial blood pressure of 6 ± 1 mmHg and heart rate of 30 ± 16 beats min\(^{-1}\) 2 min after injection (baseline blood pressure and heart rate values are given in Table 3.1). This was associated with a significant fall in mesenteric conductance (nadir 19 ± 2 %, after 1 min) and these changes were maintained for 5 min. There was an immediate significant increase in hindquarters conductance of 22 ± 8 % which had returned to baseline levels after 3 min. There was no significant change in renal conductance (Figure 27). A representative trace of this response is shown in Figure 28. Higher doses of 5-HT \([40 \text{ (} n=11 \text{)} \text{ and } 120 \text{ (} n=8 \text{)} \text{ nmol kg}^{-1} \text{ i.c.v.}]\) caused immediate increases in arterial blood pressure which reached maxima between 2-3 min of 15 ± 1 and 15 ± 2 mmHg, respectively. The rise in blood pressure was maintained for 5 min and then returned to baseline levels after 10 min (Figure 27). In addition, 5-HT \([40 \text{ and } 120 \text{ nmol kg}^{-1}]\) caused significant falls in heart rate of 63 ± 10 and 83 ± 10 beats min\(^{-1}\), respectively after 5 min. These heart rate changes had returned to near baseline values after 15 min. After 30 min 5-HT 120 nmol kg\(^{-1}\) caused a significant tachycardia of 33 ± 12 beats min\(^{-1}\) (Figure 27). Representative tracings of the effects of 5-HT 40 and 120 nmol kg\(^{-1}\) are illustrated in Figures 29 and 30 and baseline values are given in Table 3.2. 5-HT 40 and 120 nmol kg\(^{-1}\) caused small reductions in renal conductance (nadir 10 ± 2 % and 9 ± 2 %, respectively) 2-3 min after i.c.v. administration. 5-HT 40 and 120 nmol kg\(^{-1}\) also caused immediate, marked reductions in mesenteric
Figure 27 Conscious Long-Evans rats: a comparison of the changes from baseline values over time (min) caused by i.c.v. saline (○; 5 μl; n=8), 5-HT 4 nmol kg⁻¹ (▼; n=8), 5-HT 40 nmol kg⁻¹ (■; n=11) and 5-HT 120 nmol kg⁻¹ (●; n=8) in mean arterial blood pressure (MAP), heart rate (HR) and renal, mesenteric and hindquarters vascular conductance. Each point represents the mean value and the vertical lines show s.e.mean. * P < 0.05 and ** P < 0.01 compared (ANOVA) to i.c.v. saline.
Figure 28 Representative traces showing the effects of i.c.v. 5-HT 4 nmol kg\(^{-1}\) on phasic and mean arterial blood pressure (BP), heart rate (HR) and renal, mesenteric and hindquarters blood flow (as measured by Doppler shift) in a conscious Long-Evans rat.
Figure 29 Representative traces showing the effects of i.c.v. 5-HT 40 nmol kg⁻¹ on phasic and mean arterial blood pressure (BP), heart rate (HR) and renal, mesenteric and hindquarters blood flow (as measured by Doppler shift) in a conscious Long-Evans rat.
Figure 30 Representative traces showing the effects of i.c.v. 5-HT 120 nmol kg⁻¹ on phasic and mean arterial blood pressure (BP), heart rate (HR) and renal, mesenteric and hindquarters blood flow (as measured by Doppler shift) in a conscious Long-Evans rat.
conductance of 28 ± 3 and 38 ± 5 %, respectively. The mesenteric vasoconstriction was maintained for 5-10 min. 5-HT caused a dose-related peak followed by a maintained increase in hindquarters conductance. Peak increases of 15 ± 9 and 37 ± 11 % occurred 1 min after administration of 5-HT 40 and 120 nmol kg\(^{-1}\) and these changes were followed by maintained increases reaching maxima after 10 min of 30 ± 7 and 47 ± 13 %, respectively (Figure 27). Therefore, 5-HT caused differential haemodynamic changes; renal and mesenteric vasoconstriction were associated with dilatation of hindquarters vasculature. The mesenteric constriction and the pressor response were temporally matched (see Figure 27).

**Effect of pretreatment with d(CH\(_2\))\(_5\)Tyr(Me)AVP on the response to 5-HT**

The effect of vasopressin V\(_1\)-receptor blockade on the response to 5-HT in Long-Evans rats was examined. The dose of 5-HT selected for the antagonist study was 40 nmol kg\(^{-1}\) as this dose showed all aspects of the 5-HT response and was submaximal. Pretreatment (10 min) with d(CH\(_2\))\(_5\)Tyr(Me)AVP (10 µg kg\(^{-1}\) bolus; 10 µg kg\(^{-1}\)h\(^{-1}\) infusion; i.v.; n = 7) which has previously been shown to block V\(_1\)-receptors (Gardiner et al, 1989) did not significantly (Student’s paired t test) change baseline values for blood pressure, heart rate or mesenteric conductance. However, there was a small increase in renal and hindquarters conductance (see Table 3.3). In the presence of d(CH\(_2\))\(_5\)Tyr(Me)AVP the maximum rise in blood pressure caused by 5-HT 40 nmol kg\(^{-1}\) i.c.v. was not significantly affected (Figure 31). However, the bradycardia associated with the pressor response was significantly attenuated (Figure 31). Pretreatment with d(CH\(_2\))\(_5\)Tyr(Me)AVP significantly reduced the mesenteric vasoconstriction caused by 5-HT and increased the initial hindquarters dilatation. The initial renal vasoconstriction was attenuated (Figure 31).
Figure 31 Conscious Long-Evans rats: a comparison of the changes from baseline or post-pretreatment values over time (min) caused by 5-HT (40 nmol kg\(^{-1}\), i.c.v.) in the absence (■; \(n = 11\)) and the presence of dC\(_2\)H\(_5\)Tyr(Me)AVP (bolus 10 \(\mu g\) kg\(^{-1}\), infusion 10 \(\mu g\) kg\(^{-1}\) h\(^{-1}\), i.v.; □; \(n = 7\)) in mean arterial blood pressure (MAP), heart rate (HR) and renal, mesenteric and hindquarters vascular conductance. Each point represents the mean value and the vertical lines show s.e.mean. * \(P < 0.05\) and ** \(P < 0.01\) compared (ANOVA) to 5-HT in non-pretreated animals.
**Effect of i.c.v. administration of 5-HT in Brattleboro rats**

Administration of 5-HT 4 nmol kg\(^{-1}\) (n=8) i.c.v. caused a significant rise in arterial blood pressure of 7 ± 4 mmHg associated with a tachycardia of 86 ± 23 beats min\(^{-1}\), 3 min following administration (Figure 32). These changes were accompanied by significant reductions in renal and mesenteric conductances of 11 ± 5 % and 14 ± 3 %, respectively. The onset of these changes was immediate and all variables had returned near to baseline values after 5 min (Figure 32). 5-HT 4 nmol kg\(^{-1}\) caused an immediate significant increase in hindquarters conductance which reached a maxima after 1 min of 37 ± 9 % (Figure 32). A representative trace diagram of the response to 5-HT 4 nmol kg\(^{-1}\) is illustrated in Figure 33 and baseline values are given in Table 3.2. Higher doses of 5-HT [40 (n=8) and 120 (n=8) nmol kg\(^{-1}\) i.c.v.] caused significant reductions in arterial blood pressure (8 ± 3 and 14 ± 2 mmHg, respectively) which were maximal between 10-15 min (Figure 32). 5-HT (40 and 120 nmol kg\(^{-1}\)) caused initial significant increases in heart rate of 33 ± 10 and 41 ± 15 beats min\(^{-1}\), respectively after 1 min. The tachycardia was followed by bradycardia and maximum falls (nadir 21 ± 7 and 33 ± 9 beats min\(^{-1}\), respectively) were observed 10 min after injection of 5-HT (Figure 32). These changes were associated with initial (1-5 min) reductions in mesenteric conductance which reached significance. Maximum falls were observed 3 min after i.c.v. injection of 5-HT 40 and 120 nmol kg\(^{-1}\) and were 13 ± 4 and 19 ± 3 %, respectively. A significant, dose related increase in hindquarters conductance was observed immediately following administration of 5-HT (40 and 120 nmol kg\(^{-1}\)); maximum changes occurred within 5 min of injection and were 37 ± 12 and 60 ± 5 %, respectively. The hindquarters dilatation was maintained for 20 min and the duration of this change was similar to the duration of the depressor response (Figure 32). 5-HT (40 and 120 nmol kg\(^{-1}\)) caused little change in renal conductance. Representative tracings of the effect of 5-HT 40 and 120 nmol kg\(^{-1}\) are depicted in
Figure 32 Conscious Brattleboro rats: a comparison of the changes from baseline values over time (min) caused by i.c.v. saline (O; 5 μl; n=8), 5-HT 4 nmol kg⁻¹ (▲; n=8), 5-HT 40 nmol kg⁻¹ (■; n=8) and 5-HT 120 nmol kg⁻¹ (●; n=8) in mean arterial blood pressure (MAP), heart rate (HR) and renal, mesenteric and hindquarters vascular conductance. Each point represents the mean value and the vertical lines show s.e.mean. * P < 0.05 and ** P < 0.01 compared (ANOVA) to i.c.v. saline.
Figure 33 Representative traces showing the effects of i.c.v. 5-HT 4 nmol kg\(^{-1}\) on phasic and mean arterial blood pressure (BP), heart rate (HR) and renal, mesenteric and hindquarters blood flow (as measured by Doppler shift) in a conscious Brattleboro rat.
Figure 34 Representative traces showing the effects of i.c.v. 5-HT 40 nmol kg\(^{-1}\) on phasic and mean arterial blood pressure (BP), heart rate (HR) and renal, mesenteric and hindquarters blood flow (as measured by Doppler shift) in a conscious Brattleboro rat.
Figure 35 Representative traces showing the effects of i.c.v. 5-HT 120 nmol kg\(^{-1}\) on phasic and mean arterial blood pressure (BP), heart rate (HR) and renal, mesenteric and hindquarters blood flow (as measured by Doppler shift) in a conscious Brattleboro rat.
Figures 34 and 35 and baseline values are shown in Table 3.2. Therefore, in Brattleboro rats 5-HT produced dose-related changes in blood pressure; the low dose (4 nmol kg\(^{-1}\)) caused a pressor response whereas higher doses (40 and 120 nmol kg\(^{-1}\)) caused falls in blood pressure. All doses of 5-HT caused a tachycardia and this was followed by bradycardia at higher doses. 5-HT caused differential conductance changes. Vasoconstriction was observed in the mesenteric vasculature and this was accompanied with a marked hindquarters dilatation. Renal constriction was observed with the low dose of 5-HT.

Effect of pretreatment with ICI 118551 on the response to 5-HT in Brattleboro rats.

In four animals the effect of \(\beta_2\)-adrenoceptor blockade on the response to 5-HT was examined. Brattleboro rats were pretreated (1 h) with ICI 118551 (0.2 mg kg\(^{-1}\) bolus; 0.1 mg kg\(^{-1}\) infusion; i.v.). This regimen has previously been shown to selectively block \(\beta_2\)-adrenoceptors (see Gardiner and Bennett, 1988). ICI 118551 did not significantly affect baseline values for blood pressure, heart rate and renal conductance. However, 1 h after administration there was a significant reduction in mesenteric and hindquarters conductance (see Table 3.4). In the presence of ICI 118551 the changes in blood pressure, heart rate, renal conductance and mesenteric conductance caused by 5-HT 120 nmol kg\(^{-1}\) i.c.v. were not significantly affected. However, the initial (1-2 min) increase in hindquarters conductance was significantly attenuated. The maintained increase in this variable was not significantly changed (see Figure 36).
### Table 3.3
Baseline values and changes in MAP, HR and renal, mesenteric and hindquarters vascular conductance following administration of d(CH$_2$)$_5$Tyr(Me)AVP (n = 4) in conscious Long-Evans rats. Values are the mean ± s.e.mean. Significant changes (Student’s paired $t$ test) in baseline values are shown as * $P < 0.05$.

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>d(CH$_2$)$_5$Tyr(Me)AVP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose</td>
<td>Bolus 10 µg kg$^{-1}$; Infusion 10 µg kg$^{-1}$ h$^{-1}$</td>
</tr>
<tr>
<td>Injection route</td>
<td>i.v.</td>
</tr>
<tr>
<td>Pretreatment time</td>
<td>10 min</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Basal</th>
<th>Change 5 min</th>
<th>Change 10 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mmHg)</td>
<td>104 ± 3</td>
<td>-2 ± 1</td>
<td>-3 ± 1</td>
</tr>
<tr>
<td>HR (beats min$^{-1}$)</td>
<td>341 ± 9</td>
<td>4 ± 4</td>
<td>-4 ± 4</td>
</tr>
<tr>
<td>Vascular conductance (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renal</td>
<td>0</td>
<td>12 ± 5*</td>
<td>4 ± 5</td>
</tr>
<tr>
<td>Mesenteric</td>
<td>0</td>
<td>0 ± 1</td>
<td>-1 ± 2</td>
</tr>
<tr>
<td>Hindquarters</td>
<td>0</td>
<td>7 ± 2*</td>
<td>8 ± 2*</td>
</tr>
</tbody>
</table>

### Table 3.4
Baseline values and changes in MAP, HR and renal, mesenteric and hindquarters vascular conductance following administration of ICI 118551 (n = 4) in conscious Brattleboro rats. Values are the mean ± s.e.mean. Significant changes (Student’s paired $t$ test) in baseline values are shown as * $P < 0.05$.

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>ICI 118551</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose</td>
<td>Bolus 0.2 mg kg$^{-1}$; Infusion 0.1 mg kg$^{-1}$ h$^{-1}$</td>
</tr>
<tr>
<td>Injection route</td>
<td>i.v.</td>
</tr>
<tr>
<td>Pretreatment time</td>
<td>1 h</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Basal</th>
<th>Change 10 min</th>
<th>Change 60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mmHg)</td>
<td>113 ± 2</td>
<td>2 ± 4</td>
<td>5 ± 4</td>
</tr>
<tr>
<td>HR (beats min$^{-1}$)</td>
<td>344 ± 6</td>
<td>-1 ± 10</td>
<td>19 ± 10</td>
</tr>
<tr>
<td>Vascular conductance (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renal</td>
<td>0</td>
<td>1 ± 12</td>
<td>9 ± 17</td>
</tr>
<tr>
<td>Mesenteric</td>
<td>0</td>
<td>-8 ± 4</td>
<td>-15 ± 3*</td>
</tr>
<tr>
<td>Hindquarters</td>
<td>0</td>
<td>-5 ± 6</td>
<td>-18 ± 2*</td>
</tr>
</tbody>
</table>
Figure 36 Conscious Brattleboro rats: a comparison of the changes from baseline or post-pretreatment values over time (min) caused by 5-HT (120 nmol kg$^{-1}$, i.c.v.) in the absence (●; n=8) and the presence of ICI 118551 (bolus 0.2 mg kg$^{-1}$, infusion 0.1 mg kg$^{-1}$ h$^{-1}$, i.v.; ■; n=4) in mean arterial blood pressure (MAP), heart rate (HR) and renal, mesenteric and hindquarters vascular conductance. Each point represents the mean value and the vertical lines show s.e.mean. * P < 0.05 and ** P < 0.01 compared (ANOVA) to 5-HT in non-pretreated animals.
3.3.3 Behavioural responses caused by 5-HT in Long-Evans and Brattleboro rats

There were no behavioural changes associated with i.c.v. administration of saline in Long-Evans and Brattleboro rats; animals usually remained settled during the injection and for the duration of the experiment. Changes in behaviour were observed following administration of 5-HT but these changes were not quantitatively analysed. Similar behavioural changes were observed in Long-Evans and Brattleboro rats and behavioural changes were observed in all animals. 5-HT 4 nmol kg\(^{-1}\) caused an initial arousal response. Animals were usually resting when injections were made and following injection of 5-HT the animals became more alert and exhibited exploratory behaviour. This response had a duration of about 5 min. Higher doses of 5-HT (40 and 120 nmol kg\(^{-1}\)) caused initial (1-4 min) arousal after which flat body posture with hind limb abduction was observed. This response lasted for 15-20 min and was more pronounced following injection of 5-HT 120 nmol kg\(^{-1}\). Interestingly, in Long-Evans rats the time course for the flat body posture and the maintained increase in hindquarters conductance were temporally matched. In Brattleboro rats a similar pattern was observed and this was also related temporally to the depressor response caused by 5-HT 120 nmol kg\(^{-1}\) (see Figures 32 and 35). In Long-Evans and Brattleboro rats, 5-HT 4 nmol kg\(^{-1}\) caused an arousal response which was temporally related to tachycardia and a peak increase in hindquarters dilatation.

3.3.4 Regional haemodynamic changes caused by DP-5-CT in Long-Evans rats

The effect of central administration of a selective 5-HT\(_{1A}\) receptor agonist, DP-5-CT was examined in conscious Long-Evans rats. Baseline values for blood pressure and heart rate for this group of experiments are shown in Table 3.2. DP-5-CT (3, 30 nmol kg\(^{-1}\) i.c.v.; n = 4) did not significantly
Figure 37 Conscious Long-Evans rats: a comparison of the changes from baseline values over time (min) caused by i.c.v. saline (O; 5 μl; n = 4), DP-5-CT 3 nmol kg⁻¹ (▼; n = 4), DP-5-CT 30 nmol kg⁻¹ (■; n = 4) and DP-5-CT 100 nmol kg⁻¹ (●; n = 4) in mean arterial blood pressure (MAP), heart rate (HR) and renal, mesenteric and hindquarters vascular conductance. Each point represents the mean value and the vertical lines show s.e.mean. * P < 0.05 and ** P < 0.01 compared (ANOVA) to i.c.v. saline.
change arterial blood pressure. A higher dose of DP-5-CT (100 nmol kg\(^{-1}\) i.c.v.; \(n = 4\)) caused a delayed (3 min) increase in blood pressure which reached a maximum of 10 ± 1 mmHg after 10 min (Figure 37). DP-5-CT 3, 30 and 100 nmol kg\(^{-1}\) caused immediate significant increases in heart rate which reached maxima between 2-5 min of 68 ± 18, 50 ± 32 and 92 ± 15 beats min\(^{-1}\), respectively (see Figure 37). These changes were associated with differential changes in renal, mesenteric and hindquarters conductances. DP-5-CT 100 nmol kg\(^{-1}\) caused a reduction in renal conductance which reached significance after 10 min. Lower doses of DP-5-CT did not significantly alter renal conductance (Figure 37). DP-5-CT (3 and 100 nmol kg\(^{-1}\) i.c.v.) caused significant reductions in mesenteric conductance of 16 ± 7 and 29 ± 7 %, respectively (Figure 37). DP-5-CT (3, 30 and 100 nmol kg\(^{-1}\)) caused an immediate increase in hindquarters conductance which reached a maximum after 15 min of 53 ± 5, 67 ± 32 and 77 ± 12 %, respectively. Representative trace diagrams for DP-5-CT 3, 30 and 100 nmol kg\(^{-1}\) are depicted in Figures 38, 39 and 40.

### 3.3.5 Behavioural responses caused by DP-5-CT in Long-Evans rats

All doses of DP-5-CT caused an arousal response immediately after i.c.v. administration. This response lasted for 3-4 min and in animals which received DP-5-CT 30 and 100 nmol kg\(^{-1}\), flat body posture and hind limb abduction were observed. This response was more pronounced with the high dose 100 nmol kg\(^{-1}\) and flat body posture was maintained for 30 min after administration.
Figure 38 Representative traces showing the effects of i.c.v. DP-5-CT 3 nmol kg\(^{-1}\) on phasic and mean arterial blood pressure (BP), heart rate (HR) and renal, mesenteric and hindquarters blood flow (as measured by Doppler shift) in a conscious Long-Evans rat.
Figure 39 Representative traces showing the effects of i.c.v. DP-5-CT 30 nmol kg\(^{-1}\) on phasic and mean arterial blood pressure (BP), heart rate (HR) and renal, mesenteric and hindquarters blood flow (as measured by Doppler shift) in a conscious Long-Evans rat.
Figure 40 Representative traces showing the effects of i.c.v. DP-5-CT 100 nmol kg$^{-1}$ on phasic and mean arterial blood pressure (BP), heart rate (HR) and renal, mesenteric and hindquarters blood flow (as measured by Doppler shift) in a conscious Long-Evans rat.
3.4 Discussion

In the present experiments in conscious Long-Evans rats 5-HT administered to the lateral ventricle caused similar cardiovascular changes to those observed in conscious Sprague-Dawley rats (see Chapter 2 and compare Figures 2 and 26). This suggests that a strain difference does not occur and allows comparison between these studies. In conscious Long-Evans rats 5-HT (4, 40, 120 nmol kg\(^{-1}\) i.c.v.) caused a pressor response which was associated with dose-related changes in heart rate. The low dose of 5-HT (4 nmol kg\(^{-1}\)) caused an increase in heart rate whereas higher doses of 5-HT (40 and 120 nmol kg\(^{-1}\)) initially caused bradycardia which was followed by tachycardia with the highest dose. In rats with genetically produced diabetes insipidus, which are deficient in vasopressin, 5-HT caused dose-related changes in blood pressure and heart rate. The low dose of 5-HT (4 nmol kg\(^{-1}\)) caused immediate tachycardia and a rise in blood pressure whereas higher doses of 5-HT (40, 120 nmol kg\(^{-1}\)) caused a delayed, dose-dependent fall in blood pressure associated with biphasic heart rate changes; an initial tachycardia was followed by a maintained bradycardia. The magnitude of the bradycardia in Brattleboro rats was markedly reduced compared to that observed in Long-Evans rats and the time course for these changes was different. However, 5-HT (40 nmol kg\(^{-1}\)) given i.c.v. in Long-Evans rats pretreated with a vasopressin \(V_1\)-receptor antagonist caused a similar change in heart rate to that observed in Brattleboro rats.

In the present experiments in conscious Long-Evans rats 5-HT administered to the lateral ventricle caused a pressor response which was of similar magnitude to that previously reported for 5-HT (Sukamoto et al., 1984; Dalton, 1986; Pergola & Alper, 1990; Dedeoglu & Fisher, 1991a; Chapter 2). In conscious Brattleboro rats a depressor response was also observed. Intracerebroventricular injection of 5-HT has previously been shown to
cause a delayed fall in blood pressure associated with a bradycardia in conscious (Dalton, 1986) and anaesthetized (Krstic & Djurkovic, 1981) rats and was similar in magnitude to that observed in Brattleboro rats in the present study. In conscious (Dalton, 1986; Pergola & Alper, 1992) and anaesthetized (see Chapter 2) rats the reduction in heart rate resulted from a combination of both an increase in vagal drive and a reduction in sympathetic drive; presumably similar mechanisms were involved in the present study.

The rise in blood pressure in Long-Evans rats was associated with differential changes in regional haemodynamics; mesenteric and renal vasoconstriction were accompanied with vasodilatation of the hindquarters vasculature. In Brattleboro rats i.c.v. 5-HT caused a small mesenteric vasoconstriction and a marked hindquarters vasodilatation with little change in renal conductance. The 5-HT induced fall in renal conductance in Long-Evans rats was not associated with a fall in renal blood flow (see appendix). Therefore, it was possible that the renal constriction was mediated by autoregulatory mechanisms in the kidney, preserving renal flow as systemic blood pressure was increased. This supposition is supported by the finding that renal conductance was not reduced in Brattleboro rats in which the pressor response was absent.

In Long-Evans rats 5-HT caused a dose-related reduction in mesenteric vascular conductance and these changes paralleled the pressor response. This suggests that the rise in blood pressure caused by i.c.v. 5-HT was mediated by mesenteric vasoconstriction. Systemic administration of vasopressin has previously been shown to cause a marked constriction of the mesenteric bed and a rise in blood pressure associated with a reflexly mediated bradycardia (Gardiner et al., 1988, 1989). In Brattleboro rats the fall in mesenteric conductance was markedly smaller than that elicited in
Long-Evans rats and there was no increase in blood pressure in following administration of higher doses (40, 120 nmol kg⁻¹) of 5-HT. These findings strongly suggest that the initial pressor response and mesenteric constriction caused by 5-HT administered i.c.v. in Long-Evans rats were mediated following the release of vasopressin. In support of this conclusion, the vasopressin V₁-receptor antagonist d(CH₂)₅Tyr(Me)AVP (Kruszynski et al., 1980; Manning & Sawyer, 1986), at a dose which has previously been shown to prevent the cardiovascular action of vasopressin (Inoue & Buñag, 1989), antagonised the mesenteric vasoconstriction in Long-Evans rats. Furthermore, the absence of an initial marked bradycardia in Brattleboro rats and Long-Evans rats pretreated with d(CH₂)₅Tyr(Me)AVP suggests that the initial bradycardia caused by 5-HT given i.c.v. in non-pretreated Long-Evans rats was mediated through activation of the baroreceptor reflex following the pressor response caused by vasopressin. Further, the bradycardia produced by 5-HT was prevented by ganglionic blockade or vasopressin V₁-receptor antagonism (Pergola & Alper, 1991) and was absent in sinoaortic denervated rats (Pergola & Alper, 1992). This data supports the view that i.c.v. 5-HT causes the release of vasopressin which produces a pressor response and a reflexly mediated bradycardia. This is consistent with the conclusions made in previous studies (Anderson et al., 1992; see Chapter 2; Pergola & Alper, 1991; 1992). The release of vasopressin by centrally administered 5-HT has previously been shown to be mediated by 5-HT₂ and/or 5-HT₁C receptors (see Chapter 2; see Van de Kar, 1991).

Interestingly, the pressor response caused by i.c.v. 5-HT was not prevented by pretreatment with a V₁-receptor antagonist in the present study in conscious Long-Evans rats and in the previous study in anaesthetized rats (see Chapter 2). This response suggests that pressure is also increased in Long-Evans rats through an additional mechanism. In this respect, 5-HT (in
the presence of 5-HT₂/5-HT₁c receptor antagonists) has been shown to cause an increase in blood pressure and heart rate which were associated with an increase in sympathetic outflow following activation of central 5-HT₁A receptors. Moreover, central administration of selective 5-HT₁A receptor agonists caused an increase in blood pressure, heart rate and generalised sympathoexcitation (Anderson et al., 1992; Chapter 2). In the present study in Long-Evans rats pretreated with the V₁-receptor antagonist d(CH₂)₅Tyr(Me)AVP and in Brattleboro rats, 5-HT i.c.v. caused significant reductions in mesenteric conductance associated with tachycardia. This effect of 5-HT administered i.c.v. is consistent with an increase in sympathetic outflow to the mesenteric vascular bed and to the heart. In this respect, 5-HT has been shown to cause a marked tachycardia in sinoaortic denervated rats mediated through β-adrenoceptors (Pergola & Alper, 1992). Moreover, the low dose (4 nmol kg⁻¹) of 5-HT caused an increase in blood pressure associated with a tachycardia and mesenteric vasoconstriction in Long-Evans and Brattleboro rats. The finding that these responses occur in Brattleboro rats (i.e. animals without vasopressin) further supports the view that these changes are a result of a sympathoexcitatory action of 5-HT. This could be evaluated by examining the effects of the α₁-adrenoceptor antagonist prazosin on the response caused by i.c.v. administration of 5-HT in Brattleboro rats or a combined pretreatment of prazosin and d(CH₂)₅Tyr(Me)AVP in Long-Evans rats. In this respect, Pergola & Alper (1991) demonstrated that a combined pretreatment with prazosin and a vasopressin V₁-receptor antagonist abolished the pressor response caused by i.c.v. 5-HT in conscious rats; neither antagonist prevented the rise in blood pressure when given alone. These studies suggest that in Long-Evans and Brattleboro rats 5-HT can cause sympathoexcitation that results in an increase in blood pressure. The pressor response caused by the low dose of 5-HT would appear to result from the sympathoexcitatory actions of 5-HT and not the release of
vasopressin since similar cardiovascular effects were observed in Long-Evans and vasopressin deficient Brattleboro rats. This conclusion is consistent with the findings of previous studies (Chapter 2; Dedeoglu & Fisher, 1991; Pergola & Alper, 1991a).

The cardiovascular changes of the selective 5-HT$_{1A}$ receptor agonist DP-5-CT (Mir et al., 1987; Doods et al., 1988; see Schoeffter & Hoyer, 1988) were investigated in Long-Evans rats. DP-5-CT administered i.c.v. caused a modest increase in blood pressure associated with a marked tachycardia and mesenteric vasoconstriction. DP-5-CT also caused a dose-related increase in hindquarters vascular conductance. Essentially the response produced by DP-5-CT was similar to that produced by 5-HT in Brattleboro rats. The mesenteric vasoconstriction and tachycardia is consistent with a sympathoexcitatory action of this 5-HT$_{1A}$ receptor agonist. These findings support the view that the sympathoexcitation caused by 5-HT in conscious rats was produced through activation of 5-HT$_{1A}$ receptors and are consistent with previous studies (Anderson et al., 1992; Dedeoglu & Fisher, 1991a; see Chapter 2). However, this conclusion would be strengthened if the effects of DP-5-CT or 5-HT (in Brattleboro rats) were shown to be abolished by pretreatment with a 5-HT$_{1A}$ receptor antagonist.

DP-5-CT caused a marked hindquarters vasodilatation in conscious Long-Evans rats. 5-HT also caused a marked vasodilatation of this vascular bed and these effects were most pronounced in the Brattleboro rat. Indeed, 5-HT i.c.v. did not cause a rise in blood pressure in the Brattleboro rat in spite of a significant mesenteric vasoconstriction, this was presumably due to the marked hindquarters vasodilator response. Moreover, the high dose of 5-HT caused a delayed reduction in blood pressure which was temporally matched to the maintained increase in hindquarters conductance.
Therefore, it is likely that this depressor response was mediated by hindquarters vasodilatation. In Long-Evans and Brattleboro rats the low dose of 5-HT caused a initial peak rise in hindquarters vascular conductance which was maintained with higher doses of 5-HT. Interestingly, the initial rise in this variable was temporally associated with tachycardia in Long-Evans and Brattleboro rats. In Brattleboro rats the initial rise in hindquarters conductance was prevented by pretreatment with the ICI 118551 at a dose that has previously been shown to selectively block \( \beta_2 \)-adrenoceptors. Adrenaline is known to cause tachycardia and hindquarters vasodilatation through activation of \( \beta_1 \)- and \( \beta_2 \)-adrenoceptors, respectively. Furthermore, the 5-HT\(_{1A}\) receptor agonist 8-OH-DPAT (i.v.) can cause the release of adrenaline by central sympathoexcitation of the adrenal glands in conscious rats (Chaouloff & Jeanrenaud, 1987; Chaouloff et al., 1990a, 1990b; Bagdy et al., 1989a; 1989b; Bouhelal & Mir, 1990, 1992). These findings suggest that the initial hindquarters vasodilatation caused by 5-HT i.c.v. was indirectly mediated through the release of adrenaline. Presumably the initial tachycardia was in part caused by adrenaline, however, this does not preclude an increase in cardiac sympathetic nerve activity. The relative influence of neural and hormonal actions in this response could be investigated by repeating 5-HT administration in adrenalectomized animals. The involvement of adrenaline in the response to DP-5-CT was not tested in this study, however, the initial tachycardia associated with hindquarters vasodilatation is consistent with the involvement of adrenaline. Furthermore, DP-5-CT has been shown to cause a marked increase in adrenal nerve activity (Chapter 2) and 5-HT\(_{1A}\) receptor agonists have the ability to release adrenaline (see above).

The maintained increase in hindquarters vasodilatation caused by higher doses of 5-HT was not sensitive to antagonism by the ICI 118551 and the involvement of adrenaline in this response is unlikely. A maintained
hindquarters vasodilatation was observed with both 5-HT and the 5-HT$_{1A}$ receptor agonist DP-5-CT and the magnitude of the changes caused by these agonists were similar. This finding suggests that this phase of the hindquarters dilator response was mediated by 5-HT$_{1A}$ receptors, although this requires conformation with antagonist studies. The mechanism mediating the maintained increase in hindquarters conductance is unknown.\footnote{See Appendix 3}

However, activation of 5-HT$_{1A}$ receptors located in the rostral ventrolateral medulla has previously been shown to cause a depressor response associated with an increase in femoral arterial conductance in rats (Lovick, 1989a, 1989b; Helke et al., 1993). Bilateral microinjection of 8-OH-DPAT into the RVLM also produced a reduction in blood pressure and lumbar sympathetic nerve activity (Nosjean & Guyenet, 1991; this data is reviewed in more detail in the General Introduction; see 1.7.1). Therefore, the hindquarters vasodilatation observed may reflect the withdrawal of a tonic sympathetic vasoconstrictor outflow to the hindquarters vasculature following the activation of 5-HT$_{1A}$ receptors in the RVLM by 5-HT and DP-5-CT. Activation of 5-HT$_{1A}$ receptors in all species tested including rats has been shown to cause a reduction in blood pressure, sympathoinhibition and an increase in vagal drive (see 1.7.1; see Ramage, 1990). The depressor response and bradycardia observed in Brattleboro rats may also be caused by an action of 5-HT on 5-HT$_{1A}$ receptors.

In $\alpha$-chloralose anaesthetized rats 5-HT causes an increase in blood pressure associated with renal sympathoexcitation in animals pretreated with d(CH$_2$)$_5$Tyr(Me)AVP (Chapter 2). It is interesting to note that i.c.v. 5-HT caused little change in renal blood flow in conscious Long Evans rats (in the absence or presence of d(CH$_2$)$_5$Tyr(Me)AVP) and Brattleboro rats. Furthermore, the selective 5-HT$_{1A}$ agonist DP-5-CT also produced an increase in renal nerve activity in anaesthetized rats but did not significantly affect renal blood flow in conscious Long-Evans rats (Chapter 2).
Alterations in efferent renal sympathetic nerve activity have been shown to produce changes in renal blood flow as well as changes in glomerular filtration rate, reabsorption of water and sodium and the release of renin (see DiBona, 1989). The lack of effect of 5-HT and DP-5-CT on renal conductance is inconsistent with the increase in renal sympathetic nerve activity caused by these agonists. The reason for this difference is not known but could be a consequence of comparisons made between conscious and anaesthetized preparations. This point could be addressed by recording renal blood flow in α-chloralose anaesthetized rats or by recording renal nerve activity in conscious rats.

5-HT and 5-HT\textsubscript{1A} receptor agonists have been shown to cause a behavioural syndrome and certain aspects of this behaviour were observed in the present study. High doses of 5-HT and DP-5-CT caused flat body posture in Long-Evans and Brattleboro rats and this response was temporally related to the maintained hindquarters vasodilatation. Flat body posture has previously been shown to be mediated by 5-HT\textsubscript{1A} receptors (Tricklebank et al., 1985). This observation prompts the notion that the cardiovascular and behavioural changes caused by 5-HT and DP-5-CT are linked. It is possible that the increase in hindquarters conductance was caused by the positioning of the animal during this behavioural change, alternatively, the cardiovascular and behavioural changes may have occurred independently of one another. This question was not resolved in the present study.

However, Connor & Higgins (1990) were able to induce both flat body posture and hypotension in conscious rats following microinjection of 5-HT\textsubscript{1A} receptor agonists into the dorsal raphé nucleus and both the cardiovascular and behavioural changes were prevented by the 5-HT\textsubscript{1A} receptor antagonist pindolol or atropine methonitrate. This study further suggests a relationship between cardiovascular and behavioural systems. In a single experiment 5-HT administered i.c.v. in a Long-Evans rat failed to
Figure 41 Traces showing the effects of i.c.v. 5-HT 40 nmol kg$^{-1}$ on phasic and mean arterial blood pressure (BP), heart rate (HR) and renal, mesenteric and hindquarters blood flow (as measured by Doppler shift) in a conscious Long-Evans rat. In this animal no behavioural changes were observed following administration of 5-HT and there was a reduction in hindquarters blood flow. When hindquarters vascular conductance was calculated for this animal a fall of 45% was observed 5 min after injection of 5-HT. Following i.c.v. administration of dye, post-mortem examination of the brain revealed that the dye was restricted to the lateral and third cerebral ventricles. This data was not included in the mean result for this group of experiments.
evoke a behavioural change, the hindquarters vasodilatation was also absent in this animal and vasoconstriction of this vascular bed was observed (see Figure 41). Post mortem examination of the brain revealed that in this animal dye injected i.c.v. was confined to the lateral and third ventricles. This suggests that the hindquarters vasodilatation and flat body posture are only produced when 5-HT diffuses to the hindbrain and emphasises differences between hindbrain and forebrain effects of 5-HT. The opposing actions of 5-HT in the forebrain and hindbrain have previously been reported (Coote et al., 1987; see Coote, 1990; see 1.6.1). An initial arousal response was also observed with 5-HT and DP-5-CT and this occurred with low doses of these agonists. This response appeared similar to that described by Higgins & Elliott (1988) following microinjection of 8-OH-DPAT into the median raphe nucleus of the rat and may reflect an action of 5-HT and DP-5-CT at this site, although this remains to be determined.

In summary, i.c.v. administration of 5-HT in conscious Long-Evans and Brattleboro rats caused differential haemodynamic changes that were dependent on dose. The pressor response elicited by 5-HT was mediated by mesenteric vasoconstriction caused in part by the release of vasopressin. Mesenteric vasoconstriction was also caused through an additional mechanism which was consistent with sympathoexcitation. 5-HT produced dilatation of the hindquarters vasculature. The 5-HT$_{1A}$ receptor agonist DP-5-CT mimicked some aspects of the response induced by 5-HT and caused a pressor response, tachycardia, mesenteric vasoconstriction and hindquarters vasodilatation. These results are consistent with earlier findings in which 5-HT was shown to cause the release of vasopressin through activation of 5-HT$_{2}$ and/or 5-HT$_{1C}$ receptors and sympathoexcitation through activation of 5-HT$_{1A}$ receptors. In addition certain cardiovascular changes caused following i.c.v. administration of
5-HT appeared to be mediated by adrenaline. Thus, the cardiovascular actions of centrally administered 5-HT are complex and appear to be the product of the release of vasopressin and adrenaline and direct sympathoexcitation. Sympathoinhibition is also inferred.
CHAPTER 4

Characterisation of the 5-HT receptors mediating the cardiovascular effects of forebrain administration of 5-HT in anaesthetized cats

4.1 Introduction

Intracerebroventricular (i.c.v.) injections of 5-HT in anaesthetized cats has been shown to cause a fall in blood pressure, bradycardia and sympathoinhibition (Baum & Shropshire, 1975; Coote et al., 1987). However, in their study Coote et al. (1987) demonstrated that low doses of 5-HT given to the lateral ventricle causes a rise in blood pressure and heart rate. In this study renal sympathetic nerve activity was recorded but 5-HT only elicited small changes in this variable. Higher doses of 5-HT produced falls in blood pressure, heart rate and renal sympathetic nerve activity which were absent when the dose of 5-HT was prevented from diffusing to the fourth ventricle (Coote et al., 1987). Furthermore, 5-HT administered to the fourth ventricle caused a reduction in blood pressure, heart rate, cardiac output and sympathetic nerve activity in anaesthetized cats (Coote et al., 1987; Shepheard et al., 1990). These studies suggest that the inhibitory actions of 5-HT are localised to the hindbrain of anaesthetized cats.

Administration of selective 5-HT$_{1A}$ receptor agonists to the fourth ventricle causes a reduction in blood pressure, heart rate and sympathetic nerve activity (Shepheard et al., 1990). This study suggests that activation of 5-HT$_{1A}$ receptors in a brain region accessible from fourth ventricular administration produces these inhibitory cardiovascular actions of 5-HT. Areas of the ventral medulla e.g. the rostral ventrolateral medulla (subretrofacial nucleus), medullary raphé nuclei, lateral tegmental field have
been implicated in the depressor effects of 5-HT\textsubscript{1A} receptor in the cat (see 1.7.1).

Activation of 5-HT\textsubscript{2} and/or 5-HT\textsubscript{1C} receptors have been shown to cause an increase in blood pressure, heart rate and sympathetic nerve activity in anaesthetized cats, probably at the level of the rostral ventrolateral medulla (McCall et al., 1987; McCall & Harris, 1988; King & Holtman, 1989; Mandal et al., 1991; Vayssettes-Courchay et al., 1991; 1992; Shepheard et al., 1991; Ramage et al., 1993; see 1.7.2). Therefore, preferential activation of different 5-HT receptor subtypes can cause opposing cardiovascular actions.

Although cardiovascular changes caused by activation of different hindbrain 5-HT receptor subtypes are well documented the identity and role of forebrain 5-HT receptors in cardiovascular control has not been studied. This chapter investigates further the nature of the 5-HT receptors and the physiological changes involved in the centrally mediated pressor response caused by forebrain administration of 5-HT via the lateral cerebral ventricle in anaesthetized cats. 5-HT and selective agonists and antagonists for the various 5-HT receptor subtypes were administered to the lateral ventricle of \(\alpha\)-chloralose anaesthetized cats in which simultaneous recordings of blood pressure, heart rate, femoral flow and renal, splanchnic and cardiac sympathetic nerve activities were made. In addition respiratory variables were monitored. To prevent the activation of 5-HT receptors located in medullary regions (see above) drugs were restricted to the lateral and third ventricles by cannulation of the Aqueduct of Sylvius (see Coote et al., 1987).
4.2 Methods

4.2.1 Anaesthetized cat

Experiments were performed on male adult cats (2.7-4 kg) anaesthetized with a mixture of α-chloralose (70 mg kg\(^{-1}\)) and pentobarbitone sodium (6 mg kg\(^{-1}\)) i.v.; supplementary doses of α-chloralose (10-15 mg kg\(^{-1}\)) were given as required. The right brachial artery was cannulated for the measurement of arterial blood pressure. Blood pressure was measured using a pressure transducer (Gould Statham P23XL) and heart rate was derived electronically, triggering beat to beat from the blood pressure signal (rate meter; Medical Electronics workshop, RFHSM). The right jugular vein was cannulated for drug administration and the right brachial vein was cannulated for drug infusion. Following tracheotomy the animals were artificially ventilated (stroke rate 25 min\(^{-1}\), stroke volume 17-20 ml) with oxygen-enriched room air by use of a positive pressure pump (Harvard ventilator 665) and neuromuscular blockade was subsequently produced with vecuronium bromide (200 μg kg\(^{-1}\) i.v.). Body temperature was monitored by a rectal probe and maintained at 37-38°C with a homeothermic blanket system (BioScience 8185). Blood samples were taken from a cannula in a branch of the left femoral artery and blood gases and pH were monitored with a Corning pH/blood gas analyser. Blood gases were maintained between 100-130 mmHg \(P_O_2\), 30-40 mmHg \(P_C_O_2\) and pH 7.3-7.4. Slow injections of sodium bicarbonate (1.0 M, 1 ml kg\(^{-1}\) i.v.) and/or adjustments of the respiratory pump volume were made as necessary to maintain blood gas and pH balance. Once ventilated, the animals were infused (6 ml kg\(^{-1}\) h\(^{-1}\)) with a solution comprising 500 ml plasma substitute (Gelofusine, Consolidated Chemicals Ltd), 500 ml distilled water, 8.4 g sodium bicarbonate, 2g glucose and vecuronium bromide (0.4-0.5 mg kg\(^{-1}\) h\(^{-1}\)) into the right brachial vein. This was to prevent the development of non-respiratory acidosis and to maintain blood volume and neuromuscular blockade. In addition, the right femoral artery was cleared.
of connective tissue and a flow probe was positioned to measure femoral arterial flow (Gould Stratham blood flow meter, SP2202 with 1 mm probe, 70082). Tracheal pressure was measured using a pressure transducer (Statham P23BC) connected via a T-piece to the tracheal cannula.

**Cannulation of the lateral ventricle**

The cats were placed in a stereotaxic frame, the dorsal surface of the skull was exposed and a hole (1 cm²) made in the bone, the centre of which was 13.5 mm anterior from the inter-aural line and 3 mm lateral from midline. A stainless steel guide cannula (22 gauge) was implanted unilaterally into the right lateral cerebral ventricle. The co-ordinates used were 13.5 mm anterior from the intra-aural line, 3 mm lateral (right) from midline and 8 mm dorsal to stereotaxic zero (10 mm ventral from the surface of the dura). These coordinates were determined using the stereotaxic atlas of Snider and Niemer (1961). When the ventricle was successfully cannulated cerebrospinal fluid filled the cannula and pulsed in time with heart rate and respiration and there was no resistance to injection of artificial cerebrospinal fluid. Drug and vehicle solutions were administered in a volume of 20 μl over a 1 min period via an i.c.v. injection cannula (28 gauge) attached by a length of polythene tubing to an Agla micrometer syringe. To prevent the passage of drug from the lateral ventricle to the fourth ventricle a cannula was placed in the Aqueduct of Sylvius. This was achieved by removing the atlanto-occipital membrane and a portion of the occipital bone and advancing a cannula (external diameter 3 mm, internal diameter 1 mm) along the midline of the dorsal surface of the medulla, under the cerebellum. When the Aqueduct of Sylvius was successfully cannulated cerebrospinal fluid flowed down the cannula. At the end of each experiment, the placement of these cannulae was confirmed by the administration of 20 μl of 2% pontamine sky blue dye. Only experiments in which dye was seen to
flow down the Aqueduct of Sylvius cannula and in which dye was observed in the lateral and third ventricles were included in the mean result.

**Whole nerve recordings**

Simultaneous recordings were made of right inferior cardiac, splanchnic and renal nerve activities plus left phrenic nerve activity. The right inferior cardiac nerve was exposed by deflecting the right scapula and removing the second rib retropleurally. The splanchnic and renal nerves were exposed by a retroperitoneal approach through the right flank, deflecting the kidney laterally. The left phrenic nerve was exposed low down in the neck at the level of the 4th & 5th spinal nerves. Whole nerve activity was recorded following the positioning of these nerves on bipolar silver hook electrodes. Renal and phrenic nerves were crushed peripherally. Cardiac, renal and splanchnic nerve activities were amplified (Digitimer NL104), filtered (Digitimer NL125; Frequency band 100-500 Hz) and quantified by integrating the signal above background noise over 20s with a solid state integrator (Medical Electronics work shop, RFHSM). Phrenic nerve activity was amplified (Digitimer NL104), filtered (Digitimer NL125; Frequency band 200-2000 Hz) and quantified by integrating the amplitude and frequency of the action potentials in each inspiratory burst (see Holtman et al., 1986). This method gave an indication of both the amount of activity in each inspiratory burst and the frequency of inspiratory bursts. The validity of the integrator threshold setting was verified at the end of the experiment after administration of pentobarbitone sodium (60 mg per animal, i.v.).

At the beginning of each experiment the baroreceptor reflex response was tested by observing whether sympathetic nerve activity and heart rate were increased by a reduction in blood pressure caused by sodium nitroprusside (2 μg kg⁻¹, i.v.). Only preparations with an intact baroreceptor reflex were used. Blood pressure, heart rate, femoral flow, tracheal pressure, and the
integrator outputs of cardiac, renal, splanchnic and phrenic nerve activities were displayed on a Grass polygraph recorder. Blood pressure, femoral flow, tracheal pressure and unfiltered nerve activity from cardiac, renal, splanchnic and phrenic nerves were recorded on electromagnetic tape using a Racal Store 7 tape recorder.

4.2.2 Experimental protocol
All variables were recorded for a 20 min stabilization period before microinjection (20 µl over 1 min) of saline. After a 10 min control period a dose of test compound or saline control was given i.c.v. Cumulative dose-response curves were constructed for 5-HT (10, 40, 160 nmol kg⁻¹), DOI (80, 160, 320 nmol kg⁻¹), 5-CT (2.5, 10, 40 nmol kg⁻¹) and DP-5-CT (2.5, 10, 40 nmol kg⁻¹) and in separate control experiments saline was administered at similar time intervals to the test compound. For each compound 3 doses were administered with a dose interval of 5 or 10 min. These times were chosen to allow the response of each dose to reach a maximum before the next injection. In DOI experiments, all animals were pretreated with the peripherally acting 5-HT₂/5-HT₁C receptor antagonist BW501C67 (Fuller et al. 1986). BW501C67 (1mg kg⁻¹) was administered i.v. 15 min before the addition of the first dose of DOI (5 min before the i.c.v. injection of saline). This was to prevent the activation of peripheral 5-HT₂ receptors following i.c.v. administration of DOI (see Shepheard et al., 1991).

In three experiments, animals were pretreated with cinanserin (265 nmol kg⁻¹ i.c.v.) 10 min before the addition of 5-HT (10, 40, 120 nmol kg⁻¹ i.c.v.).

4.2.3 Analysis of results
Baseline values were taken 1 minute before the addition of drug or vehicle. All results are expressed as changes from baseline values. The mean
femoral flow was divided by mean arterial pressure to provide estimates of femoral vascular conductance. Cardiac, renal and splanchnic nerve activities were measured as the average of the integrated values over 1 minute in arbitrary units and were expressed as the percentage change from baseline. The amplitude of phrenic nerve activity was measured as the average of the integrated values over 1 minute in arbitrary units and was expressed as the percentage change from baseline. The frequency of bursts of phrenic nerve activity was measured by counting the number of bursts in 1 min. Changes in mean arterial pressure, heart rate, femoral vascular conductance, tracheal pressure and cardiac, renal, splanchnic and phrenic nerve activities caused by the test drug were compared with time-matched saline controls using two-way analysis of variance and were subsequently analysed using the least significant difference test (Sokal & Rohlf, 1969). Changes in variables caused by antagonist pretreatments were compared to the pre-dose baseline using Student’s t test for paired data. All values are expressed as the mean ± s.e.mean, differences in the mean were taken as significant when P < 0.05.

4.2.4 Drugs and solutions

Drugs used were BW501C67 (2-anilino-N-(2-(3-chlorophenoxy)propyl) acetamide HCl); 5-carboxamidotryptamine maleate (5-CT);
N,N-di-n-propyl-5-carboxamidotryptamine maleate (DP-5-CT ; these were gifts from Wellcome Research Laboratories, Beckenham, Kent, U.K.);
cinanserin HCl (Squibb Inc., Princeton, U.S.A.); α-chloralose (Sigma Chemical Co., Poole, Dorset, U.K.); DOI, 1-(2,5-di-methoxy-4-iodophenyl)-2-aminopropane (Research Biochemicals Inc., Semat, St. Albans, U.K.);
Gelofusine (Consolidated Chem., Wrexham, Clwyd, U.K.);
5-hydroxytryptamine creatinine sulphate (5-HT; BDH, Poole, Dorset, U.K.);
pentobarbitone sodium (May & Baker Ltd., U.K.); sodium nitroprusside
All drugs were dissolved in 0.9% w/v saline. The pH of the drug solutions to be given i.c.v. were tested and back-titrated to pH 7.4 where possible. Solutions of DOI and cinanserin were found to have pH values between 5.5-6.0. Therefore, a control solution of saline was made up to the same pH as the drug solution and administered 10 min before the addition of drug. The composition of the artificial cerebrospinal fluid used to check the position of the i.c.v. cannula was 2.2 mM KH$_2$PO$_4$; 1.2mM MgSO$_4$.7H$_2$O; 2.0mM KCl, 10mM glucose; 25mM NaHCO$_3$; 115mM NaCl and 2.5mM CaCl$_2$.2H$_2$O.
4.3 Results

4.3.1 Effects of i.c.v. administration of saline

In vehicle control experiments saline (20 μl; n = 5) was administered i.c.v. at 10 min intervals. This dose interval was chosen to provide time-matched control responses to compare with responses caused following cumulative dosing with test compounds. Administration of saline (3 volumes over a 30 min period) produced little change in any of the variables being measured (see Figure 43 and 44). Baseline values for this experimental group are shown in Table 4.1.

4.3.2 Effects of i.c.v. administration of 5-HT

A representative trace diagram of the effect of administration of 5-HT is depicted in Figure 42 and baseline values for this group of experiments are given in Table 4.1. Cumulative administration of 5-HT (10, 40 and 160 nmol kg\(^{-1}\) i.c.v.; n = 5) caused an increase in arterial blood pressure which reached a maximum rise of 16 ± 3 mmHg 3 min after administration of 5-HT 10 nmol kg\(^{-1}\). The pressor response was not maintained with higher doses of 5-HT (Figure 43). This was associated with an increase in heart rate (maximum 30 ± 7 beats min\(^{-1}\)) and a reduction in femoral conductance (nadir 11 ± 5 ml min\(^{-1}\) mmHg\(^{-1}\) 10\(^{-3}\)). The onset of these changes was immediate and responses were near maximal 3 min after administration of 5-HT 10 nmol kg\(^{-1}\). The tachycardia and femoral constriction were maintained following administration of higher doses of 5-HT (40 and 160 nmol kg\(^{-1}\); Figure 43). Differential changes in renal, splanchnic and cardiac nerve activities were observed following 5-HT administration. 5-HT (10 nmol kg\(^{-1}\) i.c.v.) caused a marked increase in cardiac nerve activity which reached 94 ± 18 % after 3 min; there was also a small increase in splanchnic nerve activity (16 ± 6 %) at this time. Maximum increases in cardiac and splanchnic nerve activity (129 ± 27 and 20 ± 7 %, respectively) occurred after 5-HT 40 nmol kg\(^{-1}\). 5-HT 10, 40 and 160
<table>
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<th>Treatment Group</th>
<th>n</th>
<th>MAP mm Hg</th>
<th>HR beats min⁻¹</th>
<th>FAC ml min⁻¹mmHg⁻¹10⁻³</th>
<th>TP cm H₂O</th>
<th>Insp. rate bursts min⁻¹</th>
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<tr>
<td>Saline</td>
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<td>135 ± 7</td>
<td>229 ± 11</td>
<td>69 ± 11</td>
<td>4.3 ± 0.4</td>
<td>5.4 ± 1.1</td>
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<tr>
<td>5-HT</td>
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<td>202 ± 12</td>
<td>73 ± 13</td>
<td>4.1 ± 0.2</td>
<td>5.4 ± 1.4</td>
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<tr>
<td>DOI</td>
<td>4</td>
<td>128 ± 8</td>
<td>181 ± 9</td>
<td>75 ± 18</td>
<td>5.0 ± 0.2</td>
<td>5.8 ± 0.8</td>
</tr>
<tr>
<td>5-CT</td>
<td>4</td>
<td>119 ± 1</td>
<td>206 ± 16</td>
<td>77 ± 7</td>
<td>4.3 ± 0.5</td>
<td>6.0 ± 0.6</td>
</tr>
<tr>
<td>DP-5-CT</td>
<td>3</td>
<td>110 ± 9</td>
<td>220 ± 15</td>
<td>50 ± 8</td>
<td>6.7 ± 0.3</td>
<td>8.7 ± 1.0</td>
</tr>
</tbody>
</table>

Table 4.1. Baseline values for mean arterial pressure (MAP), heart rate (HR), femoral arterial conductance (FAC), tracheal pressure (TP) and inspiratory rate (Insp. rate) in α-chloralose anaesthetized cats. All compounds were administered i.c.v. Values are the mean ± s.e.mean. BW501C67 (1 mg kg⁻¹) was administered i.v. 15 min before the administration of DOI i.c.v. and the baseline values shown are 1 min before the administration of DOI.
Figure 42 Traces showing the effects of cumulative i.c.v. doses of 5-HT (10, 40, 160 nmol kg⁻¹) on integrated renal (RNA), splanchnic (SNA) and cardiac (CNA) nerve activity, heart rate (HR), mean arterial blood pressure (BP), femoral flow, tracheal pressure (TP) and phrenic nerve activity (PNA) in an anaesthetized cat.
Figure 43 Anaesthetized cats: a comparison of the changes from baseline values over time (min) caused by cumulative i.c.v. doses of 5-HT (■; 10, 40, 160 nmol kg\(^{-1}\); n = 5) and saline (○; 3 x 20 μl; n = 5) in renal (RNA), splanchnic (SNA) and cardiac (CNA) nerve activity, heart rate (HR), mean arterial blood pressure (MAP) and femoral arterial conductance (FAC). Each point represents the mean value and the vertical lines show s.e.mean. * P < 0.05 and ** P < 0.01 compared (ANOVA) to the corresponding values in saline treated animals. The dotted lines show the points at which 5-HT and saline were injected.
Figure 44 Anaesthetized cats: a comparison of the changes from baseline values over time (min) caused by cumulative i.c.v. doses of 5-HT (■; 10, 40, 160 nmol kg\(^{-1}\); n=5) and saline (○; 3 x 20 μl; n=5) in mean arterial blood pressure (MAP), tracheal pressure (TP), phrenic nerve activity (PNA) and inspiratory rate (Insp. rate). Each point represents the mean value and the vertical lines show s.e.mean. * P < 0.05 and ** P < 0.01 compared (ANOVA) to the corresponding values in saline treated animals. The dotted lines show the points at which 5-HT and saline were injected.
nmol kg$^{-1}$ did not significantly change renal nerve activity (Figure 43).

Figure 44 summarises the changes in respiratory variables caused by 5-HT
(10, 40 and 120 nmol kg$^{-1}$). 5-HT caused significant increases in tracheal
pressure, phrenic nerve activity and inspiratory rate which reached maxima
of $0.20 \pm 0.06$ cm H$_2$O, $153 \pm 58$ % and $1.2 \pm 0.4$ bursts min$^{-1}$,
respectively. Changes were near maximal 5-10 min after administration of
5-HT 10 nmol kg$^{-1}$ (Figure 42 and 44).

4.3.3 Effect of cinanserin on the response to 5-HT
In three animals, the effect of 5-HT$_2$/5-HT$_1$C receptor antagonism on the
response to 5-HT i.c.v. was examined. Cinanserin 265 nmol kg$^{-1}$ i.c.v. did
not significantly (Student's paired $t$ test) change baseline variables (see
Table 4.2). However, there was a tendency for arterial blood pressure and
heart rate to fall ($10 \pm 4$ mmHg and $25 \pm 6$ beats min$^{-1}$, respectively) and
femoral conductance to increase ($44 \pm 20$ ml min$^{-1}$ mmHg$^{-1} \times 10^{-3}$; Table
4.2). After a 5 min pretreatment with cinanserin, 5-HT (10, 40 and 160
nmol kg$^{-1}$) was administered i.c.v. In the presence of cinanserin the
response to 5-HT on all cardiovascular and respiratory variables recorded
was significantly attenuated (Figure 45 and 46).

4.3.4 Effect of i.c.v. administration of DOI
15 min before the addition of DOI, cats were pretreated with a peripherally
acting 5-HT$_2$/5-HT$_1$C receptors antagonist, BW501C67 (1 mg kg$^{-1}$ i.v.) to
prevent DOI activating 5-HT$_2$ receptor located on vascular and bronchial
smooth muscle (see discussion). BW501C67 pretreatment caused no
significant (Student's paired $t$ test) change in baseline values (see Table
4.3.). A representative trace showing the response produced by i.c.v.
administration of DOI in the presence of BW501C67 (also shown) is
illustrated in Figure 47. DOI 80, 160 and 320 nmol kg$^{-1}$ ($n=4$)
administered i.c.v. caused a significant, dose-related increase in blood
Table 4.2. Baseline values and changes in mean arterial pressure (MAP), heart rate (HR), femoral arterial conductance (FAC), cardiac (CNA), splanchnic (SNA), renal (RNA) and phrenic (PNA) nerve activities, tracheal pressure (TP) and inspiratory rate (Insp. rate) following the administration of cinanserin (n = 3) in α-chloralose anaesthetized cats. Values are the mean ± s.e.mean. Cinanserin did not significantly change (Student’s paired t test) in baseline values.
Figure 45 Anaesthetized cats: a comparison of the changes from baseline or post-pretreatment values over time (min) caused by cumulative i.c.v. doses of 5-HT (10, 40, 160 nmol kg\(^{-1}\)) in the absence (■; \(n = 5\)) and the presence of cinanserin (□; 265 nmol kg\(^{-1}\), i.c.v.; \(n = 3\)) in renal (RNA), splanchnic (SNA) and cardiac (CNA) nerve activity, heart rate (HR), mean arterial blood pressure (MAP) and femoral arterial conductance (FAC). Each point represents the mean value and the vertical lines show s.e.mean. * \(P < 0.05\) and ** \(P < 0.01\) compared (ANOVA) to i.c.v. 5-HT in non-pretreated animals. The dotted lines show the points at which 5-HT was injected.
Figure 46 Anaesthetized cats: a comparison of the changes from baseline or post-pretreatment values over time (min) caused by cumulative i.c.v. doses of 5-HT (10, 40, 160 nmol kg\(^{-1}\)) in the absence (■; \(n = 5\)) and the presence of cinanserin (□; 265 nmol kg\(^{-1}\), i.c.v.; \(n = 3\)) in mean arterial blood pressure (MAP), tracheal pressure (TP), phrenic nerve activity (PNA) and inspiratory rate (Insp. rate). Each point represents the mean value and the vertical lines show s.e.mean. * \(P < 0.05\) and ** \(P < 0.01\) compared (ANOVA) to i.c.v. 5-HT in non-pretreated animals. The dotted lines show the points at which 5-HT was injected.
Table 4.3. Baseline values and changes in mean arterial pressure (MAP), heart rate (HR), femoral arterial conductance (FAC), cardiac (CNA), splanchnic (SNA), renal (RNA) and phrenic (PNA) nerve activities, tracheal pressure (TP) and inspiratory rate (Insp. rate) following the administration of BW501C67 (n = 4) in α-chloralose anaesthetized cats. Values are the mean ± s.e.mean. BW501C67 did not significantly change (Student’s paired t test) in baseline values. DOI was subsequently administered to these animals.
Figure 47 Traces showing the effects of cumulative i.c.v. doses of DOI (80, 160, 320 nmol kg$^{-1}$) on integrated renal (RNA), splanchnic (SNA) and cardiac (CNA) nerve activity, heart rate (HR), mean arterial blood pressure (BP), femoral flow, tracheal pressure (TP) and phrenic nerve activity (PNA) in an anaesthetized cat pretreated with BW501C67 (1 mg kg$^{-1}$, i.v.). The left panel shows the administration of BW501C67 and the right panel shows the response produced by DOI.
Figure 48 Anaesthetized cats: a comparison of the changes from baseline values over time (min) in renal (RNA), splanchnic (SNA) and cardiac (CNA) nerve activity, heart rate (HR), mean arterial blood pressure (MAP) and femoral arterial conductance (FAC) caused by cumulative i.c.v. doses of DOI (● ; 80, 160, 320 nmol kg\(^{-1}\); n = 4) in animals pretreated with BW501C67 (1mg kg\(^{-1}\), i.v) and saline (○ ; 3 x 20 μl ; n = 5) in non-pretreated animals. Each point represents the mean value and the vertical lines show s.e.mean. * P < 0.05 and ** P < 0.01 compared (ANOVA) to the corresponding values in saline treated animals. The dotted lines show the points at which DOI and saline were injected.
Figure 49 Anaesthetized cats: a comparison of the changes from baseline values over time (min) in mean arterial blood pressure (MAP), tracheal pressure (TP), phrenic nerve activity (PNA) and inspiratory rate (Insp. rate) caused by cumulative i.c.v. doses of DOI (●; 80, 160, 320 nmol kg⁻¹; n = 4) in animals pretreated with BW501C67 (1 mg kg⁻¹, i.v) and saline (○; 3 x 20 µl; n = 5) in non-pretreated animals. Each point represents the mean value and the vertical lines show s.e.mean. * P < 0.05 and ** P < 0.01 compared (ANOVA) to the corresponding values in saline treated animals. The dotted lines show the points at which DOI and saline were injected.
pressure which reached a maximum of 22 ± 2 mmHg. The pressor response was maintained for the duration of the experiment (Figure 48). This was associated with an increase in heart rate of 26 ± 8 beats min⁻¹ and a reduction in femoral conductance of 31 ± 5 ml min⁻¹ mmHg⁻¹ 10⁻³ (Figure 48). There was a dose-related increase in cardiac and splanchnic nerve activity which reached 154 ± 24 and 49 ± 15 %, respectively after administration of DOI 320 nmol kg⁻¹. Renal nerve activity was not significantly changed (Figure 48). There were no significant changes in tracheal pressure, phrenic nerve activity or inspiratory rate after administration of DOI (Figure 49).

4.3.5 Effect of i.c.v. administration of 5-CT
A representative trace showing the effects of 5-CT is depicted in Figure 50 and the mean result from four experiments is shown in Figures 51 and 52. Baseline values for this group of experiments are given in Table 4.1. Cumulative i.c.v. administration of 5-CT (2.5, 10, 40 nmol kg⁻¹; n = 4) caused a dose-related increase in arterial blood pressure which reached a maximum rise of 12 ± 4 mmHg after administration of 10 nmol kg⁻¹ 5-CT. 5-CT also caused an increase in heart rate (maximum 25 ± 6 beats min⁻¹) and a reduction in femoral flow (nadir 12 ± 4 ml min⁻¹ mmHg⁻¹ 10⁻³; Figure 51). Dose-related increases in cardiac, splanchnic and renal nerve activity were observed following administration of 5-CT and reached maxima of 119 ± 44, 64 ± 19 and 48 ± 26 %, respectively (see Figure 51). Tracheal pressure and phrenic nerve activity were not significantly changed, however, there was an increase in inspiratory rate following administration of 5-CT (see Figure 52).

4.3.6 Effect of i.c.v. administration of DP-5-CT
Baseline values for this group of experiments are given in Table 4.1. DP-5-CT (2.5, 10, 40 nmol kg⁻¹; n = 3) caused an increase in arterial blood
Figure 50 Traces showing the effects of cumulative i.c.v. doses of 5-CT (2.5, 10, 40 nmol kg⁻¹) on integrated renal (RNA), splanchnic (SNA) and cardiac (CNA) nerve activity, heart rate (HR), mean arterial blood pressure (BP), femoral flow, tracheal pressure (TP) and phrenic nerve activity (PNA) in an anaesthetized cat.
Figure 51  Anaesthetized cats: a comparison of the changes from baseline values over time (min) caused by cumulative i.c.v. doses of 5-CT (▼; 2.5, 10, 40 nmol kg\(^{-1}\); n = 4) and saline (○; 3 x 20 μl; n = 5) in renal (RNA), splanchnic (SNA) and cardiac (CNA) nerve activity, heart rate (HR), mean arterial blood pressure (MAP) and femoral arterial conductance (FAC). Each point represents the mean value and the vertical lines show s.e.mean. * P < 0.05 and ** P < 0.01 compared (ANOVA) to the corresponding values in saline treated animals. The dotted lines show the points at which 5-CT and saline were injected.
Figure 52 Anaesthetized cats: a comparison of the changes from baseline values over time (min) caused by cumulative i.c.v. doses of 5-CT (▼; 2.5, 10, 40 nmol kg⁻¹; n=4) and saline (○; 3 x 20 μl; n=5) in mean arterial blood pressure (MAP), tracheal pressure (TP), phrenic nerve activity (PNA) and inspiratory rate (Insp. rate). Each point represents the mean value and the vertical lines show s.e.mean. * P < 0.05 and ** P < 0.01 compared (ANOVA) to the corresponding values in saline treated animals. The dotted lines show the points at which 5-CT and saline were injected.
pressure. This reached a maximum change of 17 ± 6 mmHg after administration of 10 nmol kg⁻¹ DP-5-CT (Figure 53). This was associated with an immediate increase in heart rate which reached a maximum of 32 ± 2 beats min⁻¹ after 10 nmol kg⁻¹ DP-5-CT (Figure 52). These changes in blood pressure and heart rate were maintained with DP-5-CT 40 nmol kg⁻¹. Femoral arterial conductance was significantly reduced (nadir 5.2 ± 0.7 ml min⁻¹ mmHg⁻¹ 10⁻³) following administration of 2.5 nmol kg⁻¹ DP-5-CT but changes in this variable were not maintained with higher doses of DP-5-CT. DP-5-CT caused an increase in cardiac and splanchnic nerve activity reaching maxima of 96 ± 39 and 70 ± 43 % respectively following administration of 10 nmol kg⁻¹ DP-5-CT. There was no significant change in renal nerve activity (Figure 53). There was no significant change in phrenic nerve activity or inspiratory rate. However, there was a significant increase in tracheal pressure, which reached a maximum of 0.4 ± 0.1 cm H₂O after administration of 40 nmol kg⁻¹ DP-5-CT (Figure 54).
Figure 53 Anaesthetized cats: a comparison of the changes from baseline values over time (min) caused by cumulative i.c.v. doses of DP-5-CT (▲; 2.5, 10, 40 nmol kg\(^{-1}\); n=3) and saline (○; 3 x 20 μl; n=5) in renal (RNA), splanchnic (SNA) and cardiac (CNA) nerve activity, heart rate (HR), mean arterial blood pressure (MAP) and femoral arterial conductance (FAC). Each point represents the mean value and the vertical lines show s.e.mean. * P < 0.05 and ** P < 0.01 compared (ANOVA) to the corresponding values in saline treated animals. The dotted lines show the points at which DP-5-CT and saline were injected.
Figure 54  Anaesthetized cats: a comparison of the changes from baseline values over time (min) caused by cumulative i.c.v. doses of DP-5-CT (▼; 2.5, 10, 40 nmol kg⁻¹; n = 3) and saline (○; 3 × 20 μl; n = 5) in mean arterial blood pressure (MAP), tracheal pressure (TP), phrenic nerve activity (PNA) and inspiratory rate (Insp. rate). Each point represents the mean value and the vertical lines show s.e.mean. * P < 0.05 and ** P < 0.01 compared (ANOVA) to the corresponding values in saline treated animals. The dotted lines show the points at which DP-5-CT and saline were injected.
4.4 Discussion

Administration of 5-HT to the lateral ventricle of the anaesthetized cat caused a rise in blood pressure which was associated with a tachycardia and a decrease in femoral arterial conductance. In addition, 5-HT evoked an increase in cardiac and splanchnic sympathetic nerve activity but did not change renal nerve activity. The tachycardia, cardiac sympathoexcitation and femoral vasoconstriction were maintained with the administration of higher doses of 5-HT, although the pressor response was not maintained. 5-HT administered i.c.v. also caused an increase in tracheal pressure and central respiratory drive. An increase in the amplitude of phrenic nerve activity was associated with an increase in respiratory frequency.

The magnitude of the changes in blood pressure and heart rate caused by administration of 5-HT in the present study were similar to those reported by Coote et al. (1987). In the present experiments 5-HT caused a reduction in femoral vascular conductance. The rise in blood pressure caused by 5-HT was presumably due to an increase in sympathetic vasoconstrictor outflow to the hindlimb and splanchnic vascular beds. The observed tachycardia was probably caused by an increase in cardiac sympathetic nerve activity since heart rate changes mirrored changes in cardiac nerve activity. The influence of these cardiac changes in producing the pressor response caused by 5-HT were not investigated in the present study but this could be evaluated by recording cardiac output in further experiments. The blood pressure rise caused by 5-HT was not maintained with higher doses, yet both the increase in femoral conductance and the tachycardia remained elevated. This would suggest the additional haemodynamic changes occurred with higher doses of 5-HT to limit the pressor response. Such an action may have resulted from a reduction in total peripheral resistance following dilatation of other vascular beds and/or a reduction in cardiac output, however, this requires further investigation. In the present
experiments there was little change in renal sympathetic nerve activity and this study clearly showed that 5-HT caused a differential action of sympathetic outflow. Coote et al. (1987) similarly reported that lateral ventricular administration of 5-HT (in animals in which the Aqueduct of Sylvius was cannulated) did not cause an increase in renal nerve activity.

The cardiovascular and respiratory changes caused by 5-HT were abolished by pretreatment with cinanserin and this suggests that this response was caused by activation of central 5-HT₂ and/or 5-HT₁C receptors. Interestingly, central administration of cinanserin tended to cause a reduction in blood pressure and heart rate associated with femoral vasodilatation and a reduction in cardiac nerve activity. These effects were presumably due to antagonism of 5-HT₂ and/or 5-HT₁C receptors and may reflect an inhibition of tonic sympathoexcitatory drive mediated by 5-HT₂ and/or 5-HT₁C receptors to the femoral vasculature. In this respect, cinanserin has previously been shown to cause a reduction in blood pressure and sympathetic nerve activity following i.v. administration to anaesthetized cats (McCall & Humphrey, 1982). Previous studies have demonstrated that cinanserin and other 5-HT₂/5-HT₁C receptor antagonists given i.v. caused an increase in femoral conductance associated with a slight fall in preganglionic sympathetic nerve activity in anaesthetized cats (Ramage, 1988b). An increase in femoral conductance was also observed following fourth ventricular administration of cinanserin (Shepheard et al., 1990a). These studies support the above conclusion and would suggest that this action of cinanserin was centrally mediated.

To gain further evidence that the cardiovascular and respiratory action of i.c.v. 5-HT were mediated through activation of 5-HT₂ and/or 5-HT₁C receptors, the selective 5-HT₂/5-HT₁C receptor agonist DOI (Shannon et al., 1984; Glennon et al., 1986, 1988c; Van Wijngaarden et al., 1990) was
administered i.c.v. in the present study. Central administration of DOI has previously been shown to cause femoral vasoconstriction and bronchoconstriction mediated by peripheral 5-HT$_2$ receptors in the anaesthetized cat (Shepheard et al., 1991). Therefore, to prevent an action of DOI at peripheral 5-HT$_2$ receptors in the present study, animals were pretreated with the peripherally acting 5-HT$_2$/5-HT$_1$C receptor antagonist BW501C67 (Mawson & Whittington 1970; Fuller et al., 1986) at a dose which has previously been shown to prevent these systemic actions of DOI (Ramage et al., 1993). In the presence of BW501C67, DOI administered to the lateral ventricle caused a rise in blood pressure associated with tachycardia and a reduction in femoral vascular conductance. In addition, a marked increase in cardiac and splanchnic nerve activity was observed with little change in renal nerve activity. DOI did not affect tracheal pressure or central respiratory drive. These effects were near maximal following administration of DOI 160 nmol kg$^{-1}$ and were maintained with a higher dose of DOI. The cardiovascular changes caused by DOI (160 nmol kg$^{-1}$) were essentially similar to those produced by 5-HT (10 nmol kg$^{-1}$) and the difference in potency between these agonists reflects the low efficacy (Dabire et al., 1989) and affinity (Van Wijngaarden et al., 1990) of DOI for 5-HT$_2$ receptors. As the 5-HT$_2$/5-HT$_1$C receptor agonist DOI causes similar cardiovascular effects to those mediated by 5-HT and the effects of 5-HT are blocked by the 5-HT$_2$/5-HT$_1$C receptor antagonist cinanserin, it is concluded that the rise in blood pressure, tachycardia, femoral vasoconstriction and increase in sympathetic nerve activity are mediated by activation of forebrain 5-HT$_2$/5-HT$_1$C receptors.

Activation of central 5-HT$_2$/5-HT$_1$C receptors have previously been shown to cause sympathoexcitation in the anaesthetized cat (McCall et al., 1987; McCall & Harris, 1988; Shepheard et al., 1991; Ramage et al., 1993), at the level of the rostral ventrolateral medulla (King & Holtman, 1989; Mandal...
et al., 1990, 1991; Vayssettes-Courchay et al., 1991; 1992). It is unlikely that 5-HT and DOI caused their sympathoexcitatory actions through activation of these hindbrain 5-HT$_2$/5-HT$_1$c receptors since in the present study drugs given i.c.v. were denied access to the hindbrain by a cannula placed in the Aqueduct of Sylvius, the connection between the third and fourth ventricles (see Methods). Furthermore, both 5-HT and DOI produced rapid (<1 min) cardiovascular effects following i.c.v. administration and 5-HT does not readily penetrate tissue, a factor which would limit access to the hindbrain for 5-HT. DOI is a lipophilic compound ($\log P = 2.85$: G.R. Martin, Wellcome Research Laboratories, U.K.; personal communication) and may have diffused to the hindbrain. However, the response caused by lateral ventricular administration is not consistent with that produced by fourth ventricular administration. Shepheard et al. (1991) demonstrated that fourth ventricular injection of DOI 80, 160 and 320 nmol kg$^{-1}$ (the same doses as used in the present study) caused a pressor response without changing sympathetic nerve activity. Taken together, these studies suggest that activation of 5-HT$_2$/5-HT$_1$c receptors located in the hindbrain and forebrain can cause a pressor response. The forebrain location of the 5-HT$_2$/5-HT$_1$c receptors which caused the cardiovascular changes observed in the present study are unknown. This could be investigated by microinjecting 5-HT and DOI into specific forebrain regions implicated in cardiovascular control e.g. the hypothalamus (see General Discussion).

In previous studies in the conscious and anaesthetized rat (Chapter 2; Alper, 1990; Vayssettes-Courchay et al., 1990) there was no evidence for a central 5-HT$_2$/5-HT$_1$c receptor-induced increase in sympathetic tone. Thus, it appears that the cat and rat are different in this respect. In rats 5-HT has been shown to cause an indirectly mediated increase in blood pressure through the release of vasopressin following activation of central 5-HT$_2$/5-HT$_1$c receptors (see Chapter 2 and Chapter 3). In the present
experiments in anaesthetized cats the cardiovascular response caused by 5-HT was not consistent with an indirect cardiovascular action through the release of vasopressin and no information concerning the regulation of vasopressin by 5-HT in cats could be obtained from the current literature. This action of 5-HT remains to be evaluated in cats and could be achieved by measuring plasma vasopressin levels before and after i.c.v. administration of 5-HT.

Intracerebroventricular administration of the non-selective 5-HT₁ receptor agonist 5-CT (Schoeffter & Hoyer, 1988; Van Wijngaarden et al., 1990; see Hoyer, 1991) caused a rise in blood pressure associated with tachycardia and a reduction in femoral arterial conductance. Additionally, 5-CT caused an increase in renal, splanchnic and cardiac nerve activity. These changes were accompanied with an increase in respiratory frequency. 5-CT has previously been shown to cause tachycardia in anaesthetized cats through a direct action at peripheral 5-HT receptors, which have similar pharmacology to the '5-HT₁-like' receptors mediating contraction of dog saphenous vein (Connor et al., 1986). Therefore, the tachycardia caused by 5-CT in the present study may be mediated by peripheral 5-HT receptors. However, a marked increase in cardiac nerve activity accompanied the tachycardia which would suggest that this response was mediated by central cardiac sympathoexcitation. Interestingly, in anaesthetized cats 5-CT caused a generalised sympathoexcitation and this response differed from that of 5-HT since an increase in renal nerve activity was observed with 5-CT. The reason for this difference is unknown.

The finding that a 5-HT₁ receptor agonist could also cause an increase in blood pressure and sympathetic nerve activity was unexpected since the response to 5-HT, the endogenous agonist for this receptor, was abolished by the 5-HT₂/5-HT₁C receptor agonist cinanserin. One explanation for these
findings is that both 5-HT$ _1$ and 5-HT$_2$/5-HT$_{1C}$ receptors occur in the sympathoexcitatory neuronal pathway and that 5-HT$ _1$ receptor activation leads to a response mediated by 5-HT$_2$/5-HT$_{1C}$ receptors. In this way 5-HT could activate both 5-HT$ _1$ and 5-HT$_2$/5-HT$_{1C}$ receptors to cause sympathoexcitation but in the presence of cinanserin both excitatory actions would be prevented. Alternatively, cinanserin may non-specifically prevent sympathoexcitation in this model. These points could be tested by investigating the effects of 5-CT in animals pretreated with cinanserin and other selective 5-HT$_2$/5-HT$_{1C}$ receptor antagonists (e.g. LY 53857). This type of experiment would determine if the effects of 5-CT were dependent on 5-HT$_2$/5-HT$_{1C}$ receptors and through the use of alternative 5-HT$_2$/5-HT$_{1C}$ receptor antagonists, a non-specific action by cinanserin may be discounted. A third possibility is that the sympathoexcitatory actions of 5-HT and 5-CT are mediated by 5-HT$_{1C}$ receptors. However, 5-CT has been demonstrated to have a low affinity for 5-HT$_{1C}$ receptors (5-CT has a pK$_i$ of 6.23 at 5-HT$_{1C}$ receptors; Hoyer, 1988) whereas 5-HT has a high affinity for these receptors (5-HT has a pK$_i$ of 8.37 at 5-HT$_{1C}$ receptors; Van Wijngaarden et al., 1990). In the present experiments 5-HT and 5-CT appeared equipotent; each agonist producing a maximum effect at a dose of 10 nmol kg$^{-1}$. Thus, the potency of 5-CT relative to that of 5-HT would argue against an action at 5-HT$_{1C}$ receptors.

In the present study DP-5-CT also caused a rise in blood pressure, tachycardia, femoral constriction and an increase in cardiac and splanchnic sympathetic nerve activity. DP-5-CT has been demonstrated to be a selective agonist for 5-HT$_{1A}$ receptors (Mir et al., 1987; Doods et al., 1988; Schoeffter & Hoyer, 1988; see Hoyer, 1991). Furthermore, 5-CT has agonist activity at 5-HT$_{1A}$ receptors (Schoeffter & Hoyer, 1988; Van Wijngaarden et al., 1990; see Hoyer, 1991). Therefore, the sympathoexcitatory action of these agonists could have been mediated by
activation of forebrain 5-HT₁A receptors. However, this suggestion requires confirmation with further agonist and antagonist studies. This could be achieved by administering 5-CT and DP-5-CT in animals pretreated with the 5-HT₁A receptor antagonist spiroxatrine and by investigating the actions of the selective 5-HT₁A receptor agonist 8-OH-DPAT in this model. In this respect, activation of central 5-HT₁A receptors in conscious and anaesthetised rats has previously been shown to cause a generalised sympathoexcitation and the release of adrenaline (see Chapter 2 and Chapter 3). An action at 5-HT₁A receptors may account for the rise in blood pressure and increase in sympathetic nerve activity observed in the present study in anaesthetized cats.

The sympathoinhibitory effects of 5-HT, 5-CT and DP-5-CT observed following administration of these agonists to the fourth ventricle of the anaesthetized cat (Shepheard et al., 1990a; Coote et al., 1987) were not observed in the present study. These findings support the idea that 5-HT can cause opposing cardiovascular effects depending on the central site of administration. Forebrain administration of 5-HT causes an increase in blood pressure and sympathetic nerve activity whereas hindbrain administration causes a depressor response and sympathoinhibition (see Coote et al., 1987; Coote, 1990).

In previous studies activation of 5-HT pathways has been demonstrated to increase central respiratory drive (Holtman et al., 1986a, 1986b; Dreteler et al., 1991a). Furthermore, central administration of 8-OH-DPAT has been shown to increase respiratory rate in cats (Gillis et al., 1989) and a rise in phrenic nerve activity in rats (Sporton et al., 1991; Chapter 2). Central administration of DOI caused a reduction in amplitude of phrenic nerve activity (King & Holtman, 1989) and a decrease in the frequency of respiratory bursts (King & Holtman, 1989; Shepheard et al., 1991) in cats.
The present results demonstrate that 5-HT caused an increase in tracheal pressure and phrenic nerve amplitude and frequency and these effects were blocked by cinanserin suggesting the involvement of 5-HT$_2$ and/or 5-HT$_{1C}$ receptors. However, DOI did not produce these changes which is not consistent with this conclusion. 5-CT caused an increase in central respiratory frequency. No firm conclusions as to the effects of activation of central 5-HT receptors on central respiratory drive can be made from the present study, although forebrain administration of 5-HT would appear to increase respiratory variables. However, as arterial blood gases could not be monitored continuously, it is always possible that these changes were the indirect effects of alterations in respiratory stimuli.

In conclusion, these results demonstrate that activation of forebrain 5-HT$_2$ and/or 5-HT$_{1C}$ receptors causes sympathoexcitation and a pressor response in anaesthetized cats. However, these results also suggest that 5-HT$_1$ receptors are involved in this sympathoexcitatory pathway, although the precise nature of this receptor requires further investigation.
CHAPTER 5

GENERAL DISCUSSION

The present study demonstrates that administration of 5-HT to the forebrain of conscious and anaesthetized rats and anaesthetized cats via the lateral cerebral ventricles causes a centrally mediated rise in systemic blood pressure. This confirms the findings of previous studies (see 1.6.1). The present study has shown that this rise in blood pressure in the rat was mediated by activation of 5-HT\textsubscript{1A} and 5-HT\textsubscript{2} and/or 5-HT\textsubscript{1C} receptors. The pressor response in this species was also demonstrated to be, in part, mediated by the release of vasopressin which caused vasoconstriction of the mesenteric vascular bed. This action was attenuated by systemic administration of a selective vasopressin V\textsubscript{1}-receptor antagonist and the blood pressure rise caused by 5-HT (40, 120 nmol kg\textsuperscript{-1}) was absent in vasopressin deficient Brattleboro rats. This is supported by the observations of Pergola \textit{et al.} (1993), who demonstrated that plasma vasopressin levels are elevated following i.c.v. administration of 5-HT in conscious rats. This release of vasopressin was shown to be mediated by activation of central 5-HT\textsubscript{2} and/or 5-HT\textsubscript{1C} receptors as the response caused by 5-HT i.c.v. was modified in a similar manner by both the 5-HT\textsubscript{2}/5-HT\textsubscript{1C} receptor antagonists LY 53857 and cinanserin (administered centrally), and the vasopressin V\textsubscript{1}-receptor antagonist d(CH\textsubscript{2})\textsubscript{5}Tyr(Me)AVP (administered peripherally). The rise in plasma vasopressin levels caused by i.c.v. 5-HT is also attenuated by LY 53857 (Pergola \textit{et al.}, 1993). The present findings explain the observation of previous studies in which peripheral administration of 5-HT reuptake blockers, releasing agents and certain receptor agonists were shown to increase circulating levels of vasopressin (Lovino & Steardo, 1985a; Steardo & Lovino, 1986; Brownfield \textit{et al.}, 1988; Bagdy \textit{et al.}, 1992).
The lack of selective agonists or antagonists for 5-HT\textsubscript{2} or 5-HT\textsubscript{1C} receptors prevents precise evaluation of the receptor mediating the release of vasopressin. Certain evidence favours an action at 5-HT\textsubscript{1C} receptors; however, this is based on the use of the non-selective 5-HT agonist, m-CPP which has pK\textsubscript{D} values of 7.7 and 6.7 for 5-HT\textsubscript{1C} and 5-HT\textsubscript{2} receptors, respectively (Hoyer, 1991). Nevertheless, the inability of ketanserin [which has pK\textsubscript{B} values of 8.9 and 7.0 for 5-HT\textsubscript{2} and 5-HT\textsubscript{1C} receptors, respectively (Hoyer, 1991)] to block the increase in vasopressin release by m-CPP does support a role for 5-HT\textsubscript{1C} receptors in this response (see Bagdy \textit{et al.}, 1992). Precise characterization of the receptor subtype involved in the release of vasopressin awaits the availability of more selective tools to discriminate between 5-HT\textsubscript{2} and 5-HT\textsubscript{1C} receptors.

The rise in blood pressure caused by i.c.v. 5-HT was associated with biphasic changes in heart rate and sympathetic nerve activity. An initial bradycardia with concomitant sympathoinhibition was followed by tachycardia and sympathoexcitation. This biphasic response can now be explained. The initial bradycardia and sympathoinhibition appear to be reflexly mediated following activation of the baroreceptor receptor reflex arc as the result of the peripheral vasoconstriction caused by vasopressin. Immediate tachycardia and sympathoexcitation were observed when peripheral vasopressin V\textsubscript{1}-receptors or central 5-HT\textsubscript{2}/5-HT\textsubscript{1C} receptors were blocked (Chapter 2). Furthermore, immediate tachycardia was also caused by i.c.v. 5-HT in Brattleboro rats (Chapter 3) and baroreceptor deficient rats (Pergola and Alper, 1992). The sympathoexcitatory actions of 5-HT were mediated by activation of central 5-HT\textsubscript{1A} receptors; selective 5-HT\textsubscript{1A} receptor agonists produced an increase in sympathetic tone and the excitatory actions of 5-HT (in the presence of LY 53857 to block 5-HT\textsubscript{2} and/or 5-HT\textsubscript{1C} receptors) and DP-5-CT were abolished by the 5-HT\textsubscript{1A}
Chapter 5

receptor antagonist spiroxatrine (Chapter 2). Activation of these receptors caused a generalized sympathoexcitation leading to a rise in blood pressure in anaesthetized and conscious rats (Chapter 2; 3).

The selective 5-HT$_{1A}$ receptor agonist 8-OH-DPAT has been shown to increase circulating levels of adrenaline and noradrenaline (Chaouloff & Jeanrenaud, 1987; Chaouloff et al., 1990a, 1990b; Bagdy et al., 1989a, 1989b; Bouhelal & Mir, 1990, 1992). In the present study, the release of adrenaline was indirectly demonstrated following i.c.v. administration 5-HT (Chapter 3). Furthermore, the selective 5-HT$_{1A}$ receptor agonist DP-5-CT caused an increase in adrenal sympathetic nerve activity (Chapter 2). The pressor response caused by i.c.v. 5-HT was reduced in rats with bilateral adrenalectomy (Krstic & Djorkovic, 1980) indicating the importance of catecholamine release in this response. Taken together, these studies suggest that 5-HT can cause the release of adrenaline through an action at 5-HT$_{1A}$ receptors.

In rats the complex cardiovascular profile caused by forebrain administration of 5-HT is the result of the release of vasopressin, direct sympathoexcitation and reflexly mediated sympathoinhibition. The cardiovascular changes caused by 5-HT were mediated by central 5-HT$_2$/5-HT$_{1C}$ and 5-HT$_{1A}$ receptors. A schematic summary of these effects is shown in Figure 55. 5-HT$_{1B}$ and 5-HT$_3$ receptors did not appear to be involved in the cardiovascular response caused by 5-HT i.c.v. (Chapter 2).

In anaesthetized cats, administration of 5-HT to the forebrain via the lateral ventricle caused a rise in blood pressure associated with sympathoexcitation. The changes in the cardiovascular variables were consistent with an increase in sympathetic vasoconstrictor outflow to
Figure 55 A summary of the cardiovascular effects of i.c.v. administration of 5-HT in rats. 5-HT acts at both 5-HT_{2} and/or 5-HT_{1C} receptors and 5-HT_{1A} receptors to cause the release of vasopressin and sympathoexcitation, respectively. The precise location of these receptors remains to be determined. Activation of these receptor subtypes accounts for the cardiovascular profile caused by 5-HT. When 5-HT_{2} and/or 5-HT_{1C} receptors are blocked only the sympathoexcitatory effects of 5-HT are observed. A similar response is caused when agonists at 5-HT_{1A} receptors are administered i.c.v. Combined blockade of 5-HT_{2}/5-HT_{1C} receptors and 5-HT_{1A} receptors prevents the action of i.c.v. 5-HT. + represents stimulation, - represents inhibition, ? indicates an unknown factor.
splanchnic and femoral vascular beds and cardiac sympathoexcitation leading to the observed pressor response and tachycardia. The effects of 5-HT were blocked by the 5-HT<sub>2</sub>/5-HT<sub>1C</sub> receptor antagonist cinanserin and were mimicked by the 5-HT<sub>2</sub>/5-HT<sub>1C</sub> receptor agonist DOI. Therefore, activation of 5-HT<sub>2</sub> and/or 5-HT<sub>1C</sub> receptors located in the forebrain would appear to mediate sympathoexcitation in this species. Activation of 5-HT<sub>2</sub> and/or 5-HT<sub>1C</sub> receptors located in the ventrolateral medulla have also been shown to cause a rise in blood pressure and sympathoexcitation in cats (see 1.7.2). Thus, both forebrain and hindbrain 5-HT<sub>2</sub> and/or 5-HT<sub>1C</sub> receptors appear to cause sympathoexcitation. Further characterization of the receptor subtype mediating the sympathoexcitatory response of 5-HT awaits the availability of agonists and antagonists which discriminate between 5-HT<sub>2</sub> and 5-HT<sub>1C</sub> receptors. The involvement of vasopressin in the cardiovascular response to i.c.v. 5-HT in the cat species remains to be determined; however, the cardiovascular response observed in the present study was not consistent with the release of this vasoconstrictor hormone. No evidence can be found in current literature to support an involvement of 5-HT in the regulation of vasopressin in cats; previous studies were limited to the rat.

The lack of a central cardiovascular action of the potent and selective 5-HT<sub>2</sub>/5-HT<sub>1C</sub> receptor agonist DOI in rats has been noted in previous studies (see 2.4). Similarly, in the present study in rats, DOI administered i.c.v. did not appear to have a central component to its cardiovascular response. The inability of DOI to release vasopressin when given i.c.v. to rats is unknown and remains to be investigated. Interestingly, in the anaesthetized cat, activation of central 5-HT<sub>2</sub> or 5-HT<sub>1C</sub> receptors with either DOI or the combined 5-HT<sub>2</sub>/5-HT<sub>3</sub> receptor agonist quipazine has been shown to cause sympathoexcitation (McCall & Harris, 1988; Vayssettes-Courchay et al., 1991; Shepheard et al., 1991; Ramage et al.,
In the present study and in previous studies (Alper, 1990; Vayssettes-Courchay et al., 1990) there is no evidence for a centrally mediated increase in sympathetic tone in the rat. Therefore, it appears that the cat and rat are different in this respect.

Interestingly, the 5-HT₁ receptor agonist 5-CT and the selective 5-HT₁₄ receptor agonist DP-5-CT caused modest increases in blood pressure associated with tachycardia, femoral constriction and sympathoexcitation following i.c.v. administration in anaesthetized cats. This finding suggests that 5-HT₁ receptors as well as 5-HT₂ and/or 5-HT₁c receptors causes sympathoexcitation in this species. The precise nature of the 5-HT₁ receptor mediating these effects requires further investigation (see 4.4) but initial data with DP-5-CT suggests an action at 5-HT₁₄ receptors. Fourth ventricular administration of 5-CT and DP-5-CT has previously been shown to cause reductions in blood pressure and sympathetic nerve activity in anaesthetized cats (Shepheard et al., 1990a). Therefore, these agonists can have opposing actions depending on the central site of administration; a similar profile is observed with 5-HT in cats (Coote et al., 1987; see Coote, 1990) and rats (Dalton, 1986; Lambert et al., 1978). Similarly, in rats activation of 5-HT₁₄ receptors with 8-OH-DPAT can have both sympathoinhibitory and sympathoexcitatory actions depending on the central site of administration (see Chapter 2). The reason for the ability of the same 5-HT receptor subtype to have opposing actions on sympathetic drive is presently unknown; it is possible that these actions are independent of one another and are stimulated under differing physiological conditions. The finding that 5-HT₁ receptor agonists could also cause an increase in blood pressure and sympathetic nerve activity was unexpected since the response to 5-HT, the endogenous agonist for this receptor, was abolished by the 5-HT₂/5-HT₁c receptor agonist cinanserin. One explanation for these findings is that both 5-HT₁ and 5-HT₂/5-HT₁c receptors exist in a
sympathoexcitatory neuronal pathway and that 5-HT{sub}1 receptor activation leads to a response involving 5-HT{sub}2/5-HT{sub}1c receptors. In this way 5-HT could activate both 5-HT{sub}1 and 5-HT{sub}2/5-HT{sub}1c receptors to cause sympathoexcitation but in the presence of cinanserin both excitatory actions would be prevented. This proposal could be tested by attempting to block the cardiovascular actions of 5-CT or DP-5-CT with cinanserin and other 5-HT{sub}2/5-HT{sub}1c receptor antagonists. A schematic summary of the effects of 5-HT incorporating the above proposal is shown in Figure 56. This hypothesis remains to be tested and other factors which may explain the present findings have previously been discussed (see 4.4).

The brain regions involved in the central pressor mechanisms of 5-HT in the rat and cat were not addressed in the present study. It is difficult to locate the specific central site(s) involved in the cardiovascular effect following administration of 5-HT to the ventricles, since the drug is widely distributed throughout the brain using this procedure. However, certain studies in which 5-HT has been confined to more specific brain regions have suggested that forebrain structures are responsible for the 5-HT induced pressor response (see 1.6.1; Chapter 4; Coote et al., 1987; Lambert et al., 1978). The precise site/sites in the brain where 5-HT is acting to cause these cardiovascular effects remain to be determined but the rapid onset of response would suggest a brain area close to the lateral or 3rd ventricles. Previous microinjection studies have shown that 5-HT injected into the anterior hypothalamus/preoptic area caused a rise in blood pressure through an increase in sympathetic outflow (Smits & Struyker-Boudier, 1976; Sukamoto et al., 1984). This response was antagonised by metergoline (Robinson, 1984), a non-selective antagonist with affinity at 5-HT{sub}1A receptors (pK{sub}D 8.1; Hoyer, 1991). Therefore the sympathoexcitatory effects of 5-HT observed in the present study may be mediated by 5-HT{sub}1A receptors located in the anterior hypothalamus/preoptic area. In this way,
Figure 56 A summary of the cardiovascular effects of i.c.v. administration of 5-HT in cats. 5-HT stimulates 5-HT₂ and/or 5-HT₁C receptors to cause a pressor response, tachycardia and femoral vasoconstriction. These changes are associated with an increase in cardiac and splanchnic sympathetic nerve activity. The cardiovascular response caused by 5-HT is blocked by cinanserin and a similar DOI caused a similar response to 5-HT, indicating that 5-HT₂ and/or 5-HT₁C receptors mediated this effect. 5-CT and DP-5-CT also caused a pressor response associated with sympathoexcitation suggesting that 5-HT₁ receptors are present in the sympathoexcitatory pathway. It is possible that activation of 5-HT₁ receptors leads to a pressor response mediated through activation of 5-HT₂ and/or 5-HT₁C receptors. The precise location of these receptors remains to be determined. + represents stimulation, - represents inhibition.
5-HT and the selective 5-HT receptor agonists 5-CT, DP-5-CT and 8-OH-DPAT administered i.c.v. may diffuse to this structure, which is close to the third ventricle, to cause sympathoexcitation. The involvement of 5-HT receptors located in the anterior hypothalamus/preoptic area in the response caused by i.c.v. administration of 5-HT and the selective 5-HT receptor agonists remains to be investigated. This could be tested by microinjecting 5-HT and selective agonists into the anterior hypothalamus/preoptic area in anaesthetized rats or cats to determine whether a similar response to those observed following i.c.v. administration of these agonists occurred. Direct evidence for an action at this site would be obtained by examining the effects of administration of selective 5-HT receptor antagonists into the anterior hypothalamus/preoptic area prior to i.c.v. administration of an agonist. For example, spiroxatrine could be microinjected into the anterior hypothalamus/preoptic area prior to administration of DP-5-CT i.c.v. Attenuation of the response to DP-5-CT would implicate the anterior hypothalamus/preoptic area in this response.

Electrical and chemical stimulation of the dorsal raphé nucleus has previously been shown to cause a pressor response through an action at the anterior hypothalamus/preoptic area (Smits et al., 1978; Kuhn et al., 1980b; Robinson et al., 1985; Lovick, 1992; Piper and Goadsby, 1985). The evidence for this forebrain pressor pathway has been reviewed in the general introduction (see 1.6.2). The 5-HT receptors mediating this response and the physiological changes responsible for the rise in blood pressure are presently unknown. It would be of interest to re-evaluate this response in the light of the present study. In conscious rats, stimulation of the dorsal raphé nucleus with substance P caused an increase in blood pressure and heart rate which was prevented by pretreatment (i.v.) with the 5-HT$_{1A}$ receptor antagonist 8-MeO-CIEPAT. An increase in 5-HT release was demonstrated in the hippocampus following dorsal raphé injection of
substance P; indicating that this procedure caused an increase in ascending serotonergic neuronal activity (Gradin et al., 1992). Thus, stimulation of ascending 5-HT neurones may cause an increase in blood pressure associated with tachycardia through activation of 5-HT$_{1A}$ receptors. Therefore, it is possible that activation of 5-HT containing neurones (originating in the dorsal raphé nucleus) leads to the release of 5-HT in the anterior hypothalamus/preoptic area. 5-HT could then stimulate 5-HT receptors to yield a sympathoexcitatory response. This hypothesis requires further evaluation and experiments should be designed to demonstrate:

1. The release of 5-HT in the anterior hypothalamus/preoptic area following chemical stimulation of the dorsal raphé nucleus.
2. That chemical stimulation of the dorsal raphé produces a sympathoexcitatory response in anaesthetized or conscious rats.
3. That the release of 5-HT and the sympathoexcitatory responses caused by dorsal raphé stimulation are prevented by lesion of the anterior hypothalamus/preoptic area.

These questions could be investigated in a study in which the dorsal raphé nucleus was chemically stimulated with glutamate whilst recording blood pressure, heart rate and sympathetic nerve activity in conscious or α-chloralose anaesthetized rats. It may be possible to detect the stimulated release of 5-HT in the anterior hypothalamus/preoptic area using microdialysis methodology in conjunction with HPLC analysis (using a similar approach as Gradin et al., 1992). Alternatively, 5-HT release may be demonstrated in this region through the use of fast cyclic voltammetry in vivo. The neurotoxin 5,7-DHT could be microinjected bilaterally into the anterior hypothalamus/preoptic area to cause a selective lesion of the 5-HT containing terminals in this region (the selectivity of the lesion could be enhanced by the use of desipramine; see Robinson et al., 1985 for methodology). The degree of lesion could be assessed by measuring 5-HT
levels in the anterior hypothalamus and selectivity of this procedure could be evaluated by measuring other amine levels (i.e. noradrenaline and dopamine; see Robinson et al., 1985). Attenuation of the response observed following dorsal raphé nucleus stimulation in animals with lesion of the anterior hypothalamus/preoptic area would indicate that this region was involved in the cardiovascular response. Microinjection of 5-HT receptor antagonists into the anterior hypothalamus/preoptic area prior to stimulation of the dorsal raphé nucleus would allow the determination of the 5-HT receptors responsible for the cardiovascular changes. Further description of the receptor subtype(s) mediating these changes would be provided in separate experiments in which 5-HT and selective agonists for the various 5-HT receptor subtypes were microinjected into the anterior hypothalamus/preoptic area.

The central site of the 5-HT_2 and/or 5-HT_1C receptors involved in the release of vasopressin in the rat are presently unknown. However, a direct or indirect action of 5-HT on neurones in the paraventricular nucleus (PVN) or the supraoptic nucleus (SON), sites which are important for the production and release of vasopressin, is implicit. As there are few serotonergic nerve fibres in the magnocellular subdivisions of the SON and PVN (Steinbusch, 1981), it is unlikely that 5-HT directly stimulates the vasopressinergic cells in the SON or PVN. However, this could be tested by microinjecting 5-HT or quipazine into the SON or PVN of conscious rats in which blood pressure and heart rate are measured and blood samples are taken to allow assay of plasma vasopressin concentrations.

Evidence supports a role for 5-HT in the regulation of renin-angiotensin system causing an increase in plasma renin levels leading to enhanced levels of angiotensin II (see Van de Kar, 1991). Angiotensin II can cause the release of vasopressin through a mechanism which involves the subfornical
organ (SFO; a periventricular structure situated outside the blood-brain barrier; Lovino & Steardo, 1984). However, it is unlikely that this indirect mechanism is involved in the release of vasopressin by 5-HT since the stimulated release of vasopressin by p-chloroamphetamine and quipazine was not affected by SFO lesion or by the angiotensin II receptor antagonist, saralasin (Steardo & Lovino, 1986).

Although several studies have addressed the pharmacology of the 5-HT-induced release of vasopressin (see above), few have investigated the brain regions and pathways involved in this response. However, it is clear that 5-HT pathways play a role in the regulation of vasopressin. As well as certain 5-HT agonists, blockade of 5-HT reuptake with fluoxetine (Gibbs & Vale, 1983) and release of 5-HT caused by d-fenfluramine (Lovino & Steardo, 1985) increases plasma concentrations of vasopressin. The rise in vasopressin concentrations caused by d-fenfluramine were prevented following depletion of 5-HT with the tryptophan hydroxylase inhibitor, p-chlorophenylalanine (Lovino & Steardo, 1985). Treatment with the 5-HT releaser p-chloroamphetamine caused an increase in plasma vasopressin concentrations which was blocked by posterior hypothalamic deafferentation (a procedure reported to separate 5-HT cell bodies in the mid brain from there nerve terminals in the hypothalamus; Brownfield et al., 1988). These studies suggest that serotonergic neuronal transmission is required for the release of vasopressin. 5-HT appears to regulate the release of vasopressin during water deprivation. p-Chlorophenylalanine and i.c.v. administration of 5,7-DHT decreased forebrain 5-HT concentrations and prevented the increase in plasma concentrations caused by water deprivation (Lovino & Steardo, 1985). Administration of hypotonic saline causes an increase in plasma vasopressin concentrations and this response is blocked by i.c.v. administration of 5,7-DHT, suggesting that 5-HT pathways are involved in the osmotic stimulation of vasopressin secretion.
Hypovolemia-induced increase in vasopressin secretion was not blocked by 5,7-DHT (Brownfield et al., 1987).

The 5-HT receptor subtype(s) involved in the release of vasopressin are known, as are the physiological stimuli for this response. However, the specific brain regions involved in this response remain to be determined. It is possible that 5-HT neurones originating in the dorsal raphé nucleus control the release of vasopressin. It would be of interest to determine whether stimulation of the dorsal raphé nucleus caused an increase in the release of vasopressin. This could be investigated in conscious rats in which blood pressure and heart rate were measured and blood samples were taken to allow the measurement of plasma vasopressin concentrations (using the method described by Pergola et al., 1993) before and after injection of glutamate into the dorsal raphé nucleus.

In conclusion, the present study has investigated the cardiovascular response caused by the administration of 5-HT to the forebrain of rats and cats. In these species 5-HT caused a pressor response. In rats the cardiovascular profile caused by forebrain administration of 5-HT is the result of the release of vasopressin, direct sympathoexcitation and reflexly mediated sympathoinhibition. The cardiovascular changes caused by 5-HT were mediated by central 5-HT\(_2\) and/or 5-HT\(_{1C}\) and 5-HT\(_{1A}\) receptors. In cats, activation of forebrain 5-HT\(_2\) and/or 5-HT\(_{1C}\) and 5-HT\(_1\) receptors caused sympathoexcitation resulting in vasoconstriction of the femoral and splanchnic vascular beds leading to the observed rise in blood pressure. An important discovery of the present study is that activation of 5-HT\(_{1A}\) receptors can cause sympathoexcitation as well as sympathoinhibition and that the observed response depends on the central site of administration.
REFERENCES


Ramage, A.G. (1988a). Are drugs that act both on serotonin receptors and $\alpha_1$-adrenoceptors more potent hypotensive agents than those that act only on $\alpha_1$-adrenoceptors? J. Cardiovasc. Pharmacol., 11, 30-34.


APPENDIX

1 Doppler shift values

Doppler flow probes were placed on the renal artery, mesenteric artery and abdominal aorta to measure doppler shift values (an index of blood flow; see 3.2.2). Vascular conductance values were calculated for the renal (REN), mesenteric (MES) and hindquarters (HQ) vascular beds by dividing the appropriate mean doppler shift by the mean arterial blood pressure. Drug-induced changes in vascular conductance were expressed as the percentage change from baseline. As the vascular conductance change gives an indication of vasoconstriction or vasodilatation of a particular vascular bed this variable is described in the text and figures. The regional changes in doppler shift caused following administration of drug or vehicle are tabulated below.

1.1 The effect of i.c.v. administration of saline and 5-HT on regional flow in conscious Long-Evans rats

<table>
<thead>
<tr>
<th>DOSE</th>
<th>SALINE 5 µl (8)</th>
<th>5-HT 4 nmol kg⁻¹ (8)</th>
<th>5-HT 40 nmol kg⁻¹ (11)</th>
<th>5-HT 120 nmol kg⁻¹ (8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TIME (min)</td>
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<td>MES</td>
<td>HQ</td>
<td>REN</td>
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<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
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<td>0 ± 1</td>
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<td>-2 ± 3</td>
<td>-2 ± 2</td>
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</table>

All values are expressed as mean ± s.e.mean from (n) animals. Baseline values were taken 1 minute before the addition of drug or vehicle and measurements at subsequent times are expressed as the percentage change from baseline (%).
1.2 The effect of i.c.v. administration of saline and 5-HT on regional flow in conscious Brattleboro rats

<table>
<thead>
<tr>
<th>TIME (min)</th>
<th>SALINE 5 μl (8)</th>
<th>5-HT 4 nmol kg(^{-1}) (7)</th>
<th>5-HT 40 nmol kg(^{-1}) (8)</th>
<th>5-HT 120 nmol kg(^{-1}) (8)</th>
</tr>
</thead>
<tbody>
<tr>
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<td>MES</td>
<td>HQ</td>
<td>REN</td>
</tr>
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<td>0</td>
<td>0</td>
<td>0</td>
</tr>
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</table>

All values are expressed as mean ± s.e.mean from (n) animals. Baseline values were taken 1 minute before the addition of drug or vehicle and measurements at subsequent times are expressed as the percentage change from baseline (%). REN renal flow; MES mesenteric flow; HQ hindquarters flow.
1.3 The effect of i.c.v. administration of saline and DP-5-CT on regional flow in conscious Long-Evans rats

<table>
<thead>
<tr>
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<th>SALINE 5 µl (4)</th>
<th>DP-5-CT 3 nmol kg⁻¹ (4)</th>
<th>DP-5-CT 30 nmol kg⁻¹ (4)</th>
<th>DP-5-CT 100 nmol kg⁻¹ (4)</th>
</tr>
</thead>
<tbody>
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<td></td>
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<td>MES</td>
<td>HQ</td>
<td>REN</td>
</tr>
<tr>
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<td>0</td>
<td>0</td>
<td>0</td>
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</tbody>
</table>

All values are expressed as mean ± s.e.mean from (n) animals. Baseline values were taken 1 minute before the addition of drug or vehicle and measurements at subsequent times are expressed as the percentage change from baseline (%). REN renal flow; MES mesenteric flow; HQ hindquarters flow.
1.4 The effect of i.c.v. administration of 5-HT on regional flow in conscious (A) Long-Evans rats pretreated with d(CH$_2$)$_5$Tyr(Me)AVP and (B) Brattleboro rats pretreated with ICI 118551.

### A.

<table>
<thead>
<tr>
<th>TIME (min)</th>
<th>REN</th>
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<th>HQ</th>
</tr>
</thead>
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<td>10</td>
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<td>5 ± 2</td>
</tr>
</tbody>
</table>

**DOSE**

- d(CH$_2$)$_5$Tyr(Me)AVP: bolus 10 µg kg$^{-1}$; infusion 10 µg kg$^{-1}$ h$^{-1}$; i.v.

### B.

<table>
<thead>
<tr>
<th>TIME (min)</th>
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<th>MES</th>
<th>HQ</th>
</tr>
</thead>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>1 ± 9</td>
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<td>-4 ± 4</td>
</tr>
<tr>
<td>60</td>
<td>-6 ± 15</td>
<td>-12 ± 3</td>
<td>-15 ± 3</td>
</tr>
</tbody>
</table>

**DOSE**

- ICI 118551: bolus 0.2 mg kg$^{-1}$; infusion 0.1 mg kg$^{-1}$ h$^{-1}$; i.v.

<table>
<thead>
<tr>
<th>DOSE</th>
<th>5-HT 40 nmol kg$^{-1}$; i.c.v.</th>
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<td>1</td>
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</tr>
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<td>15</td>
<td>-5 ± 5</td>
</tr>
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</tbody>
</table>

**DOSE**

- 5-HT 120 nmol kg$^{-1}$; i.c.v.

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<th>0</th>
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<tbody>
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<td>29 ± 12</td>
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<tr>
<td>20</td>
<td>1 ± 9</td>
<td>-5 ± 10</td>
<td>20 ± 7</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± s.e.mean from 7 and 4 animals, respectively. Baseline values were taken 1 minute before the addition of drug or vehicle and measurements at subsequent times are expressed as the percentage change from baseline (%). REN renal flow; MES mesenteric flow; HQ hindquarters flow.
2 Femoral flow values  An electromagnetic flow probe was placed on the right femoral artery to monitor femoral flow. Vascular conductance changes were calculated by dividing the appropriate flow value by the mean arterial pressure. Drug-induced changes in vascular conductance were expressed as the percentage change from baseline. As the vascular conductance change gives an indication of vasoconstriction or vasodilatation of the vascular bed this variable is described in the text and figures. The changes in femoral flow caused following administration of drug or vehicle are tabulated below.

2.1 The effect of i.c.v. administration of (A) saline and 5-HT in the absence and presence of cinanserin and (B) 5-CT and DP-5-CT on femoral flow in anaesthetized cats.

<table>
<thead>
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<th>TIME</th>
<th>0</th>
<th>3</th>
<th>5</th>
<th>10</th>
<th>13</th>
<th>18</th>
<th>20</th>
<th>23</th>
<th>25</th>
<th>30 (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOSE</td>
<td>basal</td>
<td>20 µl</td>
<td>20 µl</td>
<td>20 µl</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SALINE (5)</td>
<td>9.5 ± 1.9</td>
<td>0 ± 0.2</td>
<td>-0.1 ± 0.1</td>
<td>-0.1 ± 0.1</td>
<td>-0.1 ± 0.1</td>
<td>-0.6 ± 0.3</td>
<td>-0.4 ± 0.3</td>
<td>-0.5 ± 0.3</td>
<td>-0.6 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>DOSE</td>
<td>basal</td>
<td>10 nmol kg⁻¹</td>
<td>40 nmol kg⁻¹</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-HT (5)</td>
<td>8.4 ± 1.1</td>
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<td>-0.5 ± 0.3</td>
<td>-0.3 ± 0.4</td>
<td>-0.3 ± 0.3</td>
<td>-0.6 ± 0.2</td>
<td>-1.1 ± 0.4</td>
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<td>-1.1 ± 0.5</td>
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<td>cinanserin pretreatment</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>5-HT (3)</td>
<td>16.3 ± 3.8</td>
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<td>-0.1 ± 0.1</td>
<td>-0.5 ± 0.3</td>
<td>-0.7 ± 0.3</td>
<td>-0.9 ± 0.4</td>
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<td>-1.1 ± 0.5</td>
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<td>-0.6 ± 0.4</td>
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</table>

<table>
<thead>
<tr>
<th>TIME</th>
<th>0</th>
<th>3</th>
<th>5</th>
<th>8</th>
<th>10</th>
<th>15</th>
<th>18</th>
<th>20</th>
<th>25 (min)</th>
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</thead>
<tbody>
<tr>
<td>DOSE</td>
<td>basal</td>
<td>2.5 nmol kg⁻¹</td>
<td>10 nmol kg⁻¹</td>
<td>40 nmol kg⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>5-CT (4)</td>
<td>9.3 ± 0.9</td>
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<td>-0.6 ± 0.3</td>
<td>-0.5 ± 0.3</td>
<td>-0.6 ± 0.3</td>
<td>-0.7 ± 0.3</td>
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<td>-1.0 ± 0.4</td>
<td>-1.1 ± 0.4</td>
</tr>
<tr>
<td>DP-5-CT (3)</td>
<td>5.5 ± 0.6</td>
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<td>0.1 ± 0.1</td>
<td>0.2 ± 0.1</td>
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<td>0.1 ± 0.2</td>
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<td>-0.2 ± 0.1</td>
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</table>

All values are expressed as mean ± s.e.mean from (n) animals. Baseline values were taken 1 minute before the addition of drug or vehicle and measurements at subsequent times are expressed as the change from baseline (ml min⁻¹).
2.2 The effect of i.c.v. administration of DOI on femoral flow in anaesthetized cats pretreated with BW501C67 (i.v.).

<table>
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<th>5</th>
<th>8</th>
<th>10</th>
<th>13</th>
<th>15</th>
<th>20</th>
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<tbody>
<tr>
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<td>80 nmol kg⁻¹</td>
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<tr>
<td>DOI</td>
<td>9.8 ± 1.9</td>
<td>-0.8 ± 0.3</td>
<td>-1.1 ± 0.4</td>
<td>-1.9 ± 0.4</td>
<td>-2.3 ± 0.3</td>
<td>-2.5 ± 0.2</td>
<td>-2.7 ± 0.3</td>
<td>-2.8 ± 0.3</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± s.e.mean from four animals. Baseline values were taken 1 minute before the addition of drug and measurements at subsequent times are expressed as the change from baseline (ml min⁻¹).

2.3 The effect of cinanserin (i.c.v.) and BW501C67 (i.v.) on femoral flow in anaesthetized cats.

<table>
<thead>
<tr>
<th>TIME (min) after pretreatment</th>
<th>0</th>
<th>5</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW501C67 1 mg kg⁻¹ i.v. (n=4)</td>
<td>9.7 ± 2.1</td>
<td>-0.4 ± 0.1</td>
<td>0.1 ± 0.3</td>
</tr>
<tr>
<td>cinanserin 265 nmol kg⁻¹ i.c.v. (n=3)</td>
<td>11.2 ± 3.9</td>
<td>5.2 ± 2.4</td>
<td></td>
</tr>
</tbody>
</table>

All values are expressed as mean ± s.e.mean from (n) animals. Baseline values were taken 1 minute before the addition of drug and measurements at subsequent times are expressed as the change from baseline (ml min⁻¹).
3. The mechanism of the maintained hindquarters vasodilatation caused by 5-HT following i.c.v. administration is unknown. However, this response was also caused by DP-5-CT implicating the activation of 5-HT\textsubscript{1A} receptors. Activation of 5-HT\textsubscript{1A} receptors has previously been shown to cause hypothermia in several species including rats (see Green & Goodwin, 1987). The flat body posture and hindquarters vasodilatation observed with 5-HT and DP-5-CT may be involved in the heat loss process. In this respect, 5-HT has been shown to cause hypothermia following i.c.v. administration (Yamada et al., 1988) and administration into the anterior hypothalamus/preoptic area (Lin et al., 1983).


Central administration of 5-HT activates 5-HT\textsubscript{1A} receptors to cause sympathoexcitation and 5-HT\textsubscript{2}/5-HT\textsubscript{1C} receptors to release vasopressin in anaesthetized rats

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1 The effects of intracerebroventricular injections to the right lateral ventricle (i.c.v.) of 5-hydroxytryptamine (5-HT, 40 and 120 nmol kg\textsuperscript{-1}), N,N-di-n-propyl-5-carboxamidotryptamine (DP-5-CT; 3 nmol kg\textsuperscript{-1}), 5-carboxamidotryptamine (5-CT; 3 nmol kg\textsuperscript{-1}), 8-hydroxy-2-(di-N-propylamino)tetralin (8-OH-DPAT; 3, 40 and 120 nmol kg\textsuperscript{-1}) and 1-(2,5-di-methoxy-4-iodophenyl)-2-aminopropane (DOI; 40 and 120 nmol kg\textsuperscript{-1}) on renal sympathetic nerve activity, blood pressure, heart rate and phrenic nerve activity were investigated in normotensive rats anaesthetized with α-chloralose.

2 5-HT caused a long lasting pressor response which was associated with an initial bradycardia and renal sympathoinhibition followed by a tachycardia and renal sympathoexcitation. Pretreatment with the 5-HT\textsubscript{1A}/5-HT\textsubscript{1C} receptor antagonists, cinanserin (300 nmol kg\textsuperscript{-1}, i.c.v.) or LY 53857 (300 nmol kg\textsuperscript{-1}, i.c.v.) reversed the initial bradycardia and sympathoinhibition to tachycardia and sympathoexcitation. Combined pretreatment with LY 53857 (300 nmol kg\textsuperscript{-1}, i.c.v.) and the 5-HT\textsubscript{1A} antagonist, spiroxatrine (300 nmol kg\textsuperscript{-1}, i.c.v.), blocked the effects of 5-HT on all the above variables.

3 Pretreatment with the vasopressin V\textsubscript{1}-receptor antagonist, β-mercaptop-β,β-cyclopentamethylene-propionyl\textsubscript{3}, O-Me-Tyr\textsubscript{2}, Arg\textsubscript{5}-vasopressin [(d(CH\textsubscript{2})\textsubscript{3}]Tyr(Me)AVP, 10 μg kg\textsuperscript{-1}, i.v.] did not affect the magnitude but reduced the duration of the pressor response produced by i.c.v. 5-HT and reversed the initial bradycardia and renal sympathoinhibition to tachycardia and sympathoexcitation.

4 1-(2,5-Di-methoxy-4-iodophenyl)-2-aminopropane (DOI) caused a pressor effect which was associated with a bradycardia and sympathoinhibition. These effects were blocked by pretreatment with BW501C67 (0.1 mg kg\textsuperscript{-1}, i.v.), a peripherally acting 5-HT\textsubscript{2}/5-HT\textsubscript{1C} receptor antagonist. However, BW501C67 (0.1 mg kg\textsuperscript{-1}, i.v.) failed to block the effects of i.c.v. 5-HT.

5 DP-5-CT, 5-CT and 8-OH-DPAT (3 nmol kg\textsuperscript{-1}, i.c.v.) caused sympathoexcitation, tachycardia and a rise in blood pressure. Pretreatment with methiothepin (1 mg kg\textsuperscript{-1}, i.v.) or spiroxatrine (300 nmol kg\textsuperscript{-1}, i.c.v.) attenuated the response to i.c.v. DP-5-CT.

6 It is concluded that i.c.v. administration of 5-HT activates 5-HT\textsubscript{1A} receptors to cause sympathoexcitation and 5-HT\textsubscript{2} or 5-HT\textsubscript{1C} receptors to cause the release of vasopressin.

Keywords: 5-HT\textsubscript{1A} receptors; 5-HT\textsubscript{2} or 5-HT\textsubscript{1C} receptors; vasopressin V\textsubscript{1}-receptors; 8-OH-DPAT; DP-5-CT; 5-HT; DOI; blood pressure; sympathetic nerve activity; anaesthetized rat

Introduction

Intracerebroventricular (i.c.v.) injections of 5-hydroxytryptamine (5-HT) in anaesthetized rats cause a rise in blood pressure and variable effects on heart rate (Lambert et al., 1975; 1978; Kristie & Djurkovic, 1976; 1980). In conscious rats i.c.v. administration of 5-HT also causes a pressor effect but in these animals consistently produces bradycardia (Sukamoto et al., 1984; Dalton, 1986). More recently, Inoue & Bunag (1989) were able to demonstrate that i.c.v. 5-HT induced a pressor response which was associated with a consistent bradycardia and sympathoinhibition in the anaesthetized rat. These authors also demonstrated that the pressor effect was attenuated by pretreatment with a vasopressin V\textsubscript{1}-receptor antagonist. The precise nature of the 5-HT receptor involved in this central release of vasopressin was not determined, although (as in previous studies) the pressor effect of i.c.v. 5-HT could be antagonized by the non-selective 5-HT receptor antagonists, methysergide or bromolysergic acid diethylamide (see Ramage, 1985; Hoyer, 1991). In addition, it has also been reported that the rise in blood pressure produced by i.c.v. administration of 5-HT is attenuated by cervical transection of the spinal cord, adrenalectomy, adrenergic neurone blocking agents and α-adrenoceptor antagonists (Kristie & Djurkovic, 1980), suggesting that there is an additional sympathoexcitatory component to the response of 5-HT. However, Inoue & Bunag (1989) were unable to demonstrate such an action when recording from the splanchnic nerve. We therefore decided to investigate further the mechanism and nature of the receptors involved in effects of i.c.v. administration of 5-HT on blood pressure, heart rate and renal sympathetic nerve activity in rats anaesthetized with α-chloralose using more selective agonists and antagonists for the different 5-HT receptor subtypes. In addition respiratory variables were monitored. A preliminary account of these observations has been presented to the British Pharmacological Society (Anderson, 1991; Anderson et al., 1992b).

Methods

Experiments were performed on male Sprague-Dawley normotensive rats (250–350 g). Anaesthesia was induced with halothane (2.5% in oxygen) and maintained with α-chloralose (80 mg kg\textsuperscript{-1}, i.v.). Supplemenary doses of α-
chloralose (10–20 mg kg⁻¹, i.v.) were given as required. Depth of anaesthesia was assessed by the stability of cardio-
vascular and respiratory variables being recorded. The left carotid artery was cannulated for the measurement of blood pressure and for sampling arterial blood for analysis of pH and blood gases. Blood pressure was measured with a pres-
sure transducer (Gould Statham P23XL) and the heart rate was
derived electronically from the blood pressure signal (Gould Biotech Amplifier). The left jugular vein was can-
nulated for drug administration and a tracheal cannula was implanted. Body temperature was monitored by a rectal
probe and maintained at 36–38°C with a homeothermic blank system (Harvard). The animals were artificially
ventilated (rate 50 min⁻¹, stroke volume 8 ml kg⁻¹) with oxygen-enriched room air by use of a positive pressure pump
(Harvard Rodent Ventilator 683) and neuromuscular block-
ade was produced with decamethonium (5 mg kg⁻¹, i.v.). Blood samples were taken from a T-piece on the carotid
arterial cannula and blood gases and pH were monitored with a Corning pH/blood gas analyser. Blood gases were
maintained between 90–130 mm Hg with a Corning pH/blood gas analyser. Blood gases were
monitored as necessary to maintain blood gases and
arterial pH between 7.35–7.45, with a Corning pH/blood gas analyser. Blood gases were
monitored (rate 50 min⁻¹, stroke volume 8 ml kg⁻¹) with oxygen-enriched room air by use of a positive pressure pump
(Harvard Rodent Ventilator 683) and neuromuscular block-
ade was produced with decamethonium (5 mg kg⁻¹, i.v.). Blood samples were taken from a T-piece on the carotid
arterial cannula and blood gases and pH were monitored with a Corning pH/blood gas analyser. Blood gases were
monitored as necessary to maintain blood gases and

Cannulation of the lateral cerebral ventricle

The rats were placed in a stereotaxic head holder and a stainless steel guide cannula (22 gauge) was implanted into the right lateral cerebral ventricle. The co-ordinates used from bregma were 4 mm ventral, 1.5 mm lateral and 1 mm posterior. Drug and vehicle solutions were administered through an i.c.v. injection cannula (28 gauge) attached by a length of polythene tubing to a 100 μl syringe (Hamilton). At the end of the experiment, the cannula placement was confirmed by the administration of 5 μl of 2% pontamine sky blue dye.

Recording of phrenic nerve and renal nerve activity

The right phrenic nerve was exposed by deflecting the scapula forwards and dissecting the nerve clear of overlying muscle and connective tissue. The nerve was cut peripherally and placed on a bipolar silver hook electrode as described previously (Dreteler et al., 1991). Phrenic nerve activity was quantified by counting the number of action potentials above the noise level over 5 s with a spike processor (Digitimer D130). To maintain phrenic nerve activity, a measure of central inspiratory drive, the blood Pco₂ values in these animals were maintained at a slightly higher (40–50 mmHg) level than the physiological norm (35–49 mmHg). This usually locked the rate of phrenic nerve firing to the rate of the animals chest movements caused by the respiration pump and changes in phrenic nerve activity were the result of changes in the size of each inspiratory burst. The right kidney was exposed by a retroperitoneal approach and was deflected laterally to reveal the renal artery and nerve. Renal nerve activity was recorded as previously described (Ramage & Wilkinson, 1989). Renal nerve activity was quantified by integrating the signal above background noise over 5 s with a solid state integrator (Medical Electronics workshop, Royal Free Hospital School of Medicine). The noise levels were verified at the end of the experiment after the administration of pentobarbitone sodium (20 mg per animal).

At the beginning of each experiment the baroreceptor reflex response was tested by observing whether renal nerve activity and heart rate were reduced by a rise in blood pressure caused by noradrenaline (25 ng per animal, i.v.) and were raised by a reduction in blood pressure caused by sodium nitroprusside (0.6 μg per animal, i.v.). Only preparations with an intact baroreceptor reflex were used.

Experimental protocols

The preparation was allowed to stabilize for 30 min before the administration of saline (5 μl i.c.v.). After a 5 min control period a single dose of test compound or saline control was given i.c.v. and the response was followed for at least 30 min. In antagonist studies, antagonists administered i.c.v. were given 10 min before the injection of test drug. However, both LY 53857 and spiroxatrine were administered in two doses of 150 mmol kg⁻¹ alone or combined, 5 min apart and the test drug administered 5 min after the last dose of these antagonists. Vehicle for spiroxatrine (0.01 N HCl) was administered in 2 volumes of 5 μl 5 min apart and the test agonist was given 5 min after the last dose of vehicle. When antagonists were given i.v. the test drug was administered 5 min later. However, for methiothepin the test drug was administered following a stabilization period of 20 min. These pretreatment times were chosen to allow stabilization of any changes in the variables being recorded caused by administration of the antagonists. In each rat the cardiovascular response of a single dose of the test drug was recorded.

Analysis of results

Baseline values were taken 1 min before the addition of drug or vehicle. All results are expressed as changes from baseline values. Nerve activity was measured as the average of the integrated values over 1 min in arbitrary units and was expressed as the percentage change from baseline. Changes in mean blood pressure, heart rate, renal and phrenic nerve activity caused by the test drug were compared with time-matched vehicle controls by two-way analysis of variance and were subsequently analysed by the least significant difference test (Sokal & Rohlf, 1969). Biphasic responses in some variables were observed following i.c.v. administration of 5-HT and these phases were analysed separately. Thus, the maximum change for each phase of the 5-HT response was measured and compared to the maximum change in vehicle controls during the same period by Student’s t test for unpaired data. Changes in variables caused by antagonist or vehicle pretreatments were compared to the pre-dose baseline by Student’s t test for paired data. All values are expressed as the mean ± s.e.mean, differences in the mean were taken as significant when P<0.05.

Drugs and solutions

The drugs used were BW501C67, (2-aminino-N-(2-(3-chloro-
phenoxy)propyl) acetamide HCl), 5-carboxamidotryptamine
maleate (5-CT), N,N-di-n-propyl-5-carboxamidotryptamine
maleate (DP-5-CT; these were gifts from Wellcome Laborato-
ries, Beckenham, Kent), α-chloralose (Sigma Chemical Co.,
Poole, Dorset); DOI, 1-(2,5-di-methoxy-4-iodophenyl)-
2-amino-4-propanopropene (Research Biochemicals Inc.,
Semat, St. Albans); decamethonium iodide (Koch-Light,
Haverhill, Suffolk); Gelfusine (Consolidated Chemists,
Wrexham, Clwyd); 8-hydroxy-2-(di-N-propylamino)tetralin HBr (8-OP-
DPAT; Research Biochemicals Inc., Semat, St. Albans); 5-
hydroxytryptamine creatinine sulphate, 5-HT (BDH, Poole,
Dorset); halothane (ICI Pharmaceuticals Ltd, Macclesfield);

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solved in 0.9% w/v saline except for spiroxatrine and the combination of spiroxatrine and LY 53857 which were dissolved in 0.01 N hydrochloric acid (HCl). Solutions were administered in a dose volume of 5 μl over a 20 s period. All drugs given i.v. were dissolved in saline.

**Results**

**Effect of i.c.v. administration of saline**

Saline i.c.v. (5 μl; n = 6) had little effect on blood pressure, heart rate, renal or phrenic nerve activity and these variables remained stable for the duration of the experiment (see Figure 1). Baseline values for blood pressure and heart rate for this group of experiments were 122 ± 6 mmHg and 417 ± 13 beats min⁻¹ (mean ± s.e.mean).

**Effect of i.c.v. administration of 5-HT**

5-HT [40 (n = 6); 120 (n = 8) nmol kg⁻¹] caused immediate, dose-related increases in arterial blood pressure (Figures 1 for this group of experiments were 122 ± 6 mmHg and 417 ± 13 beats min⁻¹ (mean ± s.e.mean). The maximal changes in mean arterial blood pressure (MAP), heart rate (HR), renal or phrenic nerve activity and these variables were temporally matched. MAP (120 nmol kg⁻¹) caused an initial (1–5 min) significant reduction in phrenic nerve activity of 22 ± 5%. In 3 animals this was followed by a secondary rise in phrenic nerve activity (227 ± 84%, 10–20 min). However, in the remaining animals phrenic nerve activity returned to baseline levels after 5 min. Baseline values for blood pressure and heart rate in the low and high dose groups were 112 ± 6 mmHg and 417 ± 31 beats min⁻¹ and 112 ± 4 mmHg and 417 ± 18 beats min⁻¹ respectively.

**The effect of pretreatment (i.c.v.) with either cinanserin or LY 53857 on the response to 5-HT**

Cinanserin (300 nmol kg⁻¹; n = 6) or LY 53857 (300 nmol kg⁻¹; n = 6) had no significant effect on blood pressure, heart rate, renal nerve activity or phrenic nerve activity. Baseline values for blood pressure and heart rate were 102 ± 7 mmHg and 398 ± 10 beats min⁻¹ and 102 ± 7 mmHg and 417 ± 21 beats min⁻¹ respectively. Neither drug prevented the rise in blood pressure caused by 5-HT (120 nmol kg⁻¹) but the initial bradycardia and renal sympathoinhibition were reversed to tachycardia and sympathoexcitation (Figures 2 and 3). The 5-HT induced changes in phrenic nerve activity were not significantly altered (Figure 3).

**Effect of i.v. pretreatment with BW501C67 on the response to i.c.v. 5-HT**

5-HT (120 nmol kg⁻¹) administered i.c.v. in animals pretreated with BW501C67 (0.1 mg kg⁻¹; i.v.; n = 4) caused similar effects to those observed in non-pretreated animals; there was an immediate increase in blood pressure and biphasic changes in heart rate and renal nerve activity (Figure 3). However, the duration of the bradycardia and the renal sympathoinhibition was significantly prolonged. Baseline values for blood pressure and heart rate were 115 ± 11 mmHg and 420 ± 22 beats min⁻¹.

**The effect of combined pretreatment (i.c.v.) with LY 53857 and spiroxatrine on the response to 5-HT**

Combined pretreatment with LY 53857 (300 nmol kg⁻¹) and spiroxatrine (300 nmol kg⁻¹; n = 4) did not significantly change baseline values (baseline values for blood pressure and heart rate were 98 ± 7 mmHg and 380 ± 7 beats min⁻¹). However, the effect of 5-HT (120 nmol kg⁻¹, i.c.v.) on all variables was significantly reduced in animals pretreated with this combination compared to animals pretreated with LY 53857 (300 nmol kg⁻¹, i.c.v.) alone (Figure 4). The combination of LY 53857 and spiroxatrine was dissolved in 0.01 N HCl (vehicle for spiroxatrine) whereas previously LY 53857 had been dissolved in saline. Therefore, in 2 separate experiments, animals were pretreated with LY 53857 dissolved in 0.01 N HCl. In these experiments the response to 5-HT (120 nmol kg⁻¹; i.c.v.; data not shown) was similar to that observed previously in animals pretreated with LY 53857 alone dissolved in saline.
The effect of pretreatment (i.v.) with d(CH₂)₂Tyr(Me)-AVP on the response to 5-HT

d(CH₂)₂Tyr(Me)-AVP (10 µg kg⁻¹ i.v.; n = 6) had no significant effect on blood pressure, heart rate, renal or phrenic nerve activity. Baseline values for blood pressure and heart rate were 104 ± 4 mmHg and 388 ± 8 beats min⁻¹. Pretreatment with d(CH₂)₂Tyr(Me)-AVP did not prevent the rise in blood pressure caused by 5-HT (120 nmol kg⁻¹, i.c.v.) but the duration of the pressor rise was significantly attenuated, see Figure 3. The 5-HT-induced bradycardia and renal sympathoinhibition were reversed to an immediate tachycardia and sympathoexcitation in the presence of d(CH₂)₂Tyr(Me)-AVP (Figure 3). Changes in phrenic nerve activity caused by 5-HT were unaffected.

Effect of i.c.v. and i.v. administration of 1-(2,5-di-methoxy-4-iodophenyl)-2-aminopropane (DOI) in the absence and presence of BW501C67

DOI 12 nmol kg⁻¹ (i.c.v.) had no effect on blood pressure, heart rate, renal and phrenic nerve activity (data not shown). DOI (40 nmol kg⁻¹, n = 4; 120 nmol kg⁻¹, n = 6; i.c.v.) produced maximum increases in blood pressure of 7 ± 1 and 10 ± 2 mmHg respectively and decreases in heart rate and

300 nmol kg⁻¹, i.c.v.; n = 6), BW501C67 (■; 0.1 mg kg⁻¹, i.v.; n = 4) and d(CH₂)₂Tyr(Me)AVP (Δ; 10 µg kg⁻¹, i.v.; n = 6) in mean arterial blood pressure (MAP), heart rate (HR), renal nerve activity (RNA) and phrenic nerve activity (PNA). Each point represents the mean value and the vertical bars show s.e.mean *P<0.05 and **P<0.01 compared to 5-HT saline pretreatment. Statistical significance for the effects of 5-HT on HR and RNA in animals pretreated with cinanserin, LY 53857 and d(CH₂)₂Tyr(Me)AVP between 1–5 min are the same and are illustrated by a single symbol for the sake of clarity.
renal nerve activity of 18 ± 6 and 19 ± 9 beats min⁻¹ and 31 ± 9 and 53 ± 7% respectively, 5 min after injection. There was no change in phrenic nerve activity (Figure 5). Baseline values for blood pressure and heart rate were 126 ± 5 mmHg and 424 ± 21 beats min⁻¹ and 122 ± 2 mmHg and 406 ± 20 beats min⁻¹ respectively. Pretreatment with BW501C67 (0.1 mg kg⁻¹, i.v.), which had no effect per se, significantly attenuated the response to i.c.v. DOI (120 nmol kg⁻¹, n = 4; Figure 5) on these variables. Baseline values for blood pressure and heart rate were 112 ± 2 mmHg and 406 ± 20 beats min⁻¹.

DOI, 120 nmol kg⁻¹ (n = 3), given i.v. produced a rise in blood pressure of 22 ± 2 mmHg which was maintained over 20 min. Again bradycardia and renal sympahtoinhibition were observed and reached maxima of 21 ± 3 beats min⁻¹ and 54 ± 6%, respectively 1 min following injection. The bradycardia was not maintained and had returned to baseline by 10 min. However, the renal sympahtoinhibition was maintained for 20 min. Phrenic nerve activity was not measured in these animals. Baseline values for blood pressure and heart rate were 117 ± 4 mmHg and 429 ± 22 beats min⁻¹. Pretreatment with BW501C67 (0.1 mg kg⁻¹, i.v.; n = 2) abolished the rise in blood pressure caused by i.v. DOI (data not illustrated).

**Figure 4** Anaesthetized rats: a comparison of the changes from post-pretreatment values over time (min) caused by 5-HT (120 nmol kg⁻¹, i.c.v.) in the presence of LY 53857 (300 nmol kg⁻¹, i.c.v.; □; n = 6) and the combined pretreatment of LY 53857 (300 nmol kg⁻¹, i.c.v.) and spiroxatrine (300 nmol kg⁻¹, i.c.v.; ▲; n = 4) in mean arterial blood pressure (MAP), heart rate (HR), renal nerve activity (RNA) and phrenic nerve activity (PNA). Each point represents the mean value and the vertical bars show s.e.mean. *P<0.05 and **P<0.01 compared to i.c.v. saline (not illustrated for the sake of clarity).

**Figure 5** Anaesthetized rats: a comparison of the changes with over time (min) from baseline or post-pretreatment values caused by i.c.v. 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) 46 nmol kg⁻¹ (□; n = 6) and i.c.v. DOI 120 nmol kg⁻¹ in the absence (□; n = 6) and presence of BW501C67 (0.1 mg kg⁻¹, i.v.; ■; n = 4) in mean arterial blood pressure (MAP), heart rate (HR), renal nerve activity (RNA) and phrenic nerve activity (PNA). Each point represents the mean value and the vertical bars show s.e.mean. *P<0.05 and **P<0.01 compared to i.c.v. saline (not illustrated for the sake of clarity).

**Effect of i.c.v. administration of 5-CT, DP-5-CT and 8-OH-DPAT**

DP-5-CT (3 nmol kg⁻¹; n = 8) caused an immediate and significant rise in blood pressure, heart rate and renal nerve activity reaching a maximum by 5 min of 9 ± 3 mmHg, 39 ± 5 beats min⁻¹ and 83 ± 15%, respectively (Figures 2 and 6). These changes were maintained for at least 30 min. There was no change in phrenic nerve activity (Figure 6). Baseline values for blood pressure and heart rate were 114 ± 4 mmHg and 391 ± 11 beats min⁻¹.

5-CT (3 nmol kg⁻¹; n = 5) also caused an immediate significant rise in blood pressure of 17 ± 5 mmHg after 2 min. This rise in blood pressure was associated with significant increases in heart rate of 48 ± 7 beats min⁻¹ and in renal nerve activity of 77 ± 35% (Figure 6). Both the rise in heart rate and in renal nerve activity were well maintained returning to near baseline values by 30 min. However, blood pressure remained elevated for only 2 min (Figure 6) and in three experiments, between 3 and 5 min after injection, the rise in blood pressure was followed by a substantial fall of 21 ± 3 mmHg below baseline. 5-CT caused no significant changes in phrenic nerve activity. Baseline values for blood
CARDIOVASCULAR EFFECTS OF i.c.v. 5-HT

In the present experiments in a-chloralose anaesthetized rats, treated with a neuromuscular blocking agent, i.v. injection of DP-5-CT, 5-CT, 8-OH-DPAT (low dose) and 5-HT [in

pressure and heart rate were 114 ± 4 mmHg and 442 ± 2 beats min⁻¹.

8-OH-DPAT (3 nmol kg⁻¹; n = 6) caused significant increases in blood pressure, heart rate and renal nerve activity (Figure 6). The onset of these changes was immediate and was maximal at 10 min, reaching 7 ± 3 mmHg, 49 ± 11 beats min⁻¹ and 57 ± 17% respectively. Phrenic nerve activity was also significantly increased (75 ± 24%, 15 min), however the onset of this response was delayed, see Figure 6. Baseline values for blood pressure and heart rate were 96 ± 4 mmHg and 391 ± 11 beats min⁻¹. Higher doses, 40 and 120 nmol kg⁻¹, of 8-OH-DPAT produced small reductions in blood pressure and these were associated with dose-related tachycardia of 21 ± 5 and 38 ± 14 beats min⁻¹, respectively, after 10 min. These higher doses of 8-OH-DPAT did not significantly alter renal or phrenic nerve activity (data not illustrated). Baseline values were for blood pressure 122 ± 5 and 108 ± 8 mmHg and for heart rate 428 ± 10 and 411 ± 16 beats min⁻¹ respectively.

The effect of pretreatment with either methiothepin or spiroxatrine on the response to DP-5-CT

Methiothepin (1 mg kg⁻¹ i.v.; n = 4) caused a significant reduction in blood pressure of 32 ± 2 mmHg 5 min following injection. This was associated with an initial increase in renal nerve activity, reaching a maximum between 1 and 5 min of 64 ± 14% and then returning to near baseline levels after 20 min. Methiothepin did not cause any significant changes in heart rate or phrenic nerve activity. Baseline values for blood pressure and heart rate were 114 ± 5 mmHg and 424 ± 10 beats min⁻¹. The response produced by DP-5-CT (3 nmol kg⁻¹) administered i.c.v. 20 min after methiothepin was significantly attenuated (Figure 7).

The effect of DP-5-CT (3 nmol kg⁻¹, i.c.v.) on all variables was significantly attenuated in spiroxatrine (300 nmol kg⁻¹, i.c.v.; n = 6) pretreated animals compared to vehicle (0.01 N HCl i.c.v.; n = 6) pretreated animals, see Figure 7. Spiroxatrine pretreatment did not alter baseline values per se (blood pressure 109 ± 4 mmHg and heart rate 404 ± 8 beats min⁻¹). Vehicle pretreatment caused a significant rise in heart rate of 19 ± 4 beats min⁻¹ and in renal nerve activity of 28 ± 10%.

Baseline values for blood pressure and heart rate were 109 ± 4 mmHg and 404 ± 8 beats min⁻¹ respectively.

Discussion

In the present experiments in a-chloralose anaesthetized rats, treated with a neuromuscular blocking agent, i.c.v. injection of DP-5-CT, 5-CT, 8-OH-DPAT (low dose) and 5-HT [in
animals pretreated with the 5-HT\textsubscript{1A}/5-HT\textsubscript{1C} receptor antagonists cinanserin or LY 53857 (Rubin et al., 1984; Cohen et al., 1985; Doods et al., 1989; Hoyer, 1991), caused renal sympathoexcitation, tachycardia and a rise in blood pressure. As DP-5-CT is a highly selective agonist for 5-HT\textsubscript{1A} receptors (Middlemis & Fozard, 1983; Fozard et al., 1987) and 5-CT, a non-selective 5-HT\textsubscript{1} receptor agonist (Schoeffer & Hoyer, 1988; see Hoyer, 1991) also caused sympathoexcitation, tachycardia and a rise in blood pressure supporting the above conclusion. The observation that the rise in blood pressure, sympathoexcitation and tachycardia caused by 5-HT in the presence of a 5-HT\textsubscript{1A}/5-HT\textsubscript{1C} antagonist could be blocked by the addition of spiroxatrine, demonstrates that the sympathoexcitation caused by 5-HT is also due to activation of 5-HT\textsubscript{1A} receptors.

The above data indicating the activation of 5-HT\textsubscript{1A} receptors, reached by i.c.v. administration, causes sympathoexcitatory response contrast with the findings from previous studies using 8-OH-DPAT and other non-structurally related 5-HT\textsubscript{1A} agonists. These agonists given i.v. have been demonstrated to cause a centrally mediated decrease in blood pressure and sympathoinhibition in rats, cats, rabbits and dogs (see Ramage, 1990). However, in the rat there is some evidence that activation of 5-HT\textsubscript{1A} receptors can also cause a pressor response and/or sympathoexcitation. In conscious spontaneously hypertensive rats i.v. 8-OH-DPAT caused an initial tachycardia and rise in blood pressure which was then followed by a Bradycardy and hypotension (Fozard et al., 1987). 8-OH-DPAT (i.v.) in conscious and anaesthetized rats mediates the release of adrenaline by central sympathoexcitation of the adrenal glands (Chauvouet & Jemereand, 1987; Chauvouet et al., 1989a,b; Bagdy et al., 1989; Bouhelal & Mir, 1990). Microinjection of 5-HT\textsubscript{1A} agonists into the raphé ob-scurus causes a pressor response (Dreter et al., 1991). Furthermore, i.c.v. administration of low doses of 8-OH-DPAT in conscious rats also causes a pressor response which is attenuated by methiothepin (Dedeoglu & Fisher, 1991). These combined data, at the least in the rat, demonstrate that activation of 5-HT\textsubscript{1A} receptors causes sympathoexcitation as well sympathoinhibition. In the present study and that of Dedeoglu & Fisher (1991), higher doses of 8-OH-DPAT administered i.c.v. tended to cause falls in blood pressure. A possible explanation for this observation is that the sympathoexcitation is masked by the sympathoinhibitory action of 8-OH-DPAT. A high dose of 8-OH-DPAT when given i.c.v. may diffuse to the mid and hind brain of the rat where activation of 5-HT\textsubscript{1A} receptors is known to cause a fall in blood pressure and/or sympathoinhibition, in areas such as the dorsal raphé (Connor & Higgins, 1990), raphé magnus and pallidus (Valenta & Singer, 1990) and the rostral ventrolateral medulla (Nicholl & Guynet, 1991). However, it is possible that high doses of 8-OH-DPAT can have a selective action at receptors other than 5-HT\textsubscript{1A} receptors (see Dedeoglu & Fisher, 1991).

5-HT has previously been shown to regulate a number of neuroendocrine responses including the release of vasopressin in conscious rats (Steardo & Lovino, 1986; see Van de Kar, 1991). Furthermore, in anaesthetized rats the pressor response to 8-OH-DPAT is blocked by pretreatment with the vasopressin V\textsubscript{1} receptor antagonist d(CH\textsubscript{2})\textsubscript{7}Tyr(Me)AVP (Inoue & Buñag, 1989), although the associated bradycardy is only attenuated in the first 5 min. In the present study, pretreatment with d(CH\textsubscript{2})\textsubscript{7}Tyr(Me) AVP, at a dose which has previously been shown to abolish the pressor response to injected vasopressin (Buñag & Miyajima, 1984), only attenuated the duration of the rise in blood pressure caused by i.c.v. 5-HT. This phenomenon may be due to different anaesthetic used, in the present study α-chloralose was used while in that of Inoue & Buñag (1989) urethane was used. Interestingly, in the present study the bradycardy and sympathoinhibition caused by 5-HT were reversed to tachycardia and sympathoexcitation in the presence of d(CH\textsubscript{2})\textsubscript{7}Tyr(Me)AVP. A similar observation has been made in conscious rats (Pergola & Alper, 1991) and in that study the pressor response to i.c.v. 5-HT was completely blocked by combined α-1-adrenoceptor and vasopressin V\textsubscript{1} receptor blockade. In conscious rats in which the sinoaortic nerves had been cut (Pergola & Alper, 1991) i.c.v. 5-HT, although causing a pressor response, produced a marked tachycardia. Taken together these data indicate that i.c.v. 5-HT in conscious and anaesthetized rats causes the release of vasopressin. The peripherally mediated pressor response induces a baroreceptor mediated sympathoexcitation and bradycardy which masks the ability of 5-HT to cause sympathoexcitation and tachycardia through activation of 5-HT\textsubscript{1A} receptors. It is of interest that a low dose of 5-HT (4 nmol kg\textsuperscript{-1}) given i.c.v. caused a pressor response associated with a tachycardia in conscious rats, whereas a higher dose (120 nmol kg\textsuperscript{-1}) produced a pressor response and a biphasic effect on heart rate, bradycardy followed by tachycardia (Dedeoglu & Fisher, 1991; Anderson et al., 1992a). Presumably the low dose of 5-HT produced only the sympathoexcitatory component of the 5-HT response. Therefore the pattern of response caused by i.c.v. administration of 5-HT is dependent on the dose of 5-HT given, and in anaesthetized animals, the anaesthetic used.

The present data show that the release of vasopressin by i.c.v. 5-HT is mediated by 5-HT\textsubscript{2} or 5-HT\textsubscript{1C} receptors, as a similar effect is obtained by pretreatment with the 5-HT\textsubscript{1A}/5-HT\textsubscript{1C} receptor antagonists, cinanserin and LY 53857 (i.c.v.) to that with the vasopressin V\textsubscript{1} receptor antagonist. This conclusion is supported by other studies in conscious rats (Brownfield et al., 1988; Pergola & Alper, 1991; see Van de Kar, 1991).

The selective 5-HT\textsubscript{1A}/5-HT\textsubscript{1C} receptor agonist DOI given i.c.v. or i.v. caused a rise in blood pressure, bradycardy and sympathoexcitation. However, these effects were attenuated by the peripherally acting 5-HT\textsubscript{1A}/5-HT\textsubscript{1C} receptor antagonist BW501C67 (Mawson & Whittington, 1970; Fuller et al., 1986; BW501C67 has a pK\textsubscript{o} of 9.5 at 5-HT\textsubscript{1A} and a pK\textsubscript{o} of 8.5 at 5-HT\textsubscript{1C} receptors, G.R. Martin unpublished observations), whereas the response difference may be explained by the peripherally acting 5-HT\textsubscript{2} on these variables was essentially unaffected by i.v. BW501C67. Therefore, the effects of DOI on blood pressure, heart rate and renal nerve activity can be attributed to activation of peripheral 5-HT\textsubscript{2} receptors on vascular smooth muscle (Dabire et al., 1989; Alper, 1990) and not by activation of central 5-HT\textsubscript{1A} or 5-HT\textsubscript{1C} receptors to release vasopressin. In this context, DOI given i.v. has been shown not to cause the release of vasopressin (Bagdy et al., 1992; see Van de Kar, 1991). Interestingly, in the anaesthetized cat, activation of central 5-HT\textsubscript{1A} or 5-HT\textsubscript{1C} receptors has been shown to cause sympathoexcitation (McCull & Harris, 1988; Vayssettes-Courchay et al., 1991; Shepheard et al., 1991; Ramage et al., 1991). In the present study (Alper, 1990; Vayssettes-Courchay et al., 1990) there is no evidence for a centrally mediated increase in sympathetic tone in the rat. Therefore, it appears that the cat and rat are different in this respect.

In previous studies activation of 5-HT pathways has been demonstrated to increase central respiratory drive (Fuller et al., 1986a,b; Dreter et al., 1991). Furthermore, central administration of 8-OH-DPAT has been shown to increase respiratory rate (Gillis et al., 1989) and phrenic nerve activity (Sporton et al., 1991), a measure of central inspiratory drive. The present results also demonstrate that 8-OH-DPAT can cause an increase in central inspiratory drive. However, the
involvement of 5-HT₂ receptors in this action of 8-OH-DPAT is doubtful as both DP-5-CT and 5-CT failed to have any effect on phrenic nerve activity.

The precise site/sites in the brain where 5-HT is acting to cause these cardiovascular effects remain to be determined but the rapid onset of response would suggest a brain area close to the lateral or 3rd ventricles. Angiotensin II administered i.c.v. has been shown to cause a rise in blood pressure and bradycardia in conscious rats and this has been attributed to the release of vasopressin through activation of angiotensin receptors located in the subfornical organ (Iovino & Steardo, 1984; see Hartle & Brody, 1984). Therefore, it is possible that the release of vasopressin caused by 5-HT may occur following activation of 5-HT receptors located in the subfornical organ. Smits & Struyker-Boudier (1976) have demonstrated that microinjection of 5-HT into the anterior hypothalamus/pre-optic area can cause an increase in blood pressure. This area is situated close to the 3rd ventricle and 5-HT containing neurones have been shown to project to this site from the dorsal raphé nucleus (see Coote, 1990). Thus, 5-HT may be acting at the level of the hypothalamus to produce the cardiovascular response observed following i.c.v. injection. Further microinjection studies are required to determine the precise site of action of 5-HT.

In conclusion, the present study demonstrates that i.c.v. administration of 5-HT causes sympathoexcitation by activation of 5-HT₁A receptors and the release of vasopressin through activation of 5-HT₂ or 5-HT₃ receptors.

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References


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Evidence to suggest that activation of forebrain 5-HT₁ receptors causes sympathoexcitation in anaesthetized rats

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Lateral ventricular application of 5-HT causes a rise in blood pressure in the anaesthetized rat which may be due to activation of 5-HT receptors located in the anterior hypothalamus/preoptic region (see Coote, 1990). The nature of the 5-HT receptors which mediate this pressor effect were investigated.

In male Sprague-Dawley rats (250-375g) anaesthesia was induced with halothane and maintained with α-chloralose (80 mg kg⁻¹). Rats were artificially ventilated following neuromuscular blockade with decamethonium iodide (1 mg per animal). Simultaneous recordings were made of blood pressure (BP), heart rate (HR), renal (RNA) and phrenic nerve activity (PNA). 5-HT (n=6-8; 40, 120 nmol kg⁻¹), 5-CT (n=5; 3 nmol kg⁻¹), DP-5-CT (n=4-7; 0.3, 3 nmol kg⁻¹), 8-OH-DPAT (n=8-9; 40, 120 nmol kg⁻¹) and saline (n=6) were given by microinjection (5 μl over 20s) into the right lateral ventricle (i.c.v.). In separate experiments methiothepin (n=4; 1 mg kg⁻¹) was given i.v. 20 min before DP-5-CT.

5-HT, DP-5-CT and 5-CT caused rises in BP, HR and RNA of 24±3 mmHg, 63±13 beats min⁻¹, 126±33 % and 24±4 mmHg, 63±9 beats min⁻¹, 112±29 % for the highest dose of 5-HT and DP-5-CT respectively, and 14±4 mmHg, 42±4 beats min⁻¹, 142±43 % for 5-CT, 8-OH-DPAT 40 and 120 nmol kg⁻¹ only caused a rise in HR, of 28±4 and 50±13 beats min⁻¹ respectively. 5-HT differed from DP-5-CT and 5-CT in that the rise in blood pressure was associated with an initial decrease in HR and RNA. 8-OH-DPAT also differed in that it was the only compound to consistently increase PNA. Pretreatment with methiothepin attenuated the effects of DP-5-CT on all variables.

These results suggest that activation of 5-HT₁ receptors are responsible for the sympathoexcitation and pressor effects observed when 5-HT is injected into the lateral ventricle in anaesthetized rats.

I.K.A. is in receipt of an SERC CASE studentship with Dr. G.R. Martin, Wellcome Research Laboratories, Beckenham, U.K.

EVIDENCE TO SUGGEST THAT ACTIVATION OF CENTRAL 5-HT2/5-HT1C RECEPTORS CAUSES THE RELEASE OF VASOPRESSIN IN ANAESTHETIZED RATS

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In the anaesthetized rat, right lateral ventricular (i.c.v.) injection of 5-HT caused a rise in blood pressure which was associated with an initial bradycardia and sympathoinhibition followed by a tachycardia and sympathoexcitation. This latter phase was due to activation of forebrain 5-HT2 receptor receptors (Anderson, 1991). The present experiments were designed to investigate the nature of the 5-HT receptors mediating the initial phase of this 5-HT response.

In male Sprague-Dawley rats (250-375g) anaesthesia was induced with halothane and maintained with a-chloralose (80 mg kg⁻¹). Rats were artificially ventilated following neuromuscular blockade with decamethonium iodide (3 mg kg⁻¹). Simultaneous recordings were made of blood pressure, heart rate, and renal nerve activity (RNA). Drugs given i.c.v. were microinjected in a volume of 5 μl over 20s.

5-HT (120 nmol kg⁻¹; n=8) i.c.v. caused an initial rise in BP of 12±2 mmHg and falls in HR and RNA of 15±2 bpm and 40±8 % respectively, after 2 min. Neither the 5-HT2 receptor antagonist cinanserin (300 nmol kg⁻¹ i.c.v., n=6) nor the vasopressin V1 receptor antagonist, [8-mercapto-8-cyclopentamethylenelepropionyl, O-Me-Tyr⁴,Arg⁸]vasopressin (10 μg kg⁻¹ i.v., n=5), prevented the 5-HT induced rise in BP. However, they did reverse the initial bradycardia and renal sympathoinhibition to tachycardia and sympathoexcitation respectively. The 5-HT2/5-HT1C agonist DOI (1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane; 120 nmol kg⁻¹; n=6) given i.c.v. caused a rise in BP of 15±3 mmHg and falls in HR and RNA of 21±8 bpm and 53±7 % respectively, after 5min. This profile of action was similar to the initial phase of the 5-HT response. However, the action of DOI differed from that of 5-HT in that the DOI response (n=6) but not the 5-HT response (n=6) blocked by the peripherally acting 5-HT2 receptor antagonist BM501C67 (0.1 mg kg⁻¹ i.v.)

These results suggest that 5-HT can cause activation of forebrain 5-HT2/5-HT1C receptors to release vasopressin which produces a rise in systemic blood pressure and a baroreceptor reflex mediated bradycardia and sympathoinhibition. DOI did not appear to activate central 5-HT2/5-HT1C receptors to cause a release of vasopressin, but appeared to 'leak' out of the brain and activate 5-HT2 receptors on vascular smooth muscle to cause a rise in blood pressure.

I.K.A is in receipt of a SERC CASE Studentship.

In anaesthetized rats, the pressor effect of 5-HT i.c.v. is mediated by vasopressin release and sympathoexcitation due to activation of 5-HT\textsubscript{2/1C} and 5-HT\textsubscript{1A} receptors respectively (Anderson et al., 1992). In the present experiments the regional haemodynamic effects of i.c.v. 5-HT were examined in conscious Long-Evans and Brattleboro (i.e. vasopressin deficient) rats, chronically instrumented with a right lateral ventricular cannula, pulsed Doppler flow probes and an intra-arterial catheter. Surgery was carried out under sodium methohexitone anaesthesia (60 mg kg\textsuperscript{-1} i.p.) in 3 stages with at least 7 days between each stage (Gardiner et al., 1988). Each animal received an i.c.v. dose (5\mu l over 20s) of saline and of 5-HT (4, 40, 120 nmol kg\textsuperscript{-1}).

<table>
<thead>
<tr>
<th>Do\textsuperscript{e} (nmol kg\textsuperscript{-1})</th>
<th>Long-Evans (n = 8-11)</th>
<th>Brattleboro (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>6 ± 1**</td>
<td>15 ± 2**</td>
</tr>
<tr>
<td>40</td>
<td>13 ± 1**</td>
<td>19 ± 19</td>
</tr>
<tr>
<td>120</td>
<td>15 ± 2**</td>
<td>-4 ± 2</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>30 ± 16*</td>
<td>19 ± 19</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>1 ± 2</td>
<td>2 ± 6</td>
</tr>
<tr>
<td>Renal flow (%)</td>
<td>-8 ± 3**</td>
<td>-21 ± 3**</td>
</tr>
<tr>
<td>Mesenteric flow (%)</td>
<td>-19 ± 3**</td>
<td>-30 ± 5**</td>
</tr>
<tr>
<td>Hindquarters flow (%)</td>
<td>14 ± 7*</td>
<td>36 ± 8**</td>
</tr>
<tr>
<td>Renal conductance (%)</td>
<td>-5 ± 3</td>
<td>54 ± 4**</td>
</tr>
<tr>
<td>Mesenteric conductance (%)</td>
<td>-8 ± 2*</td>
<td>5 ± 6</td>
</tr>
<tr>
<td>Hindquarters conductance (%)</td>
<td>-38 ± 5**</td>
<td>-18 ± 4**</td>
</tr>
</tbody>
</table>

The pressor and mesenteric vasoconstrictor effects of 5-HT i.c.v. in Long-Evans rats are consistent with vasopressin release. This is supported by the absence of a pressor effect in Brattleboro rats. However, Brattleboro rats did show a mesenteric vasoconstrictor response to 5-HT i.c.v. consistent with sympathoexcitation; the absence of a pressor effect was, presumably, due to the concurrent marked hindquarters vasodilator response.

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In anaesthetized rats it has been demonstrated that the rise in blood pressure caused by i.c.v. 5-hydroxytryptamine (5-HT) is mediated by activation of 5-HT\(_{1A}\) receptors which cause sympathoexcitation and 5-HT\(_2\) and/or 5-HT\(_{1C}\) receptors which release vasopressin (Anderson et al., 1992). In anaesthetized cats i.c.v. 5-HT has also been reported to produce a rise in blood pressure (Coote et al., 1987). Hence, the present investigation was carried out to determine the nature of the 5-HT receptors mediating this pressor effect of i.c.v. 5-HT in anaesthetized cats.

Cats, anaesthetized with \(\alpha\)-chloralose \((70\text{ mg kg}^{-1})\) and pentobarbitone sodium \((6\text{ mg kg}^{-1})\), were artificially ventilated after neuromuscular blockade with vecuronium bromide \((200\text{ \mu g kg}^{-1})\). Simultaneous recordings were made of renal (RNA), splanchnic (SNA) and cardiac (CNA) nerve activities, blood pressure, heart rate and femoral arterial conductance (FAC). Cumulative doses of 5-HT \((10-160\text{ nmol kg}^{-1}; n = 5)\), 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI; 80-320 nmol kg\(^{-1}\); \(n = 4\)) and 5-carboxamidotryptamine (5-CT; 2.5-40 nmol kg\(^{-1}\); \(n = 4\)) were given by microinjection \((20\text{ \mu l over 1 min})\) into the lateral ventricle. Drugs were restricted to the lateral and third ventricles by cannulation of the Aqueduct of Sylvius. To prevent the activation of peripheral 5-HT\(_2\) receptors following i.c.v. administration of DOI, the peripherally acting 5-HT\(_2/5-HT_{1C}\) antagonist BW501C67 (Fuller et al., 1986; 1 mg kg\(^{-1}\)) was administered i.v. 15 min before DOI. In 3 separate animals, cinanserin \((265\text{ nmol kg}^{-1})\) was given i.c.v. 5 min before 5-HT.

5-HT, DOI and 5-CT caused maximum increases in mean BP of 16 ± 3, 22 ± 2 and 12 ± 4 mmHg, respectively associated with a tachycardia of 28 ± 5, 24 ± 5 and 18 ± 5 beats min\(^{-1}\) and a decrease in FAC of 11 ± 4, 28 ± 4 and 12 ± 4 ml mmHg\(^{-1}\) min\(^{-1}\) × 10\(^{-3}\), respectively. These changes were also associated with varying degrees of sympathoexcitation. 5-HT and DOI increased CNA \((94 ± 24\% \text{ and } 137 ± 33\%)\) and SNA \((16 ± 5\% \text{ and } 50 ± 15\%)\) without effect on RNA. 5-CT increased CNA by 99 ± 27\%, SNA by 57 ± 11\% and RNA by 42 ± 18\%. Pretreatment with cinanserin blocked the effects of 5-HT. Cinanserin alone caused a small reduction in blood pressure and heart rate associated with an increase in FAC and no change in sympathetic nerve activity.

These results demonstrate that activation of forebrain 5-HT\(_2\) and/or 5-HT\(_{1C}\) receptors causes sympathoexcitation. However, these results also suggest that 5-HT\(_1\) receptors are involved in this sympathoexcitatory pathway.

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FOREBRAIN 5-HT₁ RECEPTORS CAUSE SYMPATHOEXCITATION IN RATS. A. G. Ramage, I. K. Anderson, and G. R. Martin. Depts. Pharmacology, Royal Free Hospital School of Medicine, London, NW3 2PF and ∗Wellcome Laboratories, Kent, UK.

Lateral ventricular (i.c.v.) application of 5-HT cause a rise in blood pressure which is thought to be due to activation of 5-HT receptors located in the anterior hypothalamus/preoptic region. The nature of the receptors mediating this pressor effect were investigated.

In α-chloralose anaesthetized rats recordings were made of BP, HR, renal (RNA) and phrenic nerve activity (PNA). 5-HT (40, 120 nmol kg⁻¹), 5-CT (3 nmol kg⁻¹), DP-5-CT (0.3, 3 nmol kg⁻¹), 8-OH-DPAT (40, 120 nmol kg⁻¹) and saline were given by microinjection (5μl over 20s) i.c.v. 5-HT, DP-5-CT and 5-CT all caused rises in BP, HR and RNA of 24 ± 3 mmHg, 63 ± 13 beats min⁻¹, 126 ± 33 X and 24 ± 4 mmHg, 63 ± 9 beats min⁻¹, 112 ± 29 X for the highest dose of 5-HT and DP-5-CT respectively, and 14 ± 4 mmHg, 42 ± 4 beats min⁻¹, 142 ± 43 X for 5-CT. 8-OH-DPAT 40 and 120 nmol kg⁻¹ only caused a rise in HR of 28 ± 4 and 50 ± 13 beats min⁻¹ respectively. 5-HT differed from DP-5-CT and 5-CT in that the rise in blood pressure was associated with an initial decrease in HR and RNA. PNA was only increase by 8-OH-DPAT. Pretreatment with methiothepin attenuated the effects of DP-5-CT on all variables.

These results suggest that activation of 5-HT₁ receptors are responsible for the sympathoexcitation and pressor effects observed when 5-HT is injected i.c.v.