THE ROLE OF THE 5-HT_{1A} RECEPTOR IN CENTRAL CARDIOVASCULAR REGULATION

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DE ROL VAN DE 5-HT_{1A} RECEPTOR IN DE CENTRALE CARDIOVASCULAIRE REGULATIE

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CONTENTS

CHAPTER 1

GENERAL INTRODUCTION

1.1 Serotonin

The indole alkylamine serotonin (5-hydroxytryptamine; 5-HT) was isolated and chemically characterized about four decades ago, and is now generally accepted to function as a neurotransmitter and neuromodulatory agent. 5-HT is formed enzymatically from the essential amino acid tryptophan by hydroxylation, followed by decarboxylation (Fig. 1).

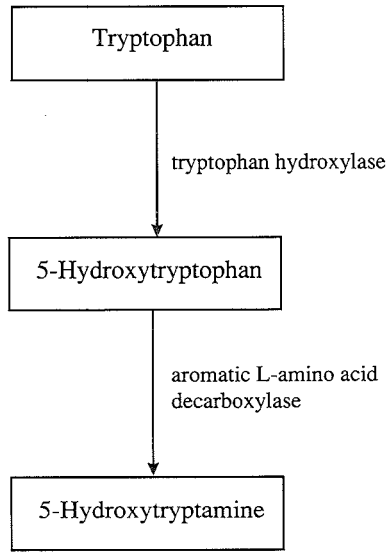


Fig. 1: Synthesis of 5-hydroxytryptamine.

1.1.1 Localisation of 5-HT

5-HT appears to be present in most plants and animals. In mammals, 5-HT is widely distributed and can be localized in nerve cells and fibers in specific brain areas (Dahlström & Fuxe, 1965; Steinbusch, 1981) as well as in several peripheral structures

like the enterochromaffin cells of the gastrointestinal mucosa, the pineal gland, blood platelets, nerves of the gastrointestinal tract and blood vessels, the blood vessel wall, in mast cells (rodents), and in lung, kidney, spleen, thyroid and heart tissue. With the exception of the platelets, 5-HT is synthesized in most tissues in which it is stored. Over 90% of the 5-HT content in the peripheral structures of the body is found in the enterochromaffin cells. From these cells 5-HT enters the circulation where it will be taken up by the platelets or removed by the liver or the endothelial cells in the lungs to be metabolized into 5-hydroxyindolacetic acid (5-HIAA) and excreted.

1.1.2 Function of 5-HT

5-HT exerts a variety of effects in the body. As the substance is not able to cross the blood brain barrier (Lexchin et al., 1977), there is an apparent discrimination between its peripheral and its central function. In the periphery 5-HT is involved in contraction of smooth muscle cells, aggregation of blood platelets and presynaptic depression of transmission in autonomic neurones. In the central nervous system 5-HT serves as a neurotransmitter and appears to play a role in the regulation of e.g. memory, appetite, anxiety, sleep, depression, body temperature, sexual behaviour and the cardiovascular system.

1.2 5-HT receptors

1.2.1 Classification

Receptor site analysis has been one of the most productive approaches to understand the different actions of 5-HT in the central and peripheral nervous systems. In 1957, Gaddum and Picarelli described two distinct 5-HT receptor subtypes i.e. the 'D' and 'M' receptor. In guinea pig ileum, 5-HT evokes a contractile response resulting from activation of two different mechanisms: a direct action on the smooth muscle and an indirect action mediated by the release of acetylcholine from parasympathetic nerve endings. Because the former of these mechanisms was blocked by dibenzylline (D) and the latter by morphine (M), the excitatory 5-HT receptors on smooth muscle and parasympathetic nerve endings were designated D- and M-receptors, respectively. Although in this study the antagonists used were not selective for 5-HT, the distinction made between these two types of 5-HT receptors is still valid.

Following the development and application of radioligand binding techniques the existence of more different 5-HT receptor types became apparent. In 1974, Bennett and Aghajanian first successfully applied these techniques to serotonin receptors using [^{3}H]LSD to label brain membrane sites. The second radioligand used to label 5-HT receptors was [^{3}H]5-HT (Bennett and Snyder, 1976; Fillion et al., 1978; Nelson et al., 1978). Further progress was made when [^{3}H]spiperone also appeared to label 5-HT recognition sites. About two decades after the characterization of the 'D' and 'M' receptors by Gaddum and Picarelli, Peroutka and Snyder (1979) reported differences between the binding characteristics of [^{3}H]5-HT, [^{3}H]LSD and [^{3}H]spiperone. Evidence was presented that [^{3}H]5-HT and [^{3}H]spiperone labelled distinct populations of 5-HT membrane recognition sites and that [^{3}H]LSD appeared to label both these sites. In this paper the authors suggested to designate the binding sites labelled by [^{3}H]5-HT as 5-HT_1 and the binding sites labelled by [^{3}H]spiperone as 5-HT_2 receptors.

The present state in the 5-HT receptor field is that there exist four main receptor populations, designated the 5-HT_1, 5-HT_2, 5-HT_3 and the recently discovered 5-HT_4 receptor. Initially, the distinction made between the 5-HT_1 and the 5-HT_2 receptor was purely based on radioligand binding studies using rather unselective drugs. Not until selective agonists and antagonists became available, could these 5-HT receptor sites be better characterized. In contrast with the 5-HT_1 and 5-HT_2 receptor types, the revelation of the 5-HT_3 and 5-HT_4 receptor types was at first based on functional receptor correlates. The development and discovery of selective drugs and, in the case of the 5-HT_3 receptor, radioligand binding studies have provided more information on these 5-HT receptor populations.

It became evident that the 5-HT_1 subdivision of the 5-HT receptor family was heterogeneous. Pedigo and co-workers (1981) discovered a high and low affinity site for spiperone, designated as 5-HT_{1A} and 5-HT_{1B} recognition sites, respectively. A third subtype, based on the high affinity displacement of [^{3}H]5-HT by the ergot derivative mesulergine, was identified in the choroid plexus of various animal species and designated as 5-HT_{1C} binding site (Pazos et al., 1985; Hoyer et al., 1985). Most recently, based on studies of the bovine caudate nucleus using [^{3}H]5-HT as radioligand in the presence of 8-OH-DPAT (5-HT_{1A}) and mesulergine (5-HT_{1C}), the existence of a 5-HT_{1D} recognition site has been reported (Heuring and Peroutka, 1987). Unfortunately, full characterization of the 5-HT_1 binding sites was hampered, since few selective and specific agonists and no specific antagonists were available. So Bradley and co-workers

(1986) suggested to designate this heterogeneous receptor group as '5-HT_1-like', representing a group of binding sites for which 5-carboxamidotryptamine (5-CT) and 5-HT show a high affinity and the effects of 5-HT cannot be blocked by 5-HT_2 or 5-HT_3 receptor antagonists. However, certain new drug tools, particularly agonists, showing high degrees of selectivity for subtypes of the '5-HT_1-like' recognition sites have recently become available (Middlemiss & Fozard, 1983; Wouters et al., 1988b). These drugs may help to unify a classification for the 5-HT_1 binding sites partly based on ligand binding studies and partly on functional responses. Sofar diverse subtypes (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} and 5-HT_{1D}) of the '5-HT_1-like' binding sites have been differentiated on the basis of the differing actions of various ligands like 8-OH-DPAT, cyanopindolol, mesulergine, etc (Table 1).

Table 1: Characteristics of 5-HT_1 binding sites

	5-HT_{1A}	5-HT_{1B}	5-HT_{1C}	5-HT_{1D}
Radioligands	[^{3}H]5-HT [^{3}H]8-OH-DPAT	[^{3}H]5-HT [^{125}I]Cyanopindolol	[^{3}H]5-HT [^{3}H]Mesulergine	[^{3}H]5-HT
High density	Raphe nuclei Hippocampus	Substantia nigra Globus pallidus	Choroid plexus	Caudate nucleus Basal Ganglia Substantia nigra
Agonists	5-CT 8-OH-DPAT Flesinoxan	5-CT RU24969	5-CT* RU24969*	5-CT RU24969
Putative antagonists	Spiperone Methiothepin Cyanopindolol Pindolol Spiroxatrine 8-MeO-ClEPAT	Methiothepin Cyanopindolol Pindolol Metergoline	Mesulergine Methiothepin Ketanserin Mianserin Metergoline	Methiothepin Metergoline
Second messenger system	cAMP	cAMP	PI	cAMP

*, low affinity.

It appeared that in isolated peripheral tissues functional responses can be induced that are mediated through the activation of specific 5-HT receptors, but are not mediated via the activation of either 5-HT_2 or 5-HT_3 receptors or the 5-HT_1 receptor subtypes that have been characterized so far. Two additional 5-HT_1-like receptor subtypes have been described (Saxena & Villalòn, 1990). Although these two receptor subtypes are still unnamed, they will be defined here (in accordance with Saxena & Villalòn, 1990) as the 5-HT_{1x} and 5-HT_{1y} receptor. The responses of the 5-HT_{1x} receptor can be characterized by the potent 5-HT_1 receptor agonist 5-CT but also the recently developed compound sumatriptan (GR 43175; Humphrey et al., 1988), 8-OH-DPAT and RU 24969 and the antagonist methiothepin. Compounds such as ipsapirone, cyanopindolol, mesulergine and metergoline seem to be inactive at this receptor type as either agonists or antagonists. The 5-HT_{1y} receptor can be characterized by the 5-HT_1 receptor agonist 5-CT and the unselective 5-HT receptor antagonists methiothepin and methysergide (Saxena & Villalòn, 1990). So these two receptor types bear certain similarities to the 5-HT_1 receptor sites but are not identical to any of the known subgroups of these sites (Heuring and Peroutka, 1987). However, receptor classification will always be dependent on the availability of selective and specific agonists and antagonists. So the discovery of selective 5-HT_1 receptor antagonists in the future may result in the adaptation of the '5-HT_1-like' classification as it stands now.

The availability of potent relatively selective 5-HT_2 receptor antagonists e.g. ketanserin (Leysen et al., 1981), cinanserin and mianserin (Peroutka and Snyder, 1981) made it possible to clearly characterize the 5-HT_2 binding site (Table 2). Subsequent studies using a variety of 5-HT receptor antagonists have provided evidence that the 5-HT_2 binding site correlates well with the 'D' site of Gaddum & Picarelli (Humphrey et al., 1982., Engel et al., 1984., Engel et al., 1985).

The 'M' receptor, described by Gaddum & Picarelli does not fall into either the 5-HT_1-like or the 5-HT_2 category, since this site can not be blocked by methiothepin or ketanserin (Bradley et al., 1986). This third type of 5-HT receptor has been designated the 5-HT_3 receptor (Table 2) (Bradley et al., 1986). With the use of selective antagonists such as cocaine, MDL 72222 and ICS 205-930 (Fozard et al., 1979; Fozard, 1984; Richardson et al., 1985) the 5-HT_3 binding site was identified in a variety of locations on peripheral neurones (Fozard, 1984). Recently, radioligand binding studies have revealed the presence of 5-HT_3 receptors also in brain tissue (Kilpatrick et al., 1987). Based on

extensive studies using selective antagonists the 5-HT_2 and 5-HT_3 receptors have well been characterized (Table 2).

Only very recently, a novel 5-HT receptor subtype has been identified, i.e. the 5-HT_4 receptor (Clarke, 1989). Full characterization of this 5-HT receptor is on its way. This site was first discovered in guinea-pig hippocampal membranes (Shenker et al., 1985; 1987) but has now also been identified in other tissues (Table 2). The 5-HT_4 receptor shows a relatively low potency for 5-HT (Dumuis et al., 1989). Dumuis et al. (1989) established the pharmacology of this receptor subtype describing antagonistic effects of high doses of ICS 205-930 and agonistic effects of several gastrointestinal prokinetic benzamide derivatives (Table 2).

Table 2: Characteristics of 5-HT_2, 5-HT_3 and 5-HT_4 receptors.

	5-HT_2	5-HT_3	5-HT_4
Radioligands	[^{3}H]Ketanserin [^{3}H]Spiperone [^{3}H]Mianserin	[^{3}H]ICS 205-930 [^{3}H]Phenylbiguanide [^{3}H]Quipazine [^{3}H]GR67330	
High density	Brain Cortex Platelets Vascular smooth muscle	Peripheral neurons Striatum Guinea pig ileum	Guinea-pig hippocampal neurons Mouse embryo colliculi neurons Guinea-pig ileum Pig heart
Agonists	DOI	Phenylbiguanide 2-Methyl-5-HT	5-HT 5-CH_3O-T α-CH_3-5-HT Renzapride
Antagonists	Ketanserin Ritanserin	ICS 205-930 MDL 72222 Zacopride	ICS 205-930
Second messenger system	PI	Direct gating of cation channel	cAMP

The development in the 5-HT receptor field seems to be a continuing story as demonstrated by the recent discovery of the 5-HT_4 receptor, which is now accepted as yet another 5-HT receptor subtype. The ongoing development of drugs with different pharmacological properties may lead to the extension of the present 5-HT receptor family.

1.2.2 Functional responses to 5-HT receptor activation

The multiplicity of 5-HT binding sites poses the question as to whether these sites really correspond to receptors, i.e. whether each subtype is coupled to an effector system. During the last years, progress has been made to define the second messenger systems that are linked to the putative 5-HT receptors (Table 1 and 2).

Except for the 5-HT_{1C} receptor, the 5-HT_1 receptor subtypes have been coupled to the modulation of adenylate cyclase. The characterization of functional responses mediated by the 5-HT_{1A} receptor is mainly based on the properties of the specific agonist 8-OH-DPAT. A more recently developed specific 5-HT_{1A} receptor agonist is flesinoxan. Until now, the 5-HT_{1A} receptor has only been identified in the CNS, mediating a variety of responses (Table 3). There is substantial evidence that 5-HT_{1A} receptors can be involved in central cardiovascular regulation, neurogenic, behavioural and metabolic and endocrine mechanisms (Table 3).

In contrast with the 5-HT_{1A} receptor subtype, no specific agonists or antagonists have been defined for the 5-HT_{1B}, 5-HT_{1C} or the 5-HT_{1D} receptor types. So the characterization of functional responses, mediated by these receptor types, has mainly been carried out by the exclusion of other receptor mechanisms. Activation of the 5-HT_{1B} receptor, which appears to be entirely confined to rat and mouse, has been identified to mediate e.g. inhibition of sympathetic neurotransmission in rat vena cava (Molderings et al., 1987), enhancement of neurogenically mediated contraction in mouse bladder (Holt et al., 1986) and inhibition of forskolin-stimulated adenylate cyclase in rat substantia nigra (Bouhelal et al., 1988). Furthermore, the 5-HT terminal autoreceptor in rat cortex has been characterized as being a 5-HT_{1B} receptor (Engel et al., 1986). In the brain, the 5-HT_{1D} receptor has been identified in those species where little or no 5-HT_{1B} binding could be demonstrated like calf, pig, guinea-pig and human tissue. Although pharmacologically distinct, 5-HT_{1B} and 5-HT_{1D} receptors show some similarity and they may be equivalent in the different species.

Table 3: Functional responses to 5-HT_{1A} receptor activation.

Functional response	Species/Preparation	References
Hypotension by a central action	Rat, Cat, Dog,	Fozard et al., 1987; Wouters et al., 1988; Di Francesco et al.,1988; Dreteler et al., 1990
Stimulation of adenylate cyclase	Rat hippocampus	Markstein et al., 1986
Inhibition of adenylate cyclase	Rat hippocampus	Schoeffter & Hoyer., 1988; Bockaert et al., 1987;
	guinea-pig hippocampus	De Vivo & Maayani, 1986
Inhibition of rhythmical slow activity	Rat hippocampus	Hirose et al., 1990
Hyperpolarisation	Rat hippocampal neurones	Colino & Halliwell, 1986
	Rat superior cervical ganglion	Gilbert & Newberry, 1987
Inhibition of 5-HT release	Rat somatodendritic terminals	Gozlan et al., 1983
Inhibition of cell firing	Rat dorsal raphe neurons	Sprouse & Aghajanian, 1986; Basse-Tomusk & Rebec, 1986
Hyperglycaemia/hypoinsulinemia	Rat	Chaouloff & Jeanrenaud, 1987
Stimulation of ACTH secretion	Rat	Gilbert et al., 1988
Stimulation of corticosterone secretion	Rat	Przegalinski et al., 1989, Haleem et al., 1989
Stimulation of adrenaline-release	Rat	Chaouloff et al., 1990
Reduction of bodytemperature	Rat	Hjorth, 1985
Induction of the 5-HT syndrome	Rat	Goodwin & Green,1985; Tricklebank et al., 1985; Molewijk et al., 1989
Anxiolytic effects	Rat, man	Traber & Glaser, 1987; Peroutka, 1985

Like the 5-HT_{1B} receptor, the 5-HT_{1D} receptor in substantia nigra (calf) is negatively coupled to adenylate cyclase (Schoeffter et al., 1988). In addition, the 5-HT terminal autoreceptor in cortex of pig, guinea-pig and human appears to be of the 5-HT_{1D} receptor subtype (Schlicker et al., 1989; Schipper & Tulp, 1988; Galzin et al., 1988). In cats, the 5-HT_{1D} site has been implicated to be involved in RU 24969 induced emesis (Lucot, 1990). Very recently, the 5-HT_{1D} receptor has also been localized peripherally in pig, subserving endothelium-dependent relaxation of coronary arteries (Schoeffter & Hoyer, 1990). The 5-HT_{1C} receptor, although differently distributed, shows remarkable similarities with the 5-HT_2 receptor, regarding molecular, pharmacological and biochemical properties (Schmidt & Peroutka, 1989). As a consequence, it is difficult to identify contributions of the 5-HT_{1C} receptor subtype to functional responses. Until now, the 5-HT_{1C} has been identified in rat, pig and human brain membranes (Pazos et al., 1985; Yagaloff & Hartig, 1985; Hoyer et al., 1986). Like the 5-HT_2 receptor, the 5-HT_{1C} receptor has been linked to phosphoinositide turnover (Conn et al., 1986).
Second messenger systems have not yet been defined for the 5-HT_{1x} and the 5-HT_{1y} receptor subtypes. The 5-HT_{1x} receptor appears to be present on certain cephalic arteries (basilar, pial), on rabbit and dog saphenous vein and on arteriovenous anastomoses in the carotid region. Activation of the 5-HT_{1x} receptor results in constriction of these vessels (Bom et al., 1989a,b; Connor et al., 1989a; van Heuven-Nolsen et al., in press; Perren et al., 1991). Furthermore, 5-HT_{1x} receptors are likely to be involved in the 5-HT induced inhibition of noradrenaline release in response to transmural stimulation of postganglionic sympathetic neurons (Göthert et al., 1990). The 5-HT_{1y} receptor is involved in 5-HT induced smooth muscle relaxation in several vessels e.g. cat isolated saphenous vein (Feniuk et al., 1983) and pig arterioles (Saxena & Verdouw, 1985). The hypotensive action of 5-CT has been described to be mediated via a 5-HT_1-like receptor (Connor et al., 1986) that corresponds to the 5-HT_{1y} receptor (Saxena & Villalòn, 1990). The 5-HT_1-like receptor that mediates tachycardia in the cat (Saxena et al., 1985, Saxena, 1988) also seems to be related to the 5-HT_{1y} receptor.

The 5-HT_2 receptor has been linked to the modulation of phosphoinositide (PI) turnover (De Chaffoy de Courcelles et al., 1985; Conn & Sanders-Bush, 1986). The 5-HT_2 receptor exists both in the periphery and the CNS (Table 1) and functional relevance of this class of receptors has now been established in a variety of preparations. Peripherally, the 5-HT_2 receptor has been identified to be involved in vascular smooth muscle contraction in e.g. canine basilar arteries (Müller-Schweinitzer & Engel, 1983)

and human coronary arteries (Connor et al., 1989b); contraction of uterine, bronchial, gastro-intestinal and bladder smooth muscle; platelet aggregation (De Clerck et al., 1984) and increased capillary permeability (Cohen et al., 1990). In the CNS 5-HT_2 receptors were identified being responsible for 5-HT induced excitation of rat brainstem neurons (Davies et al., 1988) and 5-HT induced depolarization of guinea-pig cortical pyramidal neurons (Davies et al., 1987). Some behavioural responses in rodents such as head twitch and wet-dog shakes are mediated via 5-HT_2 receptors (Lucki et al., 1984). Recent studies also indicate an important role for 5-HT_2 receptors in hallucinogenic drug action (Glennon, 1990; McKenna & Saavedra, 1987). Furthermore, 5-HT_2 receptors seem to be involved in some neuroendocrine functions like β-endorphin and corticosterone secretion in rats (Koenig et al., 1987) and prolactin release in rhesus monkeys (Heninger et al., 1987).

In contrast with the other 5-HT receptor families the 5-HT_3 receptors are directly coupled to an cation channel (Derkach et al., 1989). Functional 5-HT_3 receptors have been identified in the peripheral nervous system and (only recently) in the CNS. In the mammalian peripheral nervous system, 5-HT is known to excite a variety of neurons via 5-HT_3 receptors, mediating the release of neurotransmitters such as acetylcholine from parasympathetic neurons in e.g. the rabbit heart (Fozard, 1984) and cat urinary bladder (Saxena et al., 1985), noradrenaline from sympathetic neurons in e.g. the rabbit heart (Fozard & Mwalko, 1976) and the superior cervical ganglion (Wallis & North, 1978) and substance P from enteric neurons in e.g. the guinea pig ileum (Bradley et al., 1986). 5-HT has also been shown to stimulate sensory nerves via 5-HT_3 receptors. 5-HT_3 receptors mediate e.g. 5-HT induced pain sensation in human skin, the cardiovascular von Bezold Jarisch reflex and depolarization of C-fibers in nodose ganglion and vagus nerve (Richardson et al., 1985). Other actions involving 5-HT_3 receptors in the CNS are anxiogenic effects in mice, rats and marmosets (Tyers et al., 1987) and cytotoxic drug-induced emesis (Higgins et al., 1989).

Within the central nervous system, the 5-HT_4 receptor has been positively linked to adenylate cyclase (Dumuis et al., 1989). Peripherally, the 5-HT_4 receptor has been identified in the guinea pig ileum, where stimulation of this receptor site results in activation of cholinergically mediated contractions (Clarke et al., 1989). In addition, Villalòn and colleagues (1990; 1991) suggested that the 5-HT induced tachycardia in the pig might be mediated by the 5-HT_4 receptor.

1.3 Overall regulation of the circulation

The crucial mechanisms in general cardiovascular regulation are those that adjust total peripheral resistance and cardiac output in such a way as to maintain the blood pressure gradient required for adequate flow through the vascular system. The basic mechanisms that influence the total peripheral resistance and cardiac output include readjustments in body fluid volumes and neural or humoral (the renin- angiotensin-aldosterone system) control of vasomotor tone. In this thesis, the effects of certain pharmacological manipulations of the cardiovascular neural control system will be investigated.

1.3.1 Nervous control of the circulation

Both total peripheral resistance and cardiac output are under the control of the autonomic nervous system. The autonomic nervous system consists of two major divisions, the parasympathetic and the sympathetic system (Kuntz, 1953). Nerve trunks of both divisions contain afferent as well as efferent fibers. The latter originate from the vasomotor centres in the medulla oblongata. These centres are subject to complex excitatory and inhibitory influences from the hypothalamus and higher brain structures as well as and from afferent nerve fibers of mechanoreceptors.

Preganglionic nerve fibers emerge from the brainstem or spinal cord and relay or synapse in ganglia (Fig. 2). The ganglia contain cell bodies of postganglionic fibers which innervate the target organs. Activation of autonomic fibers results in the release of chemical substances from their terminals. These substances may then activate or inhibit target cells or another nerve fiber.

From the medullary vasomotor centers both sympathetic and parasympathetic nerve fibers project to the heart, whereas the blood vessels are predominantly supplied by sympathetic nerve fibers.

Within the circulation numerous sensors exist which detect changes in the cardiovascular system and initiate compensatory reflex adjustments. Reflexes within the cardiovascular system are the baroreceptor reflex, cardiopulmonary reflex, chemoreflexes e.g the Bezold-Jarish reflex and spinal reflexes. Activation of each of these reflexes always results in changes in sympathetic and/or parasympathetic outflow in order to modify cardiovascular function. In Fig. 3, an overall picture of the nervous control of cardiovascular function is shown.

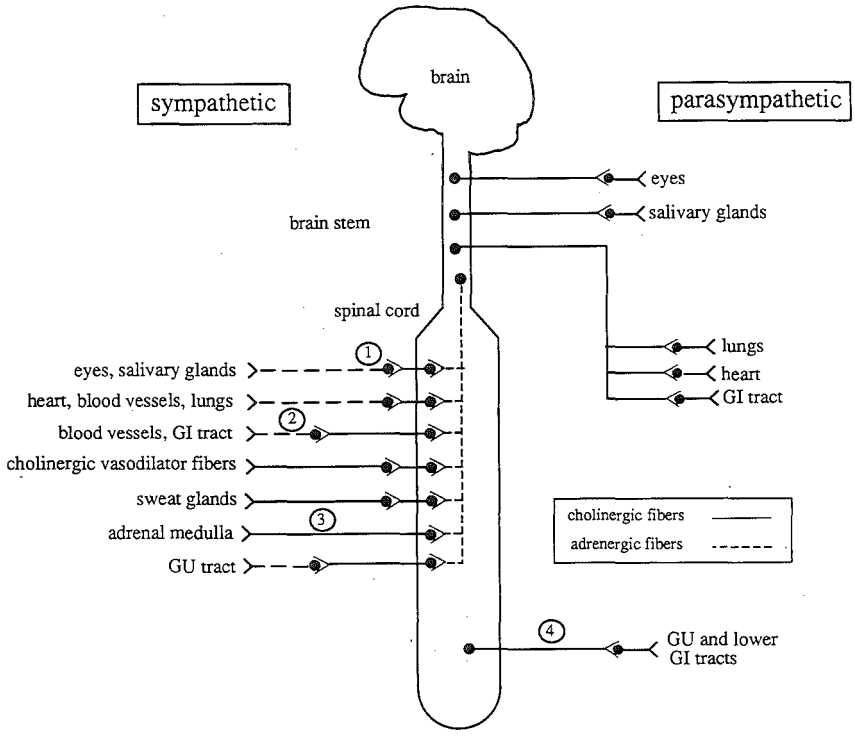


Fig. 2: Schematic representation of autonomic outflow. Roman numerals: cranial nerves. 1: synapses in ganglia of the paravertebral sympathetic chain, 2: synapses in more distal ganglia, e.g., celiac, superior and ingerior mesenteric, 3: preganglionic fibers in the splanchnic nerve, 4: sacral parasympathetic outflow. G.I. tract: gastro-intestinal tract, G.U. tract: uro-genital tract.

The neural regulation of vasomotor tone and heart function can be manipulated by drugs at different levels within the autonomic nervous system: 1. Direct action on higher brain areas and the medullary vasomotor centers. 2. Alteration of neural transmission within the ganglia. 3. Change of baroreceptor sensitivity. 4. Modulation of availability of noradrenaline at adrenergic nerve terminals by interference with synthesis, storage or

release into the synaptic cleft. 5. Influencing postjunctional receptors located on vascular smooth muscle cells (α_1 and α_2) and heart cells (β_1 and β_2).

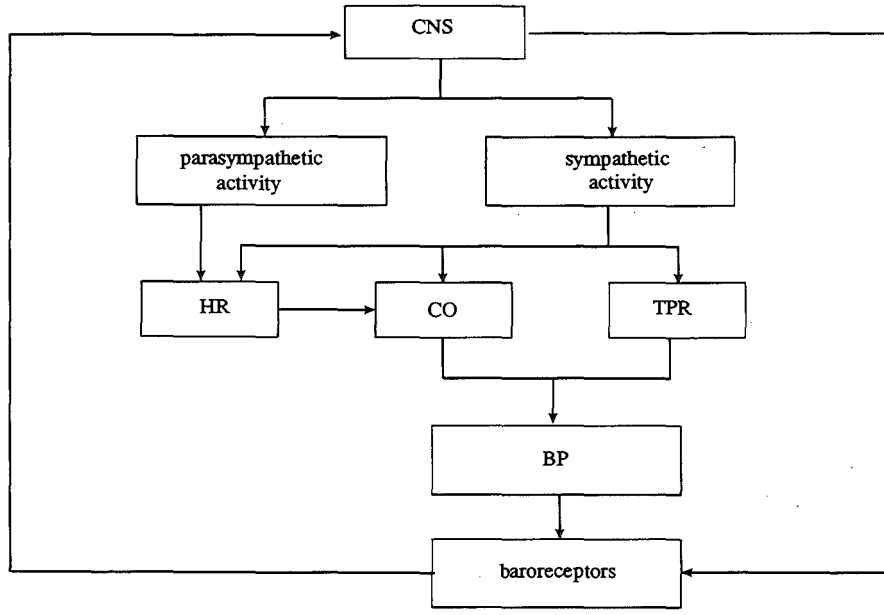


Fig. 3: Schematic presentation of the nervous control of the cardiovascular regulation. CNS = central nervous system, HR = heart rate, CO = cardiac output, TPR = total peripheral resistance, BP = blood pressure.

1.4 5-HT and cardiovascular regulation

Since 5-HT acts on different receptor subtypes, the effects of 5-HT on the cardiovascular system are, not surprisingly, complex and very variable. As mentioned in section 1.1.2, 5-HT does not readily cross the blood-brain barrier. Hence, a distinction was made between the peripheral and central cardiovascular effects of 5-HT.

1.4.1 5-HT and peripheral cardiovascular regulation

5-HT exerts marked effects throughout the cardiovascular system with diverse effects being observed in cardiac tissue, blood vessels and platelets. The diverse effects of 5-HT may be attributed to both direct and indirect effects and its interaction with multiple

receptors. In cardiac tissue and blood vessels 5-HT may exert opposing effects; e.g. both increases and decreases in heart rate and both contraction and relaxation of blood vessels (see Saxena & Villalòn, 1990) have been demonstrated. These observations point to mediation of the effects by different receptors. On the other hand, for some responses, multiple receptors may mediate the same effect.

Intravenous administration of 5-HT can induce a transient dose-dependent bradycardia. This reflex response, called the von Bezold Jarisch reflex, is due to activation of 5-HT_3 receptors located on afferent nerves in cardiac muscle, since it can be antagonized by potent 5-HT_3 receptor antagonists like MDL 72222 (Fozard, 1984), ICS 205-930 (Richardson et al., 1985), GR 38032F (Butler et al., 1988) and Zacopride (Smith et al., 1988). If the von Bezold Jarisch reflex is abolished, intravenous administration of 5-HT results in a tachycardia. The mechanism of 5-HT induced tachycardia depends on the species and preparation utilized. Acting directly on the myocardium, 5-HT induces tachycardia in the cat via 5-HT_1-like receptors (Saxena et al., 1985), in the rat (Saxena & Lawang, 1985) and dog (Feniuk et al., 1981) via 5-HT_2 and in the rabbit via 5-HT_3 receptors (Fozard, 1984).

The influence of 5-HT on blood vessels is also complex. In intact animals, an intravenous injection of 5-HT evokes a triphasic response, consisting of an initial transient depressor response, which is followed by a pressor response and then a third hypotensive phase (Salmoiraghi et al., 1956; Dalton et al., 1986). The first phase reflects the von Bezold Jarisch reflex mediated by the 5-HT_3 receptor subtype. In the subsequent pressor response, the 5-HT_2 receptor plays the key role and the hypotensive response in the third phase is initiated by excitation of vascular 5-HT_1 receptors (Saxena & Lawang, 1985).

In most blood vessels 5-HT induces contractions which are mediated by 5-HT_2 receptors. However, in some blood vessels such as the coronary and cerebral arteries multiple receptor subtypes (5-HT_1 and 5HT_2) may be responsible for the contractile response to 5-HT (Houston & Vanhoutte, 1986). In addition, 5-HT can induce endothelium dependent relaxation of certain blood vessels (Cocks & Angus, 1983; Cohen et al., 1983), which is probably mediated by 5-HT_1-like receptors, since this phenomenon is not sensitive to the 5-HT_2 and 5-HT_3 receptor antagonists ketanserin and MDL 72222, but is antagonized by methiothepin, methysergide or metergoline (Cocks & Angus, 1983; Cohen et al., 1983; Houston & Vanhoutte, 1988).

1.4.2 5-HT and central cardiovascular regulation

A large amount of evidence has accumulated to indicate that modulation of central 5-HT neurones affects the regulation of sympathetic nerve discharge and, therefore, blood pressure. Fluorescence histochemical and immunohistochemical studies have shown that 5-HT containing neurones, originating in the brainstem, project to a number of forebrain structures as well as to specific regions of midbrain, hindbrain and the intermediolateral cell column (Dahlstrom & Fuxe, 1965; Steinbusch, 1981; Loewy & Neil, 1981), which are involved in cardiovascular function.

Since 5-HT does not readily cross the blood brain barrier, either the precursor 5-hydroxytryptophan (5-HTP) must be administered peripherally in order for 'de novo' synthesis to occur centrally or 5-HT must be given directly into the brain. However, the results of these studies are often confusing. Systemically administered 5-HTP is converted to 5-HT both peripherally and centrally, which hampers interpretation of the mechanisms underlying the cardiovascular effects. Also, centrally administered 5-HT and its precursors have complex effects on cardiovascular function. Increases, decreases and biphasic effects on autonomic outflow, blood pressure and heart rate depending on the doses used, sites of injection and species, have been reported (Kuhn et al., 1980a) (Table 4).

In rats, administration of 5-HT directly into the brain via the ventricles, causes mainly pressor responses (Table 4). However, it is difficult to determine if these pressor effects are site specific, since the drug will be widely distributed throughout the brain after intracerebroventricular (i.c.v.) injection. The potential sites for the CNS mediated cardiovascular effects of 5-HT were enlighted by confining the application of 5-HT to more specific areas in the brain. In this respect, forebrain structures seem to play an important role in the mediation of the 5-HT induced pressor effect. In the cat, the prevention of 5-HT entering the 4th ventricle after i.c.v. injection, thus limiting access of the drug to the 3rd and lateral ventricle, abolished the depressor effects and even caused increases in sympathetic activity (Coote et al., 1987). Administration of 5-HT into the lateral, 3rd and 4th ventricle of the rat produced pressor responses, that were largest following administration into the 3rd ventricle. When distribution of 5-HT was confined to the 4th ventricle (no reflux of drug into the 3rd ventricle), no response at all to 5-HT was observed (Lambert et al., 1978).

Table 4: The effects of centrally administered 5-HT on blood pressure, heart rate and (if determined) sympathetic nerve activity. v: ventricle, i.c.v.: intracerebroventricular, i.c.: intracisternal, ↑: increase, ↓: decrease, ø: no effect, a: anaesthetized, c: conscious.

Treatment	Dose mg kg^{-1}	Injection route	Species	BP	HR	SA	References
5-HT	1-10	i.c.v.	dog (a)	↓	↓	↓	Bhargava & Tangri, 1959; McCubbin et al., 1960; Dhawan et al., 1967
5-HT	1.10^{-2}-1	i.c.v.	cat (a)	↓	↓	↓	Baum & Shropshire, 1975
5-HT	5.10^{-5}-25.10^{-3}	i.c.v.	rat (a)	↑	↑↓		Lambert et al., 1975
5-HT	1.10^{-6}-25.10^{-3}	3rd v., i.c.v. 4th v.	rat (a)	↑ ø	↓↑ ø		Lambert et al., 1978
5-HT	15.10^{-4}-3.10^{-3}	i.c.v. 4th v.	rat (a)	↑,↑↓	↓,↑		Krstic & Djurcovic, 1980
5-HT	5.10^{-3}-15.10^{-3}	i.c.	rat (a)	↓,↑↓	↓		Krstic & Djurcovic, 1980
5-HT	25.10^{-3}-1.10^{-2}	i.c.v.	rat (a)	↑	↓↑		Sukamoto et al., 1984
5-HT	1.10^{-3}-1.10^{-2}	i.c.v.	rat (c)	↑	↓		Sukamoto et al., 1984
5-HT	1.10^{-3}-3.10^{-2}	i.c.v.	rat (c)	↑↓	↓		Dalton, 1986
5-HT	3.10^{-3}-1.10^{-2}	i.c.v.	cat (a)	↑	↑	↑	Coote et al., 1987
5-HT	3.10^{-2}-1.10^{-1}	i.c.v.	cat (a)	↓	↓	↓	Coote et al., 1987
5-HT	1.10^{-2}-3.10^{-2}	4th v.	cat (a)	↓	↓	↓	Coote et al., 1987

Also in the rat, the preoptic-anterior hypothalamus (AH/PO), which accomodates numerous 5-HT containing terminals, has been proven to be of importance in mediating the 5-HT induced pressor response. Direct application of 5-HT into this nucleus yielded pressor responses, that could be abolished by i.c.v. administration of methysergide (Smits & Struyker-Boudier, 1976). Since the AH/PO nucleus is localized closely to the 3rd ventricle, the pressor response following i.c.v. administration of 5-HT is most likely to be explained by excitation of the vasomotor neurones in this nucleus. The main input of 5-HT neurons to the AH/PO nucleus extends from the dorsal and median raphe nuclei in the midbrain (Dahlström & Fuxe, 1965). Electrical stimulation of these raphe nuclei causes pressor responses (Smits et al., 1978), which for the dorsal raphe can be abolished by selective lesions of 5-HT-containing neurons in this nucleus by the

neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) (Robinson et al., 1985). In addition, intravenous administration of the 5-HT uptake inhibitor fluoxetine enhances the pressor response to dorsal raphe stimulation and administration of the 5-HT antagonist 2-bromolysergic diethylamide into the AH/PO attenuates the pressor response (Robinson et al., 1985). An increase in sympathetic outflow mediates part of the pressor response to dorsal raphe stimulation, since pretreatment with bretylium tosylate, which prevents the release of norepinephrine from sympathetic postganglionic nerves, attenuates it (Kuhn et al., 1980b; Robinson et al., 1985). Lesions of 5-HT neurons in the dorsal raphe but not the median raphe nucleus enlarged the pressor response to intrahypothalamic application of 5-HT. This phenomenon could be due to the development of supersensitivity of 5-HT receptors in the AH/PO or to the degeneration of nerve terminals, leading to a decreased reuptake of 5-HT and, hence, a reduced rate of termination of the neurotransmitter (Robertson et al., 1985). These observations indicate that activation of ascending 5-HT neurons from the dorsal raphe nucleus produces an increase in sympathetic activity leading to an enhanced blood pressure, which is mediated at least in part by the AH/PO.

In cats and dogs and also in rats, i.c.v. administration of 5-HT induces depressor responses (Table 4). In the cat and dog, this decrease in blood pressure probably results from the inhibition of sympathetic nerve outflow (McCubbin et al., 1960; Baum & Shropshire, 1975; Coote et al., 1987). The site of this sympatho-inhibitory effect of 5-HT is likely to be located in the brainstem or spinal cord (Coote et al., 1987), since administration of 5-HT into the 4th ventricle only produced decreases in blood pressure, heart rate and sympathetic nerve activity. Furthermore, Coote and his colleagues (1987) showed that if 5-HT was applied onto the region of the obex and nucleus tractus solitarius (NTS), of the cat, immediate reductions in blood pressure, heart rate and sympathetic nerve activity were the consequence. In the rat, however, various blood pressure responses to 5-HT injections in the NTS have been reported (Wolf et al., 1981; Laguzzi et al., 1984).

In the nucleus ambiguus of the cat, boutons containing 5-HT-like immunoreactivity have been shown to make synaptic contact with cardiac vagal motoneurons. Furthermore, the nucleus ambiguus and the dorsal motor vagal nucleus of the rat are innervated by 5-HT-like immunoreactive fibres (Steinbusch, 1981) and also contain cardiac vagal motoneurones. Microinjection of 5-HT into these areas produces a bradycardia (Izzo et al., 1988; Sporton et al., 1989). These studies show that serotonergic pathways are involved in the control of cardiac vagal motoneurons.

There is now considerable evidence that the ventrolateral medulla (VLM), which projects directly to the intermediolateral cell column (IML) of the spinal cord, the main site of origin of the sympathetic preganglionic neurons (Loewy & Neil, 1981), is densely innervated with 5-HT neurones (Steinbusch, 1981). Lovick (1988) has shown that microinjection of 5-HT into a specific area in the VLM, that has been defined as the nucleus paragigantocellularis lateralis (PGL), produces hypotension and a bradycardia. Other areas from which 5-HT neurons project directly to the intermediolateral cell column are the raphe pallidus (B1), obscurus (B2) and magnus (B3) nuclei in the medulla (Loewy & Neil, 1981). Variable changes in arterial blood pressure, heart rate and sympathetic nerve activity in response to electrical stimulation of these raphe nuclei have revealed that these areas are not homogeneous in function (Coote et al., 1988; Kuhn et-al., 1980a). There are distinct areas from which increases in sympathetic nerve activity accompanied by pressor responses and increases in heart rate are obtained, whereas at other locations in these raphe nuclei decreases in sympathetic nerve activity accompanied by depressor responses and decreases in heart rate predominate (Adair et al., 1977; Cabot et al., 1979; McCall., 1984). Amongst others, studies employing the technique of microiontophoresis confirmed the hypothesis that indeed 5-HT neurons are responsible for the excitation or inhibition of sympathetic nerve activity after electrical or chemical stimuli, applied to the medullary raphe nuclei. In rat and cat, microiontophoretic application of 5-HT to the 5-HT neurons extending from the raphe nuclei either excites or inhibits sympathetic preganglionic neurons (DeGroat & Ryall, 1967; Coote et al., 1981). These actions could be mediated via activation of 5-HT receptors located at synapses at the spinal sympathetic level. Indeed, intrathecal administration of 5-HT either caused sympatho-inhibition (high doses) or excitation (low doses) (Yusof & Coote, 1988).

1.4.3 Cardiovascular effects of central 5-HT receptor interaction

The complexity in effects of centrally administered 5-HT may reflect the presence of multiple receptor subtypes in the central nervous system, for which 5-HT is non-selective. Growing evidence shows that the inhibitory and excitatory effects of 5-HT on sympathetic neurons are mediated by different 5-HT receptor subtypes. The availability of several selective 5-HT receptor compounds has made it possible to analyse the receptor subtypes that are responsible for the above described effects of 5-HT.

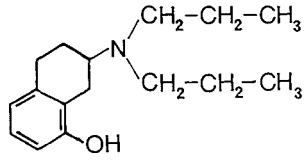
McCall (1983; 1984) showed that the sympathoexcitatory responses to microiontophoretically applied 5-HT on sympathetic preganglionic neurons or to electrical stimulation of the medullary raphe nuclei could be blocked by the 5-HT receptor antagonists methysergide and metergoline. The 5-HT_2 receptor might play a role in mediating the sympatho-excitatory effects of central 5-HT receptor activation. McCall and colleagues (1987) found that the putative 5-HT_2 receptor agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) produced a large increase in sympathetic nerve discharge and also increased blood pressure. In addition, the sympatho-excitatory effects of DOI could be blocked by the 5-HT_2 receptor antagonists ketanserin and LY 53857 (McCall & Harris, 1988). Observations in studies performed by Ramage (1985) and McCall & Harris (1987) make a role for 5-HT_2 receptors in blood pressure maintenance questionable. If 5-HT_2 receptors mediate the tonic excitatory effects of 5-HT on sympathetic neurons, then 5-HT_2 receptor antagonists should inhibit sympathetic nerve activity. However, 5-HT_2 receptor antagonists do not have an effect on sympathetic nerve discharge, indicating that central 5-HT_2 receptors are not normally under tonic activation (Ramage, 1985; McCall & Harris, 1987).
Sympatho-excitatory responses are also evoked by intrathecal administration of low doses of 5-HT or of the 5-HT_1-like receptor agonist 5-carboxamidotryptamine (5-CT). The effects of 5-HT could not be blocked by the 5-HT_2 and 5-HT_3 receptor antagonists, ketanserin and ICS 205-930, respectively, suggesting a role for 5-HT_1-like receptors in mediating 5-HT induced sympatho-excitatory effects (Yusof & Coote, 1988). Until now, the subtype of the 5-HT_1 receptor, responsible for this effect remains unclear. However, the 5-HT_{1A} receptor does not seem to be involved, since the 5-HT_{1A} receptor agonist 8-OH-DPAT has no effect (Berger & Ramirez, 1988). Furthermore, 5-CT displays a low affinity for the 5-HT_{1C} receptor (Hoyer, 1988) excluding this 5-HT_1 receptor subtype as well.

The hypotensive mechanism of 5-HT seems to be due to a decrease in sympathetic tone (Baum & Shropshire, 1975; McCall & Humphrey, 1982) and is probably the result of stimulating central 5-HT_{1A} receptors. Evidence for this hypothesis was provided by studies, in several animal species, presenting the cardiovascular effects of 8-OH-DPAT, DP-5-CT, R 28935, urapidil, ipsapirone and flesinoxan, drugs that display agonist activity at central 5-HT_{1A} receptors (Fozard et al., 1987; Ramage & Fozard, 1987; Doods et al., 1988; Wouters et al., 1988a,b; Mandal et al., 1989). By now, amongst others based on specificity and selectivity for the 5-HT_{1A} receptor and central penetration

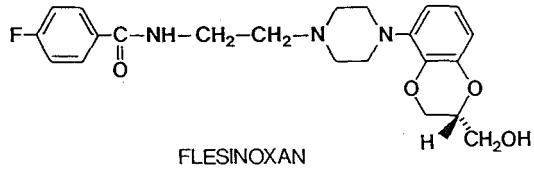
ability, 8-OH-DPAT and flesinoxan have been proven to be the leading drugs in this field.

1.5 The 5-HT_{1A} receptor agonists 8-OH-DPAT and flesinoxan

The compound 8-OH-DPAT (8-hydroxy-2-(di-n-propylamino) tetralin) (Fig. 4) was developed as part of a programme directed at finding new monoamine-receptor active drugs (Hjorth et al., 1982). This compound showed pronounced biochemical and behavioural alterations indicative of central serotoninomimetic activity. These effects were most likely due to an agonist action at a serotonin receptor, since they were resistant to prior monoamine depletion by means of reserpine (Hjorth et al., 1982). Receptor binding studies, carried out with 8-OH-DPAT, revealed that this centrally active 5-HT receptor agonist selectively binds to the 5-HT_{1A} binding site (Middlemiss & Fozard, 1983) (Table 5).



8-OH-DPAT



FLESINOXAN

Fig. 4: The chemical structures of the 5-HT_{1A} receptor agonists 8-OH-DPAT and flesinoxan.

Flesinoxan ((+)-(R)-N-[2-[4-(2,3-dihydro-2-hydroxymethyl-1,4-benzodioxin-5-yl)-1-piperazinyl]ethyl]-4-fluorobenzamide.HCl) (Fig. 4) is a representative of a series of heterobicyclicaryl-piperazine analogues (Wouters et al., 1988a). Receptor binding assays

showed a profile for flesinoxan that proved that this drug is a very selective ligand for the 5-HT_{1A} receptor (Wouters et al., 1988b) (Table 5). Furthermore, autoradiographic studies with [^{3}H]-flesinoxan revealed high affinity binding sites throughout the brain (Schipper et al., 1990), the anatomical distribution being very similar to the localization of 5-HT_{1A} binding sites labelled by [^{3}H]-8-OH-DPAT (Schipper et al., 1990). Evidence from several functional models have indicated that flesinoxan behaves as a full agonist at the 5-HT_{1A} receptor (Schipper et al., 1990).

Both 8-OH-DPAT and flesinoxan show prominent, centrally mediated cardiovascular effects, i.e. a reduction in blood pressure and heart rate, in a number of species (Wouters et al., 1988a; Fozard & Mir, 1987).

Table 5: Receptor binding affinities of 5-HT_{1A} receptor agonists and putative antagonists (M.T.M. Tulp. personal communication) used in studies described in this thesis. Affinities are expressed as pK_i values (-log K_i in nM). * Affinities are expressed as pIC_{50} values (-log IC_{50} in nM) from Fozard et al. (1987).

	5-HT_{1A}	5-HT_{1B}	5-HT_{1C}	5-HT_{1D}	5-HT_2	5-HT_3	α_1	α_2	$\beta_{1,2}$	D_1	D_2
Flesinoxan	8.8	6.3	5.3	6.8	5.4	<5	6.4	4.2	5.3	4.7	6.9
8-OH-DPAT	8.6	5.8	5.2	6.0	5.3	<5	5.6	6.5	5.3	5.1	5.7
(±)-Pindolol	7.1*	6.0*	n.d	n.d	4.2*	n.d	n.d	4.6*	9.0	n.d	n.d
Buspirone	7.8	5.5	6.1	<5	6.0	<5	6.2	<5.3	<5.3	5.1	7.4
Ipsapirone	8.3	5.5	4.9	<5	5.6	<5	6.6	5.6	<5.3	5.2	6.4
8-MeO-ClEPAT	7.4	5.2	6.7	n.d	5.7	n.d	6.9	5.6	<5	6.4	5.3
Spiroxatrine	9.1	<6	<6	<6	6.8	<6	6.9	6.5	<5	6.4	9.2
Methiothepin	7.6	7.0	8.6	6.9	9.0	5.8	9.2	6.9*	5.6	n.d	9.3

1.6 Aim of the thesis

The aim of the studies described in this thesis is to further clarify the role of the 5-HT_{1A} receptor in central cardiovascular regulation. The hypotensive action of 5-HT_{1A} receptor agonists is mainly due to differential sympatho-inhibition resulting in an increase in total peripheral vascular conductance (Wouters et al, 1988b). The first part of the thesis deals with the effects of activating the 5-HT_{1A} receptor with the selective 5-HT_{1A} receptor agonists 8-OH-DPAT and flesinoxan on the haemodynamic profile of anaesthetized cats and conscious rats. This part of the thesis will provide better insight

into which vascular beds are responsible for the increase in total peripheral conductance as caused by 5-HT_{1A} receptor activation.

In the second part, a study will be presented comparing the cardiovascular effects of the 5-HT_{1A} receptor agonists 8-OH-DPAT and flesinoxan in several rat models. Also, the results of a pharmacological study will be shown in order to provide evidence that the cardiovascular effects of 8-OH-DPAT and flesinoxan are indeed mediated by 5-HT_{1A} receptors.

The last part of the thesis will deal with the central sites, possibly involved in mediating the cardiovascular effects of 5-HT_{1A} receptor agonists. In this part the effects of central administration of 5-HT_{1A} receptor agonists on cardiovascular control will be discussed.

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CHAPTER 2

5-HT_{1A} RECEPTOR AGONIST-INDUCED CARDIOVASCULAR EFFECTS

As has been described in chapter 1, the hypotensive mechanism of 5-HT seems to be due to a decrease in sympathetic tone (Baum & Shropshire, 1975; McCall & Humphrey, 1982) and is probably the result of stimulating central 5-HT_{1A} receptors. (Fozard et al., 1987; Ramage & Fozard, 1987; Doods et al., 1988; Wouters et al., 1988b; Ramage et al., 1988; Mandal et al., 1989).

8-OH-DPAT and the more recently developed selective 5-HT_{1A} receptor agonist flesinoxan display a high affinity for the 5-HT_{1A} receptor (Middlemiss & Fozard, 1983, Wouters et al., 1988b). Enhanced hypotensive potency as observed after administration of (+)8-OH-DPAT (Doods et al., 1988) or flesinoxan (Wouters et al., 1988) via the vertebral artery of anaesthetized cats compared with the intravenous route, clearly pointed to a central site of action of these 5-HT_{1A} receptor agonists. Furthermore, studies in anaesthetized cats have also revealed that both 8-OH-DPAT and flesinoxan induce a reduction in sympathetic nerve activity (McCall et al., 1987; Ramage & Fozard, 1987; Ramage et al., 1988) together with an increase in vagal drive. The latter observation explains the drug-induced bradycardia. This combination of sympathoinhibition together with vagally mediated bradycardia was not due to activation of cardiopulmonary afferents as in the von Bezold-Jarisch reflex, since both 8-OH-DPAT and flesinoxan still caused sympathoinhibition in bi-vagotomized cats. The ability of 8-OH-DPAT and flesinoxan to cause an increase in vagal tone and prevent the expected reflex rise in central sympathetic tone in response to their hypotensive action also supports the conclusion from Wouters and colleagues (1988) about a central mechanism mediating the cardiovascular effects of these 5-HT_{1A} receptor agonists.

In the anaesthetized cat, the hypotensive action of flesinoxan and 8-OH-DPAT was accompanied with a moderate decline in thoracic preganglionic nerve activity and splanchnic nerve activity, whereas renal nerve activity was markedly decreased (Ramage et al., 1988; Ramage & Wilkinson, 1989). This differential inhibition of sympathetic nerve activity indicates that the central sympathoinhibition induced by these 5-HT_{1A} receptor agonists might be organ-dependent. In addition, results were presented indicating that the increase in total peripheral vascular conductance produced by

flesinoxan was probably not due to a generalized vasodilatation but to an organ-specific vasodilatation, since flesinoxan hardly affected femoral arterial conductance but largely increased renal vascular conductance.

In the next two chapters, studies in anaesthetized cats and conscious rats will be described that support the idea that different vascular beds contribute to a different extent to the increase in total peripheral vascular conductance induced by the 5-HT_{1A} receptor agonists 8-OH-DPAT and flesinoxan.

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CHAPTER 3

SYSTEMIC AND REGIONAL HAEMODYNAMIC EFFECTS OF THE PUTATIVE 5-HT_{1A} RECEPTOR AGONIST FLESINOXAN IN THE CAT

Summary

The systemic and regional haemodynamic effects of the centrally acting putative 5-HT_{1A} receptor agonist flesinoxan (3, 10, 30 and 100 μg kg^{-1}) were investigated in the anaesthetized cat and compared with those of 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT: 3, 10, 30 and 100 μg kg^{-1}) and clonidine (0.3, 1, 3 and 10 μg kg^{-1}). Cardiac output was measured with a precalibrated electromagnetic flow probe placed on the ascending aorta and regional blood flows and conductances were measured with radioactive microspheres.

Flesinoxan and 8-OH-DPAT caused a decrease in blood pressure (44% and 37%, respectively at 100 μg kg^{-1}) mainly resulting from an increased peripheral vascular conductance; in the case of 8-OH-DPAT, however, a reduction in cardiac output (34%) also contributed. Clonidine decreased blood pressure (12% at 10 μg kg^{-1}) by reducing cardiac output (31%). All three drugs decreased heart rate. Flesinoxan and 8-OH-DPAT decreased tissue perfusion in the heart, lungs, gastro-intestinal tract, eyes and skin, but both renal and cerebral blood flows were preserved as a result of increased vascular conductances. These two drugs also redistributed intrarenal blood flow from the outer cortex towards the inner cortex and medulla. Low doses of clonidine tended to increase but higher doses decreased organ blood flows especially to the heart, lungs, liver and eyes. Clonidine did not redistribute intrarenal blood flows. These results establish that the 5-HT_{1A} receptor agonists flesinoxan and 8-OH-DPAT elicited a systemic and regional haemodynamic profile that differs from that of the α_2-adrenoceptor agonist clonidine.

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Introduction

Flesinoxan, a phenylpiperazine derivative, lowers blood pressure and heart rate in several animal species (Wouters et al., 1988a). The cardiovascular effects of flesinoxan are largely centrally mediated. In cats, administration of the drug through the vertebral arteries increases its hypotensive potency several fold as compared with intravenous (i.v.) administration (Wouters et al., 1988b). Flesinoxan decreases sympathetic neural discharges and increases vagal tone (Ramage et al., 1988). This last phenomenon explains the bradycardia, which occurs at higher doses of the drug. The blood pressure lowering effect of flesinoxan is mainly due to an increase in peripheral vascular conductance and is only slightly due to a reduction in cardiac output (Wouters et al., 1988b).

Receptor binding studies show that flesinoxan is a potent and selective 5-HT_{1A} ligand (Wouters et al., 1988b; Ramage et al., 1988). The β-adrenoceptor antagonist pindolol, which also interacts stereoselectively with 5-HT_1 recognition sites (Middlemiss et al., 1977; Tricklebank et al., 1987), clearly prevents cardiovascular responses to flesinoxan in cats, consistent with the involvement of 5-HT_{1A} receptors (Wouters et al., 1988b).

The present study investigated the contribution of the different vascular beds in the increase in total peripheral conductance caused by flesinoxan. The effect of flesinoxan on regional blood flows and conductances was studied by the radioactive microsphere technique in anaesthetized cats. The effects of flesinoxan were compared with those of 8-OH-DPAT, which like flesinoxan lowers arterial blood pressure and heart rate via central 5-HT_{1A} receptors (Ramage & Fozard, 1987), and those of clonidine, which exerts similar effects through central α_2-adrenoceptors (Kobinger, 1978). Part of this investigation was reported to the British Pharmacological Society (Dreteler et al., 1988).

Materials and methods

General preparations

Twenty five cats of either sex (mean weight 3.1 ± 0.1 kg) were anaesthetized with pentobarbital sodium (35 mg kg^{-1}, intraperitoneally, i.p.) and kept under anaesthesia by subsequent i.v. doses as needed. After the trachea was cannulated, the animals were ventilated with an infant respirator. Cannulas for blood pressure measurements and drug

administration were inserted into the right femoral artery and vein, respectively. Another cannula for withdrawal of blood during microsphere injection was inserted into the left femoral artery. The heart was exposed after the chest was opened by an incision between the third and fourth rib, and the left atrium was cannulated to enable injection of microspheres. A precalibrated 5.0-mm electromagnetic flow probe (Skalar, Delft, The Netherlands) was placed around the ascending aorta to determine cardiac output. Blood pressure was recorded with a Statham pressure transducer (P23Ac), and heart rate was derived from the flow meter signals. Arterial blood gases were measured with an ABL-2 (Radiometer, Copenhagen, Denmark). The animals were maintained at a body temperature of about 37°C by means of a heating blanket.

Blood flow measurement

As described in detail previously (Johnston & Saxena, 1978), regional blood flows were determined in anaesthetized cats by radioactive microsphere technique (Hales, 1974; Heymann et al., 1977). Microspheres with 15-μm ($\pm$ 1 SD) diameter and labelled with ^{141}Ce, ^{113}Sn, ^{103}Ru, ^{46}Sc or ^{95}Nb (NEN Company, Dreieich, F.R.G.) were used. For each measurement, 0.2 ml of a suspension of microspheres, containing about 2 x 10^5 spheres labelled with one of the nuclides, was injected into the left atrium in 10-15 sec.

Arterial blood was withdrawn at a rate of 1.9 ml.min^{-1} from about 5 sec before until 60 seconds after the microsphere injection. At the end of each experiment, animals were killed by i.v. injection of 2 ml of a 20% (wt/vol) potassium chloride solution. The lungs, heart, a part of the gastro-intestinal tract, spleen, liver, adrenals, kidneys, brain, eyes and parts of skin and skeletal muscles were dissected out, weighed and placed in plastic vials. The radioactivity of the blood samples as well as that of the tissues was counted for 10 min in a γ-scintillation counter (Packard Minaxy), using suitable windows for discriminating the different isotopes used (Saxena et al., 1980). The systemic and microsphere data were processed by a PDP-11/70 computer using a set of specially developed programmes (Saxena et al., 1980). Tissue blood flows ($\dot{Q}_{tis}$) were calculated by the formula:

$\dot{Q}_{tis}$ (ml.min^{-1}) = (I_{tis}/I_{art}) x $\dot{Q}_{art}$, where I_{tis} is the radioactivity (cpm) in a particular tissue sample, and I_{art} and $\dot{Q}_{art}$ are the radioactivity (cpm) and the rate (ml.min^{-1}) at which the arterial blood sample was withdrawn, respectively. Regional blood flow values were normalized to 100 g tissue.

Experimental protocol

After completion of the surgical procedures, the animals were allowed to stabilize for at least 45 min. Thereafter, baseline values were obtained by injection of the first batch of microspheres and the animals were divided into four groups receiving cumulative doses of either flesinoxan (3, 10, 30 and 100 $\mu g\ kg^{-1}$; n=6), 8-OH-DPAT (3, 10, 30 and 100 $\mu g\ kg^{-1}$; n=7), clonidine (0.3, 1, 3 and 10 $\mu g\ kg^{-1}$; n=6) or physiological saline (0.2 ml four times, n=6). Subsequent injections of microspheres were administered after each dose of the drug or saline at times (about 15-20 min) yielding the maximal effect on arterial blood pressure. Just before injection of microspheres, arterial blood gases were determined and values of heart rate, arterial blood pressure and cardiac output were measured.

Drugs

The drugs used in this investigation were flesinoxan ((+)-(R)-N-[2-[4- (2,3-dihydro-2- hydroxymethyl-1, 4-benzodioxin-5-yl)-1-piperazinyl]ethyl]-4-fluorbenzamide HCl; Duphar B.V., Weesp, The Netherlands), 8-OH-DPAT (8-hydroxy-2-(di-N-propylamino) tetralin HBr; Research Biochemicals Inc., Natick) and clonidine (2-(2,6-dichlorophenyl-amino)-2-imidazoline HCL; Boehringer-Ingelheim, Ingelheim, F.R.G.). All three drugs were dissolved in saline.

Data presentation and statistical evaluation

Except as otherwise described, all data in the text and illustrations are mean values $\pm$ SEM. The significance of the differences between the values at baseline and those after different doses of drugs was evaluated by Duncan's new multiple range test once an analysis of variance (ANOVA, randomized block design) had revealed that the samples represented different populations (Steel & Torrie, 1980). A p-value ≤ 0.05 (two-tailed) was considered statistically significant.

Results

Arterial blood gases

The baseline values for arterial blood gases in the experiments of series using saline, flesinoxan, 8-OH-DPAT and clonidine were, respectively: pH,7.35 ± 0.01, 7.28 ± 0.02, 7.35 ± 0.02 and 7.34 ± 0.01; pCO_2, 29 ± 1, 30 ± 1, 27 ± 1 and 28 ± 1 mmHg; pO_2, 105 ± 6, 107 ± 4, 99 ± 5 and 114 ± 3 mmHg; and oxygen saturation, 96.5 ± 0.4%, 96.2 ± 0.2, 96.2 ± 0.4 and 97.2 ± 0.2%. The blood gas values were not appreciably changed by saline or any of the drugs.

Systemic haemodynamic variables

The baseline values for the systemic haemodynamic variables are shown in Table 1. In Fig. 1, the changes in the systemic haemodynamic variables elicited by the three drugs (flesinoxan, 8-OH-DPAT and clonidine) are compared with those observed after saline treatment.

Table 1: Baseline values (mean ± SEM) of heart rate (HR), mean arterial blood pressure (MAP), cardiac output (CO) and total peripheral conductance (TPC) in groups of anaesthetized cats subsequently treated with saline (n=6), flesinoxan (n=6) 8-OH-DPAT (n=7) or clonidine (n=6).

	Saline	Flesinoxan	8-OH-DPAT	Clonidine
MAP (mmHg)	105± 8	117± 7	124± 4	117± 3
HR (beats.min^{-1})	172±14	177±19	186±13	166±16
CO (ml.min^{-1})	506±37	440±32	577±60	449±48
TPC (ml.min^{-1}.mmHg^{-1}x100)	503±68	380±28	469±50	382±39

In cats treated with saline alone, heart rate remained constant. Mean arterial blood pressure was significantly increased from the baseline value after the first saline injection and thereafter remained unchanged until the end of the experiment. Since cardiac output remained relatively constant, systemic vascular conductance showed a decrease (Fig. 1). After the last injection of saline, the changes from baseline values for heart rate, mean arterial blood pressure, cardiac output and total peripheral conductance were 4 ± 3, 11 ± 4, -7 ± 5, and -15 ± 7%, respectively.

Cumulative doses of flesinoxan (Fig. 1) caused dose-related decreases in mean arterial blood pressure (maximum decrease 37 ± 4%). The values of blood pressure attained after the highest three doses (10, 30 and 100 $\mu g\ kg^{-1}$) were significantly different from baseline values. Heart rate showed a dose-related decrease which was significant with the two highest doses; the maximum decrease was 21 ± 7%. Flesinoxan did not affect cardiac output significantly except at the highest dose at which a slight decrease (10 ± 4%) was noticed. Total peripheral conductance was increased in a dose-dependent manner, reaching a maximum rise of 44 ± 9% after the highest dose (Fig. 1). As compared with the saline-treated group, i.v. administration of flesinoxan in the cat caused significant changes in heart rate (two highest doses), mean arterial pressure (all doses) and total peripheral conductance (three highest doses). However, the changes in cardiac output caused by flesinoxan, being no different from those noted after administration of corresponding volumes of saline, merely reflect time-dependent changes.

8-OH-DPAT caused effects qualitatively similar to those of flesinoxan on heart rate and mean arterial blood pressure (Fig. 1). At the highest dose, maximal decreases of 27 ± 10 and 44 ± 5% were reached for heart rate and mean arterial blood pressure, respectively. 8-OH-DPAT caused a dose-related reduction (maximum 34 ± 3% after the final dose) of cardiac output, which contrasts with minor effects noted after flesinoxan. As a consequence of the changes in mean arterial blood pressure and cardiac output 8-OH-DPAT increased total peripheral conductance up to a dose of 10 $\mu g\ kg^{-1}$ but no further increases were observed at higher doses. As compared with the saline-treated group, 8-OH-DPAT caused significant changes in heart rate (two highest doses), mean arterial blood pressure (all doses), cardiac output (two highest doses) and total peripheral conductance (three highest doses).

Injection of clonidine elicited a biphasic response on mean blood pressure (i.e., a short pressor phase lasting 5-10 minutes was followed by a sustained depressor phase). Heart rate showed a rapid decrease at the time blood pressure was increased; this was followed by a second sustained decrease. The pressor response and the rapid heart rate decrease represent the peripheral component of the clonidine response, and the sustained decreases in blood pressor and heart rate represent the central effects. Fig. 1 shows only the dose-responses of the sustained (central) effects. The decreases in heart rate and mean arterial pressure reached only statistical significance after the highest dose, showing maximal decreases of 15 ± 5 and 12 ± 5%, respectively. Like 8-OH-DPAT,

clonidine reduced cardiac output (maximum decrease 31 ± 6%). In contrast to both flesinoxan and 8-OH-DPAT, clonidine decreased total peripheral conductance, but these changes were not significantly different from those induced by saline. The changes in heart rate (highest dose), mean arterial pressure (two highest doses) and cardiac output (two highest doses) caused by clonidine were significantly greater than those caused by saline.

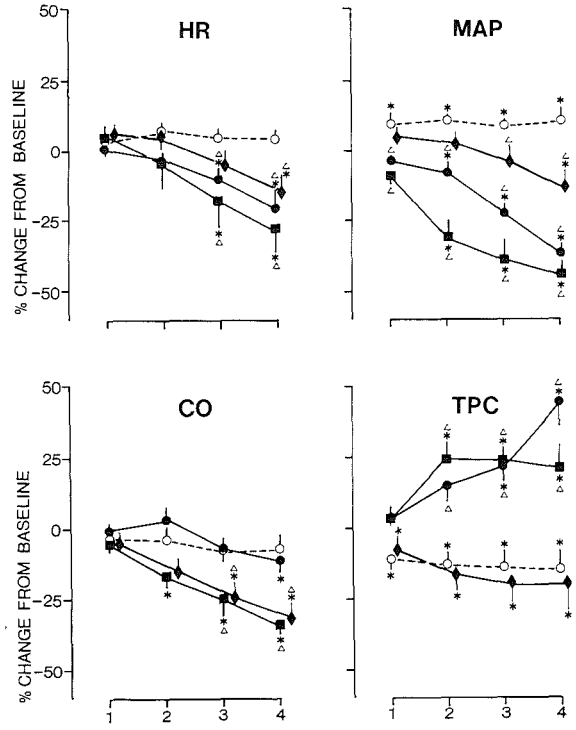

Fig. 1: Effects of sequential doses (denoted 1 to 4) of saline (○), flesinoxan (3, 10, 30 and 100 $\mu g\ kg^{-1}$; ●), 8-OH-DPAT (3, 10, 30 and 100 $\mu g\ kg^{-1}$; ■) or clonidine (0.3, 1, 3 and 10 $\mu g\ kg^{-1}$; ◆) on heart rate (HR), mean arterial blood pressure (MAP), cardiac output (CO) and total peripheral conductance (TPC), expressed as percentage changes from baseline values in anaesthetized cats.
*, significant change from baseline values ($p < 0.05$); Δ, significantly different from changes in the saline treated group ($p < 0.05$).

Regional haemodynamic variables

The baseline values for the regional haemodynamic variables, blood flows and vascular conductances, are shown in Table 2a and 2b, respectively. The effects of saline, flesinoxan, 8-OH-DPAT and clonidine on regional blood flows, conductances and intrarenal blood flows are presented in Fig. 2, 3 and 4, respectively.

Table 2a: Baseline values (mean ± SEM) normalized to 100 g tissue of regional blood flows ($ml.min^{-1}$) in groups of anaesthetized cats subsequently treated with saline, flesinoxan, 8-OH-DPAT or clonidine.

	Regional blood flow			
	Saline	Flesinoxan	8-OH-DPAT	Clonidine
Heart	158 ± 18	180 ± 38	154 ± 23	140 ± 11
Lungs	462 ± 55	536 ± 153	385 ± 47	369 ± 78
Kidneys	313 ± 45	366 ± 80	254 ± 29	234 ± 23
Brain	38 ± 5	39 ± 7	36 ± 6	31 ± 4
GIT	56 ± 11	61 ± 4	45 ± 8	43 ± 8
Spleen	79 ± 23	89 ± 24	88 ± 38	114 ± 24
Liver	61 ± 8	67 ± 10	48 ± 7	66 ± 6
Adrenals	521 ± 131	443 ± 136	475 ± 109	374 ± 65
Eyes	55 ± 6	70 ± 13	57 ± 11	59 ± 6
Muscle	4 ± 1	9 ± 3	4 ± 1	6 ± 1
Skin	8 ± 4	12 ± 4	9 ± 3	8 ± 2

The saline-treated group showed a time-related tendency to a decreased tissue perfusion in most organs, reaching significance only in the lungs and the muscle. Vascular conductance was also decreased in most organs, reaching significance in the heart, lungs, gastro-intestinal tract and muscles. Intrarenal blood flows remained unchanged except in the medulla, in which a reduction was noted.

Table 2b: Baseline values (mean ± SEM) normalized to 100 g tissue of vascular conductances ($ml.min^{-1}.mmHg^{-1}x100$) in groups of anaesthetized cats subsequently treated with saline, flesinoxan, 8-OH-DPAT or clonidine.

	Regional vascular conductances			
	Saline	Flesinoxan	8-OH-DPAT	Clonidine
Heart	147 ± 11	150 ± 22	122 ± 14	119 ± 7
Lungs	437 ± 34	431 ± 96	308 ± 32	318 ± 70
Kidneys	301 ± 42	310 ± 64	203 ± 18	203 ± 23
Brain	36 ± 4	32 ± 4	29 ± 6	28 ± 2
GIT	54 ± 10	53 ± 5	36 ± 7	37 ± 7
Spleen	72 ± 19	78 ± 23	70 ± 28	94 ± 18
Liver	63 ± 13	57 ± 7	38 ± 5	56 ± 4
Adrenals	551 ± 163	357 ± 80	378 ± 78	312 ± 46
Eyes	52 ± 4	58 ± 7	45 ± 8	50 ± 5
Muscle	4 ± 0	8 ± 3	3 ± 1	5 ± 1
Skin	6 ± 3	11 ± 4	8 ± 3	7 ± 2

As compared with baseline values, flesinoxan caused a significant decrease in perfusion of the heart, lungs, skin, eyes and gastro-intestinal tract. However, as compared with the saline-treated group, tissue perfusion was significantly decreased only in the heart and eyes. Except in the kidneys and brain, the vascular conductance pattern was similar to the regional blood flow pattern. In the kidneys and brain, vascular conductance was significantly increased, thus preserving blood flows to these organs. Administration of flesinoxan caused redistribution of intra-renal blood flows (Fig. 4). As compared with baseline values, the drug significantly decreased perfusion of the outer cortex. In contrast, blood flow to the inner cortex showed a tendency to increase and that to the medulla was significantly increased. The above changes were significantly different from those observed in the saline-treated group.

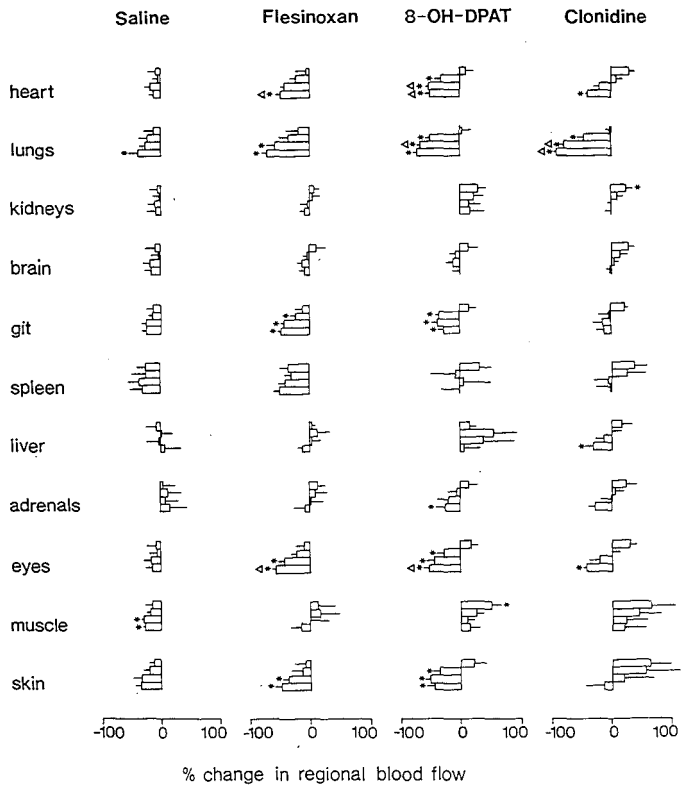

Fig. 2: Effects of saline, flesinoxan (3, 10, 30 and 100 $\mu g\ kg^{-1}$), 8-OH-DPAT (3, 10, 30 and 100 $\mu g\ kg^{-1}$) or clonidine (0.3, 1, 3 and 10 $\mu g\ kg^{-1}$) on regional blood flows expressed as percentage changes from baseline values in anaesthetized cats. The effects of the cumulative doses of each drug are represented by the four bars per organ (top to bottom).
*, significant changes from baseline values ($p < 0.05$); Δ, significantly different from changes in the saline treated group ($p < 0.05$).

8-OH-DPAT significantly increased blood flow to the muscles but reduced that to the heart, lungs, gastro-intestinal tract, skin and adrenals; the changes in the heart, lungs and eyes were greater than in the saline-treated group. Vascular conductance was decreased in the heart and lungs but was increased in the kidneys, brain, liver and muscles. 8-OH-DPAT, like flesinoxan, redistributed intrarenal blood flows from the outer

cortex to the medulla. As compared with baseline values, the drug significantly decreased blood flow to the outer cortex but increased that to the medulla. As compared with that of the saline-treated group, renal blood flow was significantly increased in the inner cortex and medulla.

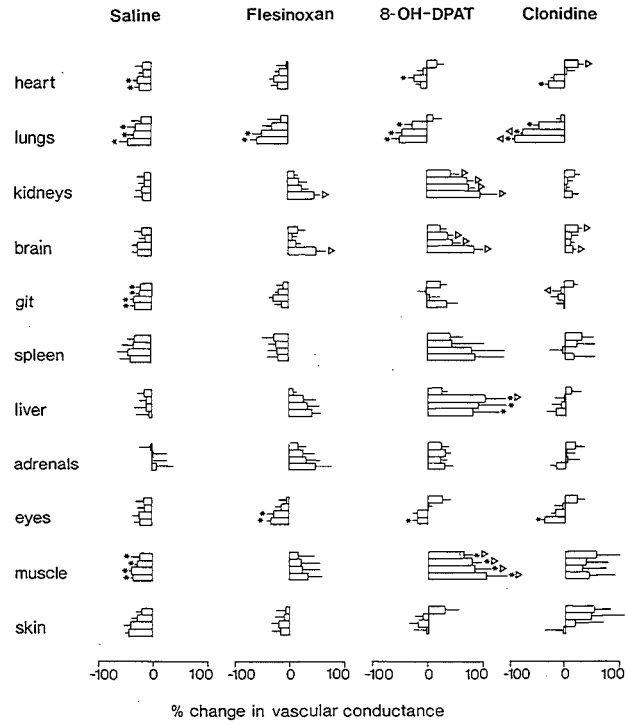

Fig. 3: Effect of saline, flesinoxan (3, 10, 30 and 100 $\mu g\ kg^{-1}$), 8-OH-DPAT (3, 10, 30 and 100 $\mu g\ kg^{-1}$) or clonidine (0,3, 1, 3 and 10 $\mu g\ kg^{-1}$) on regional vascular conductances expressed as percentage changes from baseline values. The effects of the cumulative doses of each drug are represented by the four bars per organ (top to bottom).
*, significant changes from baseline values ($p < 0.05$); Δ, significantly different from changes in the saline treated group ($p < 0.05$).

Blood flow distributions after injection of clonidine were determined at the time that sustained decreases in blood pressure and heart rate were maximal, so that we could avoid as much as possible counteracting of the central effects by the peripheral effects. Clonidine significantly reduced blood flow to the heart, lungs, liver and eyes. As compared to the saline-treated group, tissue perfusion was significantly decreased only in the lungs. Vascular conductance pattern of clonidine parallels the regional blood flow pattern. Unlike flesinoxan and 8-OH-DPAT, clonidine did not cause a redistribution of intrarenal blood flows.

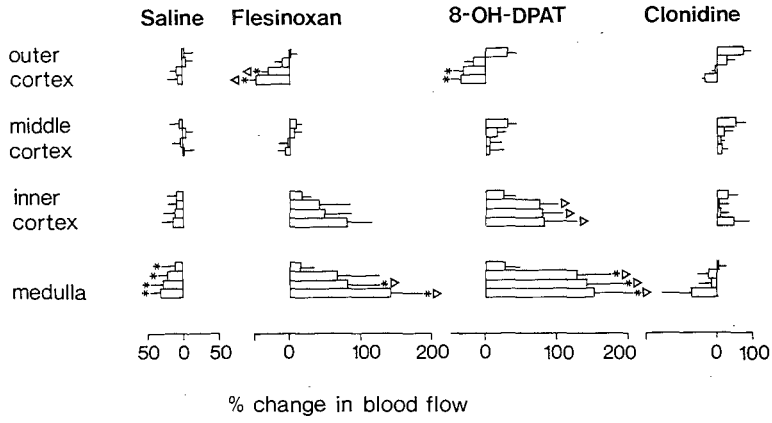

Fig. 4: Effect of saline, flesinoxan (3, 10, 30 and 100 $\mu g\ kg^{-1}$), 8-OH-DPAT (3, 10, 30 and 100 $\mu g\ kg^{-1}$) or clonidine (0,3, 1, 3, and 10 $\mu g\ kg^{-1}$) on intrarenal blood flows expressed as percentage changes from baseline values. The effects of the cumulative doses of each drug are represented by the four bars per kidney region (top to bottom).
*, significant changes from baseline values ($p < 0.05$); Δ, significantly different from changes in the saline-treated group ($p < 0.05$).

Discussion

Cumulative doses of the putative 5-HT_{1A} receptor agonists flesinoxan and 8-OH-DPAT caused dose-related decreases in mean arterial pressure and heart rate. The present results are in good agreement with data in the literature (Wouters et al., 1988b; Ramage & Fozard, 1987). Similarly, the minor effect of flesinoxan on cardiac output we

observed in open-chest cats is in good agreement with a previous report in closed-chest cats (Wouters et al., 1988b). Surprisingly, this contrasted with marked dose-dependent decreases in cardiac output observed with 8-OH-DPAT which, to our knowledge, has not been studied previously for its effects on cardiac output. The above findings demonstrate that flesinoxan lowers arterial blood pressure primarily by an increase in peripheral vascular conductance, whereas the effects of 8-OH-DPAT reflect both a reduction in cardiac output and an increase in vascular conductance. Since the baseline values of cardiac output were higher in 8-OH-DPAT-treated animals as compared with flesinoxan-treated animals, this may have rendered the 8-OH-DPAT group more susceptible to a decrease in cardiac output. However, this does not appear to explain the observed differences between 8-OH-DPAT and flesinoxan because of the following arguments. After the second dose (10 $\mu g\ kg^{-1}$) of 8-OH-DPAT and flesinoxan the absolute cardiac output values of the two groups no longer differ from each other; i.e., cardiac output in the 8-OH-DPAT group decreased from 577 to 470 ml min^{-1} and in the flesinoxan group increased from 440 to 460 ml min^{-1}. Yet, higher doses of 8-OH-DPAT caused a further decrease in cardiac output to a value of 375 ml min^{-1} and no further change in peripheral vascular conductance, whereas higher doses of flesinoxan did not cause a decrease in cardiac output but further increased peripheral vascular conductance.

The effects of clonidine on arterial blood pressure, in the doses used, were less prominent and resulted from a reduction in cardiac output. This reduction in cardiac output most probably resulted from clonidine's central effects as its time course followed that of the centrally mediated bradycardia and hypotension. Peripheral vascular conductance tended to decrease as compared with saline-treated animals. This could result from a compensatory effect set in action by the decrease in cardiac output. However, although care was taken to measure the variables at the time of maximal centrally mediated effects, the decrease in blood pressure may have been counteracted in part by a direct peripheral vasoconstriction (Kobinger & Walland, 1967; Laubie et al., 1976).

The putative 5-HT_{1A} receptor agonists flesinoxan and 8-OH-DPAT have in common with clonidine a central nervous system (CNS) site of action (Schmitt, 1977; Fozard et al., 1987; Ramage & Fozard, 1987; Wouters et al., 1988b), and these drugs exert their cardiovascular effects by a reduction of the sympathetic neural outflow and/or an increase in vagal tone. However, the sympathetic outflow to different organs has been reported to show a striking difference toward the inhibitory action of these compounds.

The sympathetic outflow to the heart was most sensitive to clonidine, whereas sympathetic outflow to the kidneys was most sensitive to the 5-HT_{1A} receptor agonists (Ramage & Fozard, 1987; Ramage et al., 1988). The main difference in systemic haemodynamics observed in this study (i.e., the finding that the α_2-adrenoceptor agonist (clonidine) lowers blood pressure by cardiac output reduction and the 5-HT_{1A} receptor agonists mainly by an increased conductance) is commensurate with the differential patterns of sympatho-inhibition exhibited by these different classes of drugs.

With respect to their effects on regional blood flow distribution, flesinoxan and 8-OH-DPAT showed a qualitatively comparable pattern. Both drugs decreased tissue blood flow to the heart, lungs, gastro-intestinal tract, skin and eyes. In contrast, and despite a prominent decrease in mean arterial blood pressure, renal and cerebral blood flows remained virtually unchanged as a result of increased vascular conductances. Whether these latter effects are due to autoregulation or to drug induced active vasodilation cannot be deduced from the present study. An increase in renal conductance with flesinoxan was also demonstrated by Ramage and colleagues (1988), who measured renal blood flow with an electromagnetic flow probe. Clonidine showed a different pattern on regional blood flows (i.e., low doses tended to increase but higher doses decreased organ flows, especially in the heart, lungs, liver and eyes). This pattern is consistent with the observed decrease in cardiac output and may reflect a compensatory vasoconstriction. However, a direct local vasoconstriction caused by clonidine through peripheral receptors again can not be fully excluded.

A further difference between the two putative 5-HT_{1A} receptor agonists (flesinoxan and 8-OH-DPAT) and the α_2-adrenoceptor agonist (clonidine) concerns intrarenal redistribution of kidney blood flow. Both flesinoxan and 8-OH-DPAT redistributed flow to the inner cortex and medulla at the expense of flow to the outer cortex. However, the measurement of intrarenal blood flow distribution by radioactive microsphere technique has two major limitations (Katz et al., 1971). First, because microspheres are trapped within the glomerular capillaries, this method essentially measures glomerular blood flow and does not detect flow in vascular elements which are in series with the glomerular capillaries. The second limitation concerns dependence of the intrarenal flow distribution on the size of the microspheres used. Microspheres of the size used in this study (15 μm) tend to overestimate blood flow to the outer cortex with respect to the inner cortex.

These limitations prevent us from making firm statements about absolute blood flow values in the different renal zones. However, proportional changes in the flow rates

within a particular zone can still be detected. The pattern of changes noted with 8-OH-DPAT and flesinoxan, i.e., a relative decrease in blood flow in the outer cortex, no effect in the middle cortex and an increase in the inner cortical flow, together with no change in total renal blood flow, closely resembles the pattern of changes noted during autoregulatory adaptations in the dog kidney (Abe, 1971; Mimran, 1987). The observed increase in medullary blood flow with the two 5-HT_{1A} receptor agonists probably reflect dilatation of non-glomerular vessels which perfuse the medulla. The lack of intrarenal blood flow effects with clonidine may suggest that the peripheral vasoconstrictor response interfered with its potential renal effects.

Microspheres which bypass the nutrient vascular bed in a particular tissue through arteriovenous anastomoses are subsequently trapped in the capillary bed of the lungs. As a consequence, "lung" blood flow largely represents arteriovenous anastomotic blood flow (Hales, 1974; Saxena et al., 1980). In this study, flesinoxan and 8-OH-DPAT decreased lung flow dose dependently, indicating a decrease in arteriovenous anastomotic flow. Indeed, a decrease in cephalic arteriovenous shunting by 8-OH-DPAT, mediated through 5-HT_1-like receptors, was recently reported in pigs (Bom et al., 1989).

In conclusion, both flesinoxan and 8-OH-DPAT show a qualitatively similar systemic and regional haemodynamic profile and this profile differs from that of clonidine. Although clonidine also has a peripheral vasoconstrictor property, the observed differences in the haemodynamic effects may be related to the observed differences in central effects of these classes of centrally acting blood pressure lowering compounds.

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CHAPTER 4

SYSTEMIC AND REGIONAL HAEMODYNAMIC EFFECTS OF THE 5-HT_{1A} RECEPTOR AGONISTS FLESINOXAN AND 8-OH-DPAT IN THE CONSCIOUS RAT

Summary

The systemic and regional haemodynamic effects of the centrally acting 5-HT_{1A} receptor agonist flesinoxan (0.5 and 2.5 mg kg^{-1}, i.a.) were investigated in the conscious freely moving spontaneously hypertensive rat (SHR) and compared with those of 8-hydroxy-2(di-n-propylamino)tetralin (8-OH-DPAT) (0.1 and 0.5 mg kg^{-1}, i.a.). In one group of animals cardiac output was measured with a precalibrated electromagnetic flow probe around the ascending aorta. In another group regional vascular conductances were measured using radioactive microspheres. Flesinoxan and 8-OH-DPAT dose dependently decreased blood pressure (22 ± 5 and $13\pm4\%$, respectively, at the highest dose) mainly resulting from an increase in total peripheral vascular conductance (34 ± 12 and $16\pm3\%$, respectively) since there was no effect on cardiac output. Both drugs reduced heart rate (17 ± 4 and $20\pm4\%$ for flesinoxan and 8-OH-DPAT, respectively, at the highest dose). Flesinoxan and 8-OH-DPAT showed a qualitatively similar pattern regarding their effects on vascular conductances. Compared to a saline treated group increases in vascular conductances were seen in the heart and skeletal muscles. Vascular conductances in the lungs were markedly increased by both flesinoxan and 8-OH-DPAT, which may indicate that the conductance in the arteriovenous shunt vessels was enhanced. These results demonstrate that flesinoxan and 8-OH-DPAT elicit a qualitatively similar systemic and regional haemodynamic profile in the conscious SHR. Furthermore, the increase in total peripheral conductance seems to be due mainly to vasodilatation in the skeletal muscles.

after: Dreteler, G.H., Wouters, W., Toorop, G.P., Jansen, J.A.P. & Saxena P.R. (in press) J. Cardiovasc. Pharmacol.

Introduction

Flesinoxan and 8-OH-DPAT, selective agonists at 5-hydroxytryptamine$_{1A}$ (5-HT$_{1A}$) receptors (Schoeffter & Hoyer, 1988), lower blood pressure and heart rate in several animal species via a central mechanism (Fozard et al., 1987; Ramage & Fozard, 1987; Wouters et al., 1988; Laubie et al., 1989; Dreteler et al., 1990). The decrease in blood pressure is mediated via a reduction in sympathetic activity, whereas the bradycardia is mainly a consequence of an increased vagal activity (Ramage et al., 1987; Ramage et al., 1988). Flesinoxan only slightly reduces cardiac output in anaesthetized animals. The hypotensive effect of flesinoxan is mainly due to an enhanced peripheral conductance (Wouters et al., 1988; Dreteler et al., 1989; Hof & Fozard, 1989). In the case of 8-OH-DPAT, however, a reduction in cardiac output also contributes to the hypotensive effect (Dreteler et al., 1989). Microsphere studies in the anaesthetized cat have shown that flesinoxan and 8-OH-DPAT increased vascular conductances predominantly in the kidneys, brain and and skeletal muscles (Dreteler et al., 1989). These effects were also found in the anaesthetized rabbit, in which an additional vasodilatation in the splanchnic circulation was observed (Hof & Fozard, 1989).

Both flesinoxan and/or 8-OH-DPAT have also been described to lower blood pressure and heart rate in conscious spontaneously hypertensive (Martin et al., 1985; Fozard et al., 1987; Bouhelal et al., 1990; Connor et al., 1990; Dreteler et al., 1990), normotensive (Dreteler et al., 1990; Gradin et al., 1985) and sinoaortic denervated rats (8-OH-DPAT: Petty et al., 1988). As yet, however, there has been no detailed analysis of the haemodynamic effects of flesinoxan and 8-OH-DPAT in the conscious rat. Thus, the present study was designed to investigate the systemic and regional haemodynamic effects of flesinoxan and 8-OH-DPAT in the conscious freely moving spontaneously hypertensive rat (SHR). In addition to the standard haemodynamic measurements of systemic effects in one group of animals, the radioactive microsphere technique has been used to assess the effects on regional haemodynamics in another group of rats.

Material and methods

General

Male spontaneously hypertensive rats (SHR; Charles River, Sulzfeld, Germany), weighing 250-350 g, were used. The rats were divided into two groups. One group was used for systemic haemodynamic measurements, the other group for measuring regional haemodynamic effects. In both groups, blood pressure was recorded from a femoral artery by means of a pressure transducer (Statham) and heart rate was derived from the pulse pressure. In order to limit the number of chronically implanted catheters, drugs were administered via the femoral artery catheter.

Systemic haemodynamic studies

General procedures

In twelve rats anaesthesia was induced with an intraperitoneal injection of a mixture of Hypnorm (fentanyl: 0.3 mg kg^{-1} and fluanisone: 15 mg kg^{-1}) and Dormicum (midazolam: 7.5 mg kg^{-1}) to allow intubation of the trachea. During surgery the animals were kept under anaesthesia by ventilation with a mixture of N_2O/O_2 (1:1) and halothane (1%) using an infant respirator. The thorax was opened between the third and the fourth rib and a precalibrated 2.4-2.5 mm flow probe (Skalar Medical) was placed around the ascending aorta for cardiac output measurements. The flow probe cable was secured to the ribs and the connector subcutaneously tunnelled to the head of the animal where it was fixed to the skull. After surgery the animals were treated with the antibacterial drug Amplicox (ampicilline/cloxacilline: 50 mg kg^{-1}, i.m.), the analgesic drug Temgesic (buprenorfine: 0.3 mg kg^{-1}, i.m.) and a supportive fluid therapy using Duphalite (vitamine B complex/electrolytes/amino acids/dextrose: 2.5 ml, s.c.). After a seven day recovery period a cannula (medical PVC) was implanted into a femoral artery under halothane (1.5-1.75%) mixed with N_2O/O_2 (2:1) anaesthesia. The free end of the cannula emerged at the nape of the neck of the rat. The animals were allowed to recover from this surgical procedure for two to three days.

Experimental protocol

After the recovery period the animals were trained for the experimental protocol. The rats were divided into three groups that received either flesinoxan (0.5 and 2.5 mg kg^{-1}, n=7), 8-OH-DPAT (0.1 and 0.5 mg kg^{-1}, n=6) or physiological saline (1 ml kg^{-1}, n=7) in random order. The first dose of each drug or saline was given after an acclimatisation period of at least one hour. The second dose was given cumulatively half an hour after the first dose. The cardiovascular effects of the drugs were observed until 90 minutes after administration of the second dose. If possible, the rats were used for the two drugs and the saline experiment. The time between experiments was at least one week.

Regional haemodynamic studies

General procedures

Twenty five rats were cannulated (medical PVC) via a femoral artery and the left ventricle of the heart under halothane (1.5-1.75% in N_2O/O_2, 2:1) anaesthesia. The cannulae were tunnelled to the nape of the neck of the animals. Animals were allowed to recover from surgery at least two to three days.

Microsphere technique

Regional blood flows were determined, as described in detail previously (Johnston & Saxena, 1978), in conscious SHR by means of the radioactive microsphere technique (Hales, 1974; Heymann et al., 1977). Microspheres with a 15-μm ($\pm$1 S.D.) diameter and labeled with ^{141}Ce, ^{103}Ru or ^{95}Nb (NEN Company, Dreieich, F.R.G.) were used. For each measurement, 0.15 ml of a suspension of microspheres, containing ~2.10^5 spheres labeled with one of the nuclides, was injected over a period of 10-15 s into the left ventricle of the heart.

Arterial blood was withdrawn at a rate of 0.5 ml min^{-1} from ~5 s before until 60 s after the microsphere injection. At the end of each experiment, the animals were killed by i.a. injection of an overdose of pentobarbital sodium. Subsequently, the animal was dissected and all tissues were weighed and placed in plastic vials. The radioactivity of the blood samples as well as that of the tissues was counted for 5 min in a γ-scintillation counter (Packard, Minaxi Autogamma 5000), using suitable windows for discriminating the different isotopes used (Saxena et al., 1980). The microsphere data were processed

by a PDP-11/70 computer using a set of specially developed programs (Saxena et al., 1980). Tissue blood flows ($\dot{Q}_{tis}$) were calculated by the formula: $\dot{Q}_{tis}$(ml min^{-1}) = (I_{tis}/I_{art}).$\dot{Q}_{art}$, where I_{tis} is the radioactivity (cpm) in a particular tissue sample, and I_{art} and $\dot{Q}_{art}$ are the radioactivity (cpm) and the rate (ml min^{-1}) at which the arterial blood sample was withdrawn, respectively. Values of regional vascular conductances were calculated from regional blood flows and normalized to 100 g tissue.

Experimental protocol

The rats were divided into three groups receiving intra-arterial cumulative doses of either flesinoxan (0.5 and 2.5 mg kg^{-1}, n=10), 8-OH-DPAT (0.1 and 0.5 mg kg^{-1}, n=8) or saline (1 ml kg^{-1}, n=7). Microspheres were injected before administration of the first dose of drug or saline and after each dose of drug or saline at times (~15-30 min) yielding the maximal effect on arterial blood pressure. Just before the injection of microspheres, values of arterial blood pressure and heart rate were determined.

Drugs

The drugs used in this study were flesinoxan ((+)-(R)-N-[2-[4-(2.3-dihydro-2-hydroxymethyl-1,4-benzodioxin-5-yl)-1-piperazinyl]ethyl]-4-fluorbenzamide.HCl; Duphar B.V.) and 8-OH-DPAT (8-hydroxy-2-(di-N-propylamino)tetralin.HBr; Research Biochemicals Inc.). Both drugs were dissolved in saline.

Data presentation and statistical evaluation

Data are given as mean values ± standard error of the mean (SEM). The significance of the differences between the values at baseline and those after different doses of the drugs was evaluated by Duncan's new multiple-range test once an analysis of variance (ANOVA, randomized block design) had revealed that the samples represented different populations (Steel & Torrie, 1980). The significance of the differences between the changes in the saline treated group and the groups treated with either flesinoxan or 8-OH-DPAT was estimated with the unpaired t-test. For both assessments a p-value ≤ 0.05 (two-tailed) was considered statistically significant.

Results

Animals implanted with aortic flow probes

The baseline values for the systemic haemodynamic variables as measured in the conscious SHR with implanted flow probes are presented in Table 1. In Fig. 1, the changes in the systemic haemodynamic parameters elicited by the two drugs (flesinoxan and 8-OH-DPAT) are compared with those after saline treatment. In the animals treated with saline heart rate, blood pressure, cardiac output and total peripheral conductance remained constant during the course of the experiment.

Table 1: Baseline values (mean ± SEM) of heart rate (HR), mean arterial blood pressure (MAP), cardiac output (CO) and total peripheral conductance (TPC) in groups of conscious SHR (instrumented with aortic flow probes) subsequently treated with saline (n=7), flesinoxan (n=7) or 8-OH-DPAT (n=6).

	Saline	Flesinoxan	8-OH-DPAT
HR (beats min^{-1})	366 ± 11	382 ± 13	386 ± 24
MAP (mmHg)	169 ± 10	171 ± 11	166 ± 10
CO (ml min^{-1})	79 ± 4	88 ± 5	81 ± 2
TPC (ml min^{-1} $mmHg^{-1}$×100)	48 ± 5	53 ± 5	50 ± 3

With respect to baseline values flesinoxan (0.5 and 2.5 mg kg^{-1}) decreased blood pressure dose dependently, reaching significance at the highest dose with a maximum decrease of 22±5%, 30-40 min after administration. Heart rate decreased significantly at both doses, by up to 17±4% at the highest dose. The onset of the effects was immediate and at the end of the observation period (90 min after administration of the highest dose) blood pressure and heart rate were still about 10% below their basal values. Flesinoxan did not affect cardiac output at the doses administered. Total peripheral conductance was significantly increased at the highest dose of flesinoxan (34±12%).

8-OH-DPAT caused qualitatively similar changes to those of flesinoxan in the cardiovascular variables measured. Administration of 8-OH-DPAT (0.1 and 0.5 mg kg^{-1}) caused a dose related decrease in blood pressure and heart rate reaching significant

reductions at the highest dose of 13±4 and 20±4%, respectively. The effects were rapid in onset and maximum changes were seen about 30 min after drug administration (highest dose). At the end of the observation period blood pressure and heart rate had returned to their basal values. Cardiac output was not affected with either dose of 8-OH-DPAT. Total peripheral conductance was significantly increased (16±3%) at the highest dose of 8-OH-DPAT administered.

Administration of 8-OH-DPAT induced overt behavioural effects. 8-OH-DPAT caused flattening of body posture and locomotor activity was markedly increased especially after the highest dose. The behavioural effects caused by flesinoxan were similar to those observed after 8-OH-DPAT, but at the dose range used much less evident.

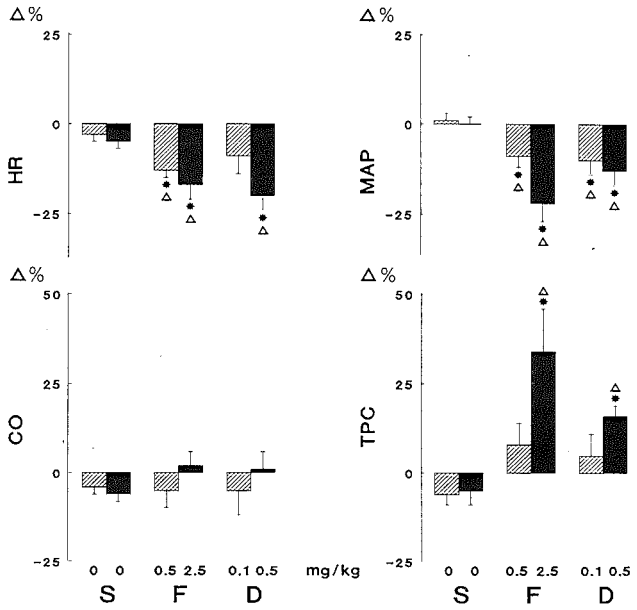

Fig. 1: Effects of subsequent doses of saline (S), flesinoxan (F; 0.5 and 2.5 mg kg^{-1}) and 8-OH-DPAT (D; 0.1 and 0.5 mg kg^{-1}) on heart rate (HR), mean arterial blood pressure (MAP), cardiac output (CO) and total peripheral conductance (TPC), expressed as percentage changes from baseline values in conscious SHR (instrumented with aortic flow probes).
*, significant changes from baseline values ($p<0.05$); Δ, significantly different from the changes in the saline treated group ($p<0.05$).

Animals given radioactive microspheres

Systemic haemodynamic variables

In the animals used for determination of regional haemodynamic effects by means of the radioactive microsphere technique, the baseline values and the effects of administration of drug or saline on blood pressure and heart rate are presented in Table 2. In the rats treated with saline, both heart rate and blood pressure remained constant. Administration of cumulative doses of flesinoxan caused dose related decreases in both heart rate and blood pressure with maximum decreases of 22±3 and 27±3%, respectively at the highest dose. Injection of 8-OH-DPAT caused qualitatively similar effects to flesinoxan on heart rate and blood pressure, although the blood pressure effects were less pronounced. At the highest dose maximal decreases of 24±5 and 18±3% were reached for heart rate and blood pressure, respectively. The described drug induced changes in heart rate and blood pressure were all statistically significant as compared to both baseline values and the saline treated group.

Table 2: Baseline values and effects of subsequent doses (D_1, D_2) of saline, flesinoxan (0.5 and 2.5 mg kg^{-1}) or 8-OH-DPAT (0.1 and 0.5 mg kg^{-1}) on heart rate (HR; bpm) and mean arterial blood pressure (MAP; mmHg) in the conscious SHR (radioactive microspheres).

Treatment	Variable	Baseline	% change from baseline D_1	D_2
Saline	HR	395 ± 7	-1 ± 2	-2 ± 2
	MAP	175 ± 6	-2 ± 3	-2 ± 3
Flesinoxan	HR	412 ±10	-12 ± 3*	-22 ± 3*
	MAP	177 ± 5	-15 ± 2*	-27 ± 3*
8-OH-DPAT	HR	386 ±11	-11 ± 4*	-24 ± 5*
	MAP	174 ± 5	-13 ± 2*	-18 ± 3*

Data are shown as mean ± SEM. *: significant change from baseline value ($p<0.05$); Δ: significantly different from changes in the saline treated group ($p<0.05$).

Regional haemodynamic variables

The baseline values for the regional vascular conductances are shown in Table 3. The effects of saline, flesinoxan and 8-OH-DPAT on regional vascular conductances are shown in Fig. 2.

Table 3: Baseline values (mean ± SEM) normalized to 100 g of tissue, of regional vascular conductances (ml min^{-1} $mmHg^{-1}$x100) in groups of conscious SHR, subsequently treated with saline (n=7), flesinoxan (n=10) or 8-OH-DPAT (n=8).

Organ	Saline	Flesinoxan	8-OH-DPAT
heart	312 ± 24	307 ± 20	300 ± 27
lungs	20 ± 4	16 ± 3	23 ± 3
kidneys	735 ± 41	640 ± 56	594 ± 61
brain	58 ± 5	72 ± 3	66 ± 4
GIT	128 ± 10	133 ± 11	143 ± 13
spleen	236 ± 24	198 ± 18	221 ± 32
liver	32 ± 8	28 ± 5	24 ± 8
adrenals	525 ± 72	496 ± 62	464 ± 102
fat	23 ± 3	18 ± 3	22 ± 3
muscles	12 ± 1	11 ± 1	13 ± 2
skin	11 ± 1	11 ± 1	12 ± 1

GIT: gastro-intestinal tract.

The saline treated group showed a significant decrease in tissue vascular conductances in the lungs, kidneys, gastro-intestinal tract, fat, muscles and skin.

Compared with baseline values flesinoxan significantly decreased vascular conductances in the gastro-intestinal tract, spleen, fat and skin. Significant increases in vascular conductances were observed in the heart, lungs and skeletal muscles. Vascular conductances were significantly different from the saline treated group in the heart, lungs, kidneys and skeletal muscles.

8-OH-DPAT showed a qualitatively similar pattern for regional vascular conductances to flesinoxan. Compared with baseline values 8-OH-DPAT significantly decreased vascular conductances in the kidneys, spleen and skin but increases in conductance were seen in the heart, lungs and skeletal muscles.

In these groups of rats treated with either flesinoxan or 8-OH-DPAT the same behavioural effects as described above were observed.

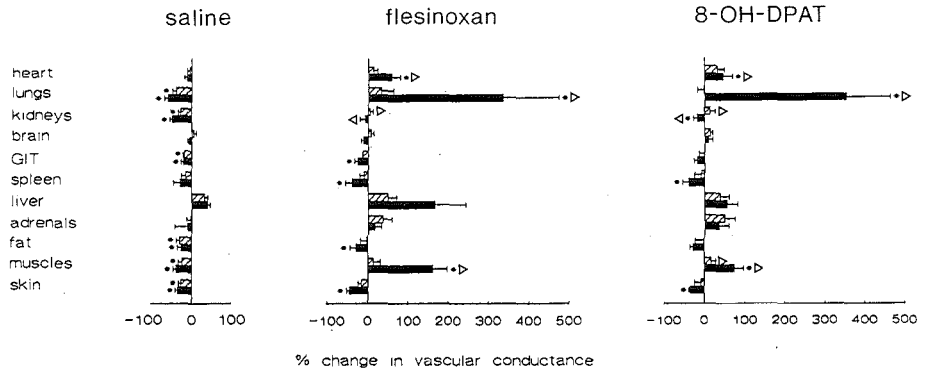

Fig. 2: Effects of saline, flesinoxan (0.5 and 2.5 mg kg^{-1}) and 8-OH-DPAT (0.1 and 0.5 mg kg^{-1}) on regional vascular conductances expressed as percentage changes from baseline values. The effects of the subsequent doses of each drug are represented by the two bars per organ. Hatched bar: first dose. Filled bar: second dose.
*, significant changes from baseline values ($p<0.05$); Δ, significantly different from changes in the saline treated group ($p<0.05$).

Discussion

The 5-HT_{1A} receptor agonist flesinoxan decreased heart rate and mean arterial blood pressure dose dependently in the conscious freely moving SHR. 8-OH-DPAT caused a moderate reduction in blood pressure and a larger dose related decrease in heart rate. These results are in good agreement with previous results obtained with equivalent doses of these drugs in conscious SHR (Dreteler et al., 1990). The decrease in mean arterial blood pressure caused by flesinoxan and 8-OH-DPAT and the lack of an effect on cardiac output in the conscious SHR consequently resulted in an enhanced total peripheral vascular conductance. The lack of an effect on cardiac output after administration of flesinoxan resembles the previously reported effects of this drug in anaesthetized open

and closed-chest cats (Wouters et al., 1988; Dreteler et al., 1989) and open-chest rabbits (Hof & Fozard, 1989). In the present experiments 8-OH-DPAT, like flesinoxan, did not appear to have any effect on cardiac output. This observation is in conformation with data obtained in the open-chest rabbit (Hof & Fozard, 1989) but in contrast with the results from studies performed in the open-chest cat (Dreteler et al., 1989). In the latter model, 8-OH-DPAT dose dependently reduced cardiac output. A possible explanation for these differences in results, other than the use of an anaesthetic or a deviation in sympathetic tone of the animals used in the different models, could be the behavioural effects caused by 8-OH-DPAT in the conscious rat. In the present study, at the highest dose of 8-OH-DPAT, locomotor activity was appreciably increased. Over the dose range used, the behavioural effects observed after administration of flesinoxan were much less pronounced. This is in accordance with recent reports (Kalkman et al., 1989; H.E. Molewijk, personal communication) stating that 8-OH-DPAT is far more active than flesinoxan in inducing behavioural effects.

The microsphere experiments were used to detect which organs were responsible for the increases in total peripheral vascular conductance induced by flesinoxan and 8-OH-DPAT. In this group of animals flesinoxan and 8-OH-DPAT caused reductions in arterial blood pressure and heart rate comparable with those seen in the group that had been instrumented with an electromagnetic flow probe. Flesinoxan and 8-OH-DPAT had qualitatively comparable effects on regional haemodynamics. Both 5-HT_{1A} agonists hardly affected vascular conductances in the kidneys and the brain, which contrasts the increases observed in anaesthetized animals (Ramage et al., 1988; Dreteler et al., 1989; Hof & Fozard, 1989). Both flesinoxan and 8-OH-DPAT increased vascular conductances in the heart and skeletal muscles in the conscious SHR. The increase in vascular conductance in the skeletal muscles has also been demonstrated in the anaesthetized cat (Dreteler et al., 1989) and rabbit (Hof & Fozard, 1989). So the vasodilatation in this tissue points to a specific effect induced by these centrally acting blood pressure lowering compounds and is not likely to be due to the enhanced locomotor activity as displayed by the freely moving rats. The increase in vascular conductance in the skeletal muscles largely contributes to the increase in total peripheral vascular conductance since in the rat about 16% of the cardiac output is delivered to the vascular bed in this tissue.

A fraction of the microspheres, injected into the systemic circulation, is not trapped in the nutrient vascular bed. These microspheres pass via arteriovenous anastomoses and veins to the right side of the heart and subsequently get trapped in the pulmonary

capillary bed. As a consequence, the vascular conductance of the lungs largely represents the conductance of the arteriovenous shunt vessels (Hales, 1974; Heymann et al., 1977; Johnston & Saxena, 1978; Saxena et al., 1980). The effect of flesinoxan and 8-OH-DPAT on the arteriovenous shunting system in conscious rats differs largely from previously reported data obtained in anaesthetized animals. In anaesthetized rabbits neither 5-HT_{1A} receptor agonist affected the conductance of the shunt vessels (Hof & Fozard, 1989), whereas a marked dose related decrease was observed in the cat (Dreteler et al., 1989) and pig (8-OH-DPAT: Bom et al., 1989). In the present study using the conscious SHR both flesinoxan and 8-OH-DPAT caused large increases in conductance of the arteriovenous shunt vessels. However, it should be realized that, compared to that in the conscious animal, arteriovenous anastomotic blood flow and conductance are much higher in pentobarbital anaesthetized animals (P.D. Verdouw et al., unpublished results), perhaps due to a decrease in the activity of the sympathetic nervous system. In the anaesthetized animals, therefore, the effects of 8-OH-DPAT (Bom et al., 1989) and, perhaps, flesinoxan, would be more marked on the vasoconstrictor 5-HT_1-like receptors (Saxena, 1989) than on the central 5-HT_{1A} receptor of which activation results in a decrease in sympathetic outflow (McCall et al., 1987; Ramage et al., 1987; Ramage et al., 1988; Saxena et al., 1990).

In conclusion, this study provides haemodynamic data on the 5-HT_{1A} receptor agonists flesinoxan and 8-OH-DPAT in the conscious freely moving SHR. The systemic and regional haemodynamic effects of these compounds are qualitatively similar. Both drugs decrease heart rate and blood pressure. Since neither flesinoxan nor 8-OH-DPAT changes cardiac output, blood pressure lowering activity results from an increased peripheral vascular conductance. The above observations further confirm the hypothesis that the increase in total peripheral conductance is not due to a generalised vasodilatation (Ramage et al., 1988). In the conscious freely moving SHR the increase in total peripheral conductance mainly results from vasodilatation in the skeletal muscles.

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CHAPTER 5

COMPARISON OF THE CARDIOVASCULAR EFFECTS OF THE 5-HT_{1A} RECEPTOR AGONIST FLESINOXAN WITH THAT OF 8-OH-DPAT IN THE RAT

Summary

The cardiovascular response to flesinoxan and 8-OH-DPAT, 5-HT_{1A} receptor agonists, has been investigated in anaesthetized Wistar rats and spontaneously hypertensive rats (SHR) and in conscious SHR. Flesinoxan and 8-OH-DPAT potently lowered blood pressure and heart rate in these models. In conscious SHR, atropine reversed the bradycardia induced by flesinoxan partially and that induced by 8-OH-DPAT completely. In pithed rats with vasopressin-raised blood pressure, neither flesinoxan nor 8-OH-DPAT lowered blood pressure or heart rate. Intracisternal administration of either flesinoxan or 8-OH-DPAT was less efficacious than intravenous administration. The cardiovascular responses to flesinoxan and 8-OH-DPAT in the anaesthetized Wistar were inhibited by the putative 5-HT_{1A} antagonists methiothepin, buspirone, spiroxatrine and 8-MeO-ClEPAT. 8-MeO-ClEPAT appeared to be the most suitable antagonist in this model. The 5-HT_{1C}, 5-HT_2 antagonist ritanserin or the 5-HT_3 antagonist GR 38032F had no effect on the responses to flesinoxan or 8-OH-DPAT. In conscious SHR however, 8-MeO-ClEPAT did not antagonize these cardiovascular responses. This study confirms the involvement of central 5-HT_{1A} receptors in the cardiovascular effects of flesinoxan and 8-OH-DPAT.

after: Dreteler, G.H., Wouters, W. & Saxena, P.R. (1990) Eur. J. Pharmacol., 180, 339-349.

Introduction

There is accumulating evidence that central serotonergic neurons play an important role in the regulation of cardiovascular function. Injection of 5-hydroxytryptamine (5-HT; serotonin) into the central nervous system causes either a pressor or a depressor response, depending upon the dose and site of administration (Kuhn et al., 1980). The centrally mediated hypotensive action of 5-HT is due to a decrease in sympathetic tone (Coote et al., 1987) and most probably results from activation of central 5-HT_{1A} receptors, since the 5-HT_{1A} agonist 8-OH-DPAT decreases blood pressure by a central action (Fozard et al., 1987; Ramage and Fozard, 1987). Further evidence for the relevance of central 5-HT_{1A} receptors in the cardiovascular effects of 5-HT is provided by the effects of DP-5-CT, R 28935, urapidil and ipsapirone, drugs that possess a high affinity for central 5-HT_{1A} receptors (Ramage and Fozard, 1987; Doods et al., 1988; Kolassa et al., 1989). Although selective 5-HT_{1A} receptor antagonists are still lacking, investigations with antagonists like (±)-pindolol, buspirone and spiroxatrine confirm the hypothesis that the 5-HT_{1A} subtype mediates the central hypotensive effect of 5-HT (Dabiré et al., 1987; Fozard et al., 1987; Doods et al., 1988).

Flesinoxan, a potent and selective 5-HT_{1A} receptor agonist, has been found to lower blood pressure and heart rate in several animal species by a central action (Wouters et al., 1988a,b). In cats, administration of flesinoxan causes sympathoinhibition and also increases vagal tone. This last phenomenon explains the bradycardia, which occurs at higher doses of the drug. The blood pressure decrease resulting from the sympathoinhibition is mainly due to a reduction in peripheral resistance (Wouters et al., 1988b; Dreteler et al., 1989; Hof and Fozard, 1989).

Here, we present a study on the cardiovascular effects of flesinoxan in both anaesthetized Wistar and spontaneously hypertensive rats (SHR) and in conscious freely moving SHR. In addition, we report the effects of several putative 5-HT receptor antagonists on the cardiovascular response to flesinoxan in anaesthetized Wistar rats. The most suitable 5-HT receptor antagonist was also used in the conscious SHR. The 5-HT_{1A} receptor agonist 8-OH-DPAT, has been used as a reference compound.

Material and methods

General in vivo procedures

Male Wistar rats (Harlan-Cpb, Zeist, The Netherlands) and SHR (Cpb, Zeist, The Netherlands; Charles River, Sulzfeld, Germany), weighing 250-350 g, were used throughout this study. Animals to be used consciously got a carotid artery cannula (medical PVC) and/or jugular vein cannula (medical PVC) implanted under halothane anaesthesia. The free ends of the implanted cannulae emerged at the nape of the neck of the animals. Animals were allowed to recover from surgery at least 1-2 days. In experiments with anaesthetized animals (sodium pentobarbitone 70 mg kg^{-1}, intraperitoneally, i.p.) the animals were provided with cannulae in a carotid artery and in a jugular vein. Pithed animals were first anaesthetized by halothane to allow the implantation of cannulae in the carotid artery, jugular vein, femoral vein and trachea to perform the pithing. The pithed animals were artificially respirated with room air (frequency 80 cycles per min, tidal volume 3 ml). Vasopressin (30 mIU kg^{-1} min^{-1}) was infused via the femoral vein to increase mean arterial blood pressure to at least 100 mmHg (Kalkman et al., 1983).

In all experiments blood pressure was recorded from the left common carotid artery by means of a pressure transducer (Statham) and heart rate was derived from the pulse pressure.

Drugs were given orally by gavage (5 ml kg^{-1}, in tragecanth), subcutaneously (s.c., 2 ml kg^{-1}), intravenously (i.v., 1 ml kg^{-1}) or intracisternally (i.c., volume < 10 μl). In the latter case the animals were placed in a stereotaxic frame. The occipito-alantoid membrane was exposed by retraction of the skin and the muscles located at the base of the skull.

Blood pressure and heart rate measurements in the anaesthetized rat

Wistar rats and SHR were instrumented for blood pressure and heart rate measurements and drug administration (see section 2.1). After completion of these surgical procedures the animals were allowed to stabilize for at least 20 min. The Wistar rats were divided into three groups receiving single i.v. doses of either flesinoxan (30, 100, 300 or 1000 μg kg^{-1}), 8-OH-DPAT (8, 32 or 128 μg kg^{-1}) or saline (1 ml kg^{-1}). The SHR were similarly divided into three groups receiving flesinoxan (100, 300 or 1000 μg kg^{-1}), 8-OH-DPAT (3, 10, 30 or 100 μg kg^{-1}) or saline. For both Wistar rats and SHR

blood pressure and heart rate effects were followed till at least 30 min after drug administration.

Blood pressure and heart rate measurements in the conscious SHR

SHR were instrumented for blood pressure and heart rate measurements and if applicable, drug administration (see section 2.1). The animals were divided into two groups. One group received flesinoxan either i.v. (0.3, 1.0, 3.0 mg kg^{-1} or vehicle) or orally (1.0, 2.0, 5.0, 10.0 mg kg^{-1} or vehicle). The other group received 8-OH-DPAT i.v. (0.032, 0.128, 0.516 mg kg^{-1} or vehicle) or s.c. (1.0, 2.5 mg kg^{-1} or vehicle). The animals were allowed a habituation period of about one hour. Thereafter, baseline values of blood pressure and heart rate were measured for one hour, drugs administered, and the cardiovascular effects observed till 2-4 hours afterwards.

Studies with putative 5-HT receptor antagonists

Male Wistar or SHR were pretreated with a putative 5-HT receptor antagonist or the vehicle, subsequently anaesthetized with sodium pentobarbitone (70 mg kg^{-1}, i.p.) and instrumented for cardiovascular recording and drug administration (see section 2.1). Sixty minutes after the pretreatment the rats were challenged for blood pressure and heart rate responses with either flesinoxan or 8-OH-DPAT.

In the experiments in which the antagonists were given i.c., the agonists flesinoxan (100 mg kg^{-1}) or 8-OH-DPAT (32 mg kg^{-1}) were given i.v. 20 minutes after the antagonist.

In conscious SHR the cardiovascular response to flesinoxan or 8-OH-DPAT was also established sixty minutes after the pretreatment with either the putative 5-HT-antagonist or the vehicle.

Drugs

Flesinoxan ((+)-(R)-N-[2-[4-[D,3-dihydro-2-hydroxymethyl-1, 4-benzodioxin-5-yl)-1-piperazinyl] ethyl]-4-fluorobenzamide.HCl), Duphar B.V.; 8-OH-DPAT (8-hydroxy-2-(di-N-propylamino) tetralin.HBr), Research Biochemicals Inc.; 8-MeO-ClEPAT (8-methoxy-2-(N-2-chloroethyl-N-n-propyl)) amino tetralin), synthesized at Duphar B.V.; buspirone.HCl (Bristol Meyers); and atropine methonitrate (Sigma) were dissolved in saline. Methiothepin maleate (Hoffman La Roche) was dissolved in distilled water. Spiroxatrine (synthesized at Duphar B.V.) was dissolved in 0.01N HCl. (±)-Pindolol (Sigma) and GR38032F (synthesized at Duphar B.V.) were dissolved in water acidified

with a few drops of 0.1N HCl. Ritanserin (AS) (Janssen) was dissolved in 43% ethanol. Vasopressin (Sandoz) was further diluted with saline. Sodium pentobarbitone was used either undiluted or diluted in saline.

Statistics

Data are expressed as means ± s.e.m. Student's t-test was used to estimate the significance of a difference between mean values; $p < 0.05$ was considered statistically significant.

Results

Effects of flesinoxan and 8-OH-DPAT on blood pressure and heart rate in the anaesthetized rat (Fig. 1)

In the Wistar rat the baseline values of the groups treated with flesinoxan (30, 100, 300 and 1000 $\mu g\ kg^{-1}$, i.v.) varied from 120 ± 6 to 135 ± 6 mmHg for blood pressure and from 374 ± 14 to 402 ± 13 beats per minute (bpm) for heart rate. Upon administration of flesinoxan both blood pressure and heart rate decreased rapidly. The largest reduction in blood pressure (36 ± 2%) was seen at the highest dose, whereas heart rate showed a maximal decrease (27 ± 3%) at the 300 $\mu g\ kg^{-1}$ dose (Fig. 1). In the SHR the baseline values for blood pressure and heart rate varied from 170 ± 7 to 185 ± 12 mmHg and 428 ± 15 to 431 ± 11 bpm, respectively. A rapid onset of reduction in both blood pressure and heart rate was seen after flesinoxan (100, 300 and 1000 $\mu g\ kg^{-1}$, i.v.). At the highest dose of flesinoxan both blood pressure and heart rate showed the largest reductions (34 ± 3% and 18 ± 3%, respectively) (Fig. 1). In the Wistar rat as in well as in the SHR the maximal decreases in blood pressure and heart rate were seen 3 to 5 minutes after administration of flesinoxan. The duration of action of flesinoxan exceeded the observation period of 30 min.

In the Wistar rat the values of blood pressure and heart rate before administration of 8-OH-DPAT (8, 32 and 128 $\mu g\ kg^{-1}$, i.v.) varied from 120 ± 6 to 132 ± 6 mmHg and 371 ± 16 to 393 ± 7 bpm respectively. 8-OH-DPAT caused rapid dose dependent decreases in both blood pressure and heart rate reaching the largest reductions of 31 ± 2% and 21 ± 2%, respectively at the highest dose (Fig. 1).

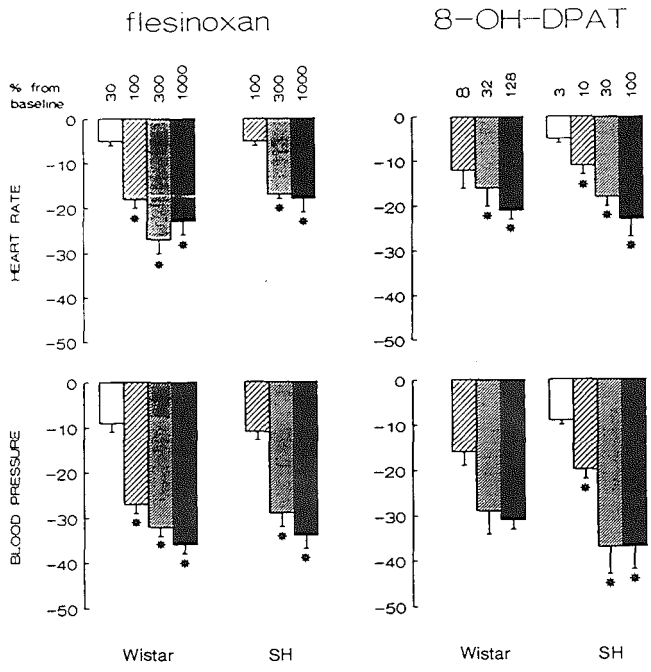

Fig. 1: Effects of i.v. administered flesinoxan and 8-OH-DPAT on mean arterial pressure and heart rate in anaesthetized Wistar rats and SHR. Figures above bars indicate doses used in $\mu g\ kg^{-1}$. Data represent maximal percentage changes from baseline values (mean ± s.e.m.; n= 5-8).
*, significantly different from vehicle treated group ($p < 0.05$).

Values of baseline blood pressure and heart rate in the SHR ranged from 175 ± 7 to 197 ± 7 mmHg and from 413 ± 15 to 436 ± 10 bpm, respectively. 8-OH-DPAT (3, 10, 30 and 100 $\mu g\ kg^{-1}$, i.v.) caused dose dependent decreases in blood pressure as well as heart rate. For blood pressure, the reduction was maximal at the 30 $\mu g\ kg^{-1}$ dose (37 ± 6%).

However, the reduction in heart rate was largest at the highest dose (23 ± 4%). In the Wistar as well as the SHR the maximal decreases in blood pressure and heart rate were seen about 5 minutes after 8-OH-DPAT. The cardiovascular effects of 8-OH-DPAT outlasted the observation period of 30 min. In these anaesthetized rats (Wistar and SHR) administration of either flesinoxan or 8-OH-DPAT always caused rapid dose dependent decreases in blood pressure and in heart rate. However, at a given dose the reduction in blood pressure exceeded the reduction in heart rate. To obtain direct evidence that these

cardiovascular effects of flesinoxan and 8-OH-DPAT are centrally mediated, as has previously been shown in cats, efforts were made to produce a dose response curve for flesinoxan and 8-OH-DPAT when given i.c. in the Wistar rat. I.c. administration of flesinoxan up to doses of 100 μg kg^{-1} did not affect blood pressure or heart rate. Injection of 300 μg kg^{-1} of flesinoxan caused a slight reduction in blood pressure (15%) but did not change heart rate. Administration of 8-OH-DPAT (8, 32 and 128 μg kg^{-1}, i.c.) caused reductions in blood pressure and heart rate with maxima of only 22 $\pm$ 4% and 13 $\pm$ 2%, respectively. Clearly, i.c. administration is less efficacious than i.v. administration of these drugs.

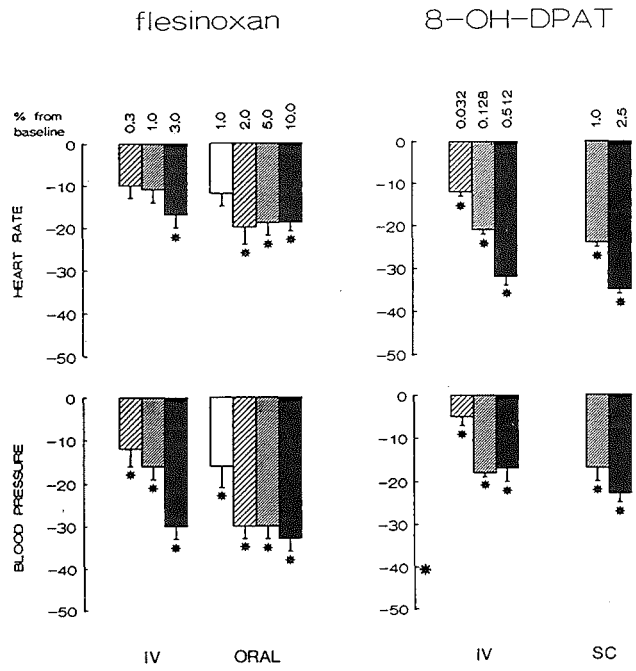

Fig. 2: Effects of flesinoxan and 8-OH-DPAT on mean arterial pressure and heart rate in conscious SHR. Flesinoxan was administered i.v. or orally; 8-OH-DPAT was given i.v. or s.c. Figures above bars indicate doses used in mg kg^{-1}. Data represent maximal percentage changes from baseline values (mean $\pm$ s.e.m.; n= 5-6).

*, significantly different from baseline values ($p < 0.05$).

Effects of flesinoxan and 8-OH-DPAT on blood pressure and heart rate in the conscious SHR (Fig. 2)

Administration of the vehicle solution to the conscious rat did not affect blood pressure and heart rate. The values of resting blood pressure and heart rate for the groups of SHR to which flesinoxan was given i.v. ranged from 142 ± 5 to 146 ± 5 mmHg and from 374 ± 12 to 422 ± 10 bpm, respectively. The groups of rats to which flesinoxan was administered orally showed baseline values that varied from 145 ± 7 to 165 ± 6 mmHg for blood pressure and from 370 ± 9 to 417 ± 16 bpm for heart rate.

I.v. administration of flesinoxan (0.3, 1 and 3 mg kg^{-1}) caused a dose dependent decrease in blood pressure (Fig. 2) which, 20 min after the highest dose, reached a maximum of 30 ± 3%. After the highest dose of flesinoxan blood pressure was still 15% below its basal level at the end of the observation period (120 min after drug administration). After oral administration of flesinoxan (1, 2, 5 and 10 mg kg^{-1}) again a profound decrease in blood pressure was seen, reaching maximal reductions 30-45 min later and leveling off at about 65-70% of the pretreatment levels (Fig. 2). After oral administration of the higher doses, flesinoxan showed a duration of action exceeding the 4 hours observation period. Flesinoxan also caused decreases in heart rate, but only to a limited extent and hardly dose related. Tachycardia was never observed.

Baseline values of the groups of SHR to which 8-OH-DPAT was given i.v. ranged from 136 ± 6 to 150 ± 3 mmHg for blood pressure and from 393 ± 3 to 398 ± 12 bpm for heart rate. For the groups that received 8-OH-DPAT s.c. baseline values for blood pressure and heart rate varied from 153 ± 6 to 164 ± 7 mmHg and from 339 ± 9 to 372 ± 7 bpm, respectively. 8-OH-DPAT at i.v. doses of 0.032 and 0.128 mg kg^{-1} caused a limited but dose-related decrease in blood pressure (maximum 18 ± 1%). The highest dose used (0.512 mg kg^{-1}) did not further decrease blood pressure. After the highest i.v. dose, 8-OH-DPAT showed a duration of action of about 70 min. High s.c. doses of 8-OH-DPAT (1 and 2.5 mg kg^{-1}) caused a dose dependent decrease in blood pressure with a maximum reduction of 23 ± 2% at the highest dose. The effects of 8-OH-DPAT on heart rate were much more pronounced and decreases of 32 ± 2% after 0.512 mg kg^{-1} i.v. and of 35 ± 2% after 2.5 mg kg^{-1} s.c. were observed. As percentage changes, the decreases in heart rate always exceeded the decreases in blood pressure at all doses tested.

Effect of atropine on the bradycardia induced by flesinoxan or 8-OH-DPAT in the conscious SHR (Fig. 3)

To see whether the bradycardia in the conscious SHR as observed in section 3.2 was caused by vagal activation, animals were treated with either saline or atropine (0.1 mg kg^{-1}, i.v.) 30 minutes after flesinoxan (3 mg kg^{-1}, i.v.) or 8-OH-DPAT (0.512 mg kg^{-1}, i.v.).

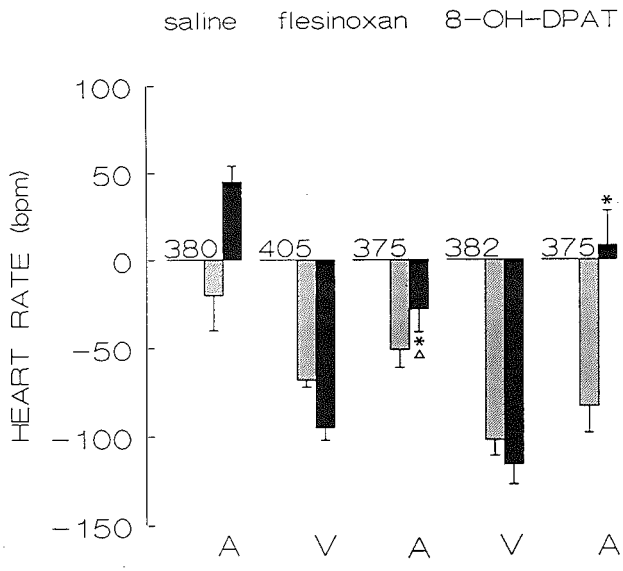

Fig. 3: Effects of saline or 0.1 mg kg^{-1} atropine on heart rate in conscious SHR pretreated with saline, flesinoxan (3 mg kg^{-1}, i.v.) or 8-OH-DPAT (0.512 mg kg^{-1}; i.v.). Figures denote baseline heart rates. The first bar of each set gives the change in heart rate just before treatment with saline or atropine; the second bar indicates the change in heart rate at the time of maximal atropine effect. Data represent mean ± s.e.m.; n= 4-6. V: Vehicle; A: Atropine.
*, $p < 0.05$ versus vehicle treated group: Δ, $p < 0.05$ versus baseline value.

Flesinoxan caused a decrease in heart rate of about 50 bpm. Subsequent treatment with atropine induced a maximum increase in heart rate of 24 bpm 20 min after administration. Animals subsequently treated with saline showed a further decrease in heart rate of 27 bpm. 8-OH-DPAT caused a strong reduction in heart rate of about 80 bpm. This bradycardia was completely abolished after administration of atropine and

heart rate was increased with 90 bpm. The animals subsequently treated with saline showed a further decrease in heart rate of about 15 bpm. In the saline pretreated group atropine also caused a marked increase in heart rate of about 80 bpm.

Effect of flesinoxan and 8-OH-DPAT on mean arterial blood pressure in the pithed rat

Pithed rats had a mean blood pressure of about 45 mmHg. Upon infusion of 30 m I.U. kg^{-1} min^{-1} vasopressin, the blood pressure was raised to about 100 mmHg. Flesinoxan up to a dose of 1 mg kg^{-1} i.v. did not affect mean arterial blood pressure in these pithed rats with artificially raised blood pressure. High doses of 8-OH-DPAT (0.128 and 0.512 mg kg^{-1},i.v.) caused transient increases in blood pressure but did not cause a reduction in blood pressure.

Effects of putative antagonists on the responses to flesinoxan and 8-OH-DPAT

Effects of the putative 5-HT_1-like receptor antagonists (±)-pindolol, buspirone, spiroxatrine, methiothepin and 8-MeO-ClEPAT in the anaesthetized Wistar rat (Table 1)

In the anaesthetized Wistar rat flesinoxan and 8-OH-DPAT were given i.v. in submaximal doses of 100 μg kg^{-1} and 32 μg kg^{-1}, respectively.
(±)-Pindolol (1 mg kg^{-1}, s.c.) almost completely inhibited the fall in blood pressure and heart rate evoked by either flesinoxan or 8-OH-DPAT. However, the β-adrenoceptor partial agonist significantly reduced baseline blood pressure by 30% whereas baseline heart rate was increased by about 20% relative to the vehicle treated animals. Buspirone (0.1, 0.25 and 1.0 mg kg^{-1}, s.c.) caused limited falls in blood pressure and heart rate and dose-dependently suppressed both the hypotension and bradycardia caused by flesinoxan as well as 8-OH-DPAT. Spiroxatrine (0.3 and 1.0 mg kg^{-1}) also caused limited decreases in both blood pressure and heart rate and dose-dependently inhibited the cardiovascular response to either flesinoxan or 8-OH-DPAT. I.p. administration of methiothepin (1 mg kg^{-1}) caused 10 to 30% decreases in baseline blood pressure and heart rate. The cardiovascular response to either flesinoxan or 8-OH-DPAT was significantly inhibited. A dose of 1 mg kg^{-1} 8-MeO-ClEPAT, given i.v., hardly affected the baseline values of both blood pressure and heart rate and produced blockade of the cardiovascular effects of flesinoxan and 8-OH-DPAT.

Table 1: Effects of pretreatment with putative (5-HT_1-like) antagonists or vehicle on baseline values of mean arterial pressure (MAP; mmHg) and heart rate (HR; bpm) and on responses to 100 μg kg^{-1} flesinoxan or 32 μg kg^{-1} 8-OH-DPAT in anaesthetized Wistar rats. Values denoted as mean ± s.e.m. *, $p < 0.05$; **, $p < 0.01$ versus vehicle pretreatment. nd: not determined.

		Baseline		Flesinoxan		
Antagonist	dose mg kg^{-1}	MAP	HR	MAP	HR	n
Vehicle		119±2	399± 9	-32±4	-63±11	6
(±)-pindolol	1.0	77±4**	482±13**	- 7±2**	-10± 4**	5
Vehicle		116±3	366±17	-26±2	-48± 7	6
Buspirone	0.1	107±3	366±19	-17±2**	-61± 5	6
	0.25	109±4	351±14	-15±2**	-26± 6*	6
	1.0	102±6	315±10*	- 9±2**	-13± 4**	6
Vehicle		126±3	395±10	-36±4	-73± 5	7
spiroxatrine	0.3	119±4	396± 9	-18±2**	-35±10**	7
	1.0	104±3**	349±10**	- 3±1**	- 9± 5**	6
Vehicle		136±2	396±15	-37±6	-60±18	6
methiothepin	1.0	94±3**	288± 8**	- 5±2**	-17± 4*	6
Vehicle		114±4	364± 8	-33±4	-94± 8	6
8-MeO-ClEPAT	0.3	110±5	357± 8	-29±2	-60± 9*	6
	1.0	117±7	358±10	- 8±2**	-12± 4**	6

		Baseline		8-OH-DPAT		
Antagonist	dose mg kg^{-1}	MAP	HR	MAP	HR	n
Vehicle		122±5	400±15	-27±2	-55±5	6
(±)-pindolol	1.0	88±4**	494±22**	- 7±2**	- 3±4**	7
Vehicle		122±5	400±15	-27±2	-55±5	6
Buspirone	0.1	117±5	403±17	-23±3	-43±10	6
	0.25	100±4**	347±10*	-16±1**	-14±6**	6
	1.0	104±3**	340± 7**	-11±2**	-16±5**	6
Vehicle		125±2	393±13	-27±3	-63±6	7
spiroxatrine	0.3	121±5	373±14	-26±5	-27±5**	7
	1.0	110±4**	336±12**	-10±6*	-21±3±*	7
Vehicle		128±3	375±10	-27±2	-59±10	5
methiothepin	1.0	103±4**	336±12*	- 9±3**	-29±8*	5
Vehicle		114±4	398±10	-34±4	-75±10	6
8-MeO-ClEPAT	0.3		nd			
	1.0	111±4	357±14*	-11±2**	-32±11*	6

Effects of i.c. administration of the putative $5HT_1$-like receptor antagonists (±)-pindolol, 8-MeO-ClEPAT and spiroxatrine in the anaesthetized Wistar rat

Administration of (±)-pindolol (30 $\mu g\ kg^{-1}$) into the cisterna magna caused large changes in baseline values of blood pressure and heart rate. Therefore no further attempt was made to study any antagonistic effect on the cardiovascular responses to flesinoxan or 8-OH-DPAT.

8-MeO-ClEPAT (10 $\mu g\ kg^{-1}$) per se decreased baseline values of blood pressure and heart rate by 10 to 20% and partially blocked the cardiovascular response to 8-OH-DPAT. Spiroxatrine (100 $\mu g\ kg^{-1}$) administered via the cisterna magna induced increases of upto 10% in blood pressure and heart rate and blocked the cardiovascular responses to both flesinoxan and 8-OH-DPAT by 50 to 70% (Table 2).

Table 2: Effects of i.c. pretreatment with 0.1 mg kg^{-1} spiroxatrine or vehicle on baseline values of mean arterial pressure (MAP; mmHg) and heart rate (HR; bpm) and on responses to 100 $\mu g\ kg^{-1}$ flesinoxan or 32 $\mu g\ kg^{-1}$ 8-OH-DPAT in anaesthetized Wistar rats. Values denoted as mean ± s.e.m. *, $p < 0.05$; **, $p < 0.01$ versus vehicle pretreatment.

		Baseline		Flesinoxan		
Antagonist	dose mg kg^{-1}	MAP	HR	MAP	HR	n
Vehicle		115±3	381±19	-28±3	-34±4	5
Spiroxatrine	0.1	128±4*	382±19	-15±3*	-17±6*	5
		Baseline		8-OH-DPAT		
Antagonist	dose mg kg^{-1}	MAP	HR	MAP	HR	n
Vehicle		128±3	378±10	-35±5	-53±7	5
Spiroxatrine	0.1	138±6	399±12	-16±5*	-12±4**	5

Effects of the $5\text{-}HT_2$ receptor antagonist ritanserin and the $5\text{-}HT_3$ receptor antagonist GR 38032 F in the anaesthetized Wistar rat (Table 3)

Ritanserin, a potent antagonist at $5\text{-}HT_2$ and $5\text{-}HT_{1C}$ receptors, given at a dose of 0.3 mg kg^{-1} s.c. neither affected baseline blood pressure and heart rate nor inhibited the cardiovascular response to both flesinoxan and 8-OH-DPAT. GR 38032 F (1 mg kg^{-1},

s.c.) a potent and selective antagonist at the 5-HT_3 receptor (Butler et al., 1988) did not alter the hypotension and bradycardia caused by either flesinoxan or 8-OH-DPAT.

Table 3: Effects of pretreatment with the 5-HT_2 antagonist ritanserin and the 5-HT_3 antagonist GR 38032F on baseline values of mean arterial pressure (MAP; mmHg) and heart rate (HR; bpm) and on responses to 100 μg kg^{-1} flesinoxan or 32 μg kg^{-1} 8-OH-DPAT in anaesthetized Wistar rats. Values denoted as mean ± s.e.m. *, $p < 0.05$ versus vehicle pretreatment.

		Baseline		Flesinoxan		
Antagonist	dose mg kg^{-1}	MAP	HR	MAP	HR	n
Vehicle		113±3	415±13	-33±2	-73±11	5
Ritanserin	0.3	113±5	403±10	-33±4	-80± 9	5
Vehicle		126±3	362±11	-32±4	-66± 5	5
GR 38032F	1.0	125±3	382±11	-25±2	-56±11	5
		Baseline		8-OH-DPAT		
Antagonist	dose mg kg^{-1}	MAP	HR	MAP	HR	n
Vehicle		123±5	425± 4	-39±3	-68± 9	5
Ritanserin	0.3	112±2	414±20	-30±2*	-75±11	5
Vehicle		123±3	394±19	-29±4	-64± 3	5
GR 38032F	1.0	126±4	391± 8	-32±5	-71± 8	5

The effect of 8-MeO-ClEPAT on the cardiovascular response to flesinoxan or 8-OH-DPAT in the SHR (Table 4)

The anaesthetized SHR was challenged for blood pressure and heart rate responses with submaximal doses of either flesinoxan (200 μg kg^{-1}, i.v.) or 8-OH-DPAT (15 μg kg^{-1}, i.v.). I.v. administration of the putative 5-HT_{1A} receptor antagonist 8-MeO-ClEPAT (1 mg kg^{-1}) induced a 15% decrease in resting blood pressure and a 10% decrease in heart rate.

In these anaesthetized SHR 8-MeO-ClEPAT only blocked the blood pressure and heart rate response to flesinoxan by 60% and 40%, respectively and that to 8-OH-DPAT by 40% and 40%, respectively, whereas in the anaesthetized Wistar rat 8-MeO-ClEPAT

blocked the blood pressure and heart rate responses to flesinoxan by 75 and 90% and to 8-OH-DPAT by 70 and 50%, respectively.

In the conscious SHR the cardiovascular response to flesinoxan and 8-OH-DPAT was induced by i.v. administration of 3 mg kg^{-1} and 0.512 mg kg^{-1} of these drugs, respectively. Pretreatment with 8-MeO-ClEPAT (3 mg kg^{-1}, i.v.) caused a 20% reduction in baseline blood pressure and heart rate and did not inhibit the cardiovascular effects of flesinoxan and 8-OH-DPAT.

Table 4: Effects of pretreatment with 1 mg kg^{-1} 8-MeO-ClEPAT or saline on baseline values of mean arterial pressure (MAP; mmHg) and heart rate (HR; bpm) and on responses to 200 μg kg^{-1} flesinoxan or 15 μg kg_{-1} 8-OH-DPAT in anaesthetized SHR. Values denoted as mean ± s.e.m. *, $p < 0.05$; **, $p < 0.01$ versus vehicle pretreatment.

		Baseline		Flesinoxan		
Antagonist	dose mg kg^{-1}	MAP	HR	MAP	HR	n
Vehicle		159±3	391±8	-36±6	-32±9	6
8-MeO-ClEPAT	1	139±5**	353±5**	-13±2**	-19±3	5
		Baseline		8-OH-DPAT		
Antagonist	dose mg kg^{-1}	MAP	HR	MAP	HR	n
Vehicle		169±10	404±9	-40±3	-49±8	6
8-MeO-ClEPAT	1	139±5*	377±5*	-20±2**	-25±5*	5

Discussion

I.v. administration of flesinoxan or 8-OH-DPAT in anaesthetized Wistar and SHR caused dose-related decreases in mean arterial blood pressure and heart rate. In conscious SHR, the effects of flesinoxan given either orally or i.v. differed from those observed in anaesthetized rats in that the dose-dependent fall in blood pressure was associated with only a small decrease in heart rate. I.v. administration of 8-OH-DPAT in conscious SHR caused a moderate decrease in blood pressure and a much larger dose-

dependent reduction in heart rate. Furthermore, the highest dose of 8-OH-DPAT used caused a further fall in heart rate, but this was not associated with a further fall in blood pressure. This apparent lack of a further decrease in blood pressure might be explained by the pronounced behavioural effects observed in these rats at this dose of 8-OH-DPAT. Over the dose range used, flesinoxan did not induce overt behavioural effects in the present experiments. This is in accordance with our previous observations (Wouters et al., 1988a) and with those of others, who also found 8-OH-DPAT to be far more potent than flesinoxan in inducing behavioural effects (Kalkman et al., 1989). In a recent detailed comparative study, evaluating the "5-HT syndrome" induced by s.c. administered 8-OH-DPAT and flesinoxan, 8-OH-DPAT was found to be about ten times more potent than flesinoxan (H.E. Molewijk; personal communication). I.c. administration of both 8-OH-DPAT and flesinoxan was less efficient than i.v. administration. These results differ from those seen in cats, where i.c. administration was clearly more efficacious than i.v. administration with respect to the cardiovascular effects of flesinoxan. However, in those experiments it was noticed that the time needed to reach the maximum effects on blood pressure was clearly longer after i.c. than after i.v. administration of flesinoxan (Wouters et al., 1988b).

In rats, intracerebroventricular administration of 8-OH-DPAT has proven to be no more effective than i.v. administration (Gradin et al., 1985; Martin and Lis, 1985). From these observations it can be concluded that neither flesinoxan nor 8-OH-DPAT exert their cardiovascular effects at cerebral sites readily accessible from either the cisterna magna or the lateral cerebral ventricle. In agreement herewith is the recent observation in dogs that the sympatho-inhibitory effects of 8-OH-DPAT were due to stimulation of 5-HT_{1A} receptors located in the ventrolateral medullary pressor area (Laubie et al., 1989).

Further evidence for a central mechanism of action was provided by the observations that neither flesinoxan nor 8-OH-DPAT lowered blood pressure in pithed rats in with the blood pressure was raised to normal levels by vasopressin. For 8-OH-DPAT these results are in agreement with earlier findings (Gradin et al., 1985; Fozard et al., 1987). Flesinoxan differed from 8-OH-DPAT in that it did not cause a pressor response.

In the present experiments with conscious SHR administration of atropine completely reversed the profound bradycardia induced by 8-OH-DPAT. These results indicate, that in contrast with earlier findings (Gradin et al., 1987) in the conscious SHR, the heart rate effects of 8-OH-DPAT seem to be totally due to vagal activation. The moderate decreases in heart rate caused by flesinoxan were only partially reversed by atropine,

indicating that an increase in vagal tone was not the sole cause of the bradycardia. In anaesthetized cats the bradycardia induced by flesinoxan or 8-OH-DPAT could be abolished by bilateral vagotomy, whereas administration of atropine counteracted the bradycardia induced by flesinoxan (Ramage et al., 1988).

The pharmacological analysis of the cardiovascular response to flesinoxan and its reference compound 8-OH-DPAT was carried out to provide (more) evidence that this response is mediated via 5-HT_{1A} receptors. Previous pharmacological analyses of the cardiovascular response to 8-OH-DPAT in both conscious (Gradin et al., 1985; Martin and Lis, 1985) and anaesthetized rats (Dabiré et al., 1987; Fozard et al., 1987) have been reported.

Various 5-HT receptor antagonists e.g. metergoline and methiothepin have failed to antagonize the cardiovascular effects of 8-OH-DPAT in conscious rats (Gradin et al., 1985; Martin and Lis, 1985). However, in anaesthetized animals attempts to block the effects of 8-OH-DPAT on blood pressure and heart rate with putative 5-HT_{1A} receptor antagonists like (±)-pindolol, buspirone, 8-MeO-ClEPAT or spiroxatrine have been more succesfull (Dabiré et al., 1987; Fozard et al., 1987). Until now no single compound has been proven to be a really useful and selective antagonist for the 5-HT_{1A} receptor. Thus, in the present study we used a combinaton of putative 5-HT_{1A} receptor antagonists like (±)-pindolol, buspirone, spiroxatrine, 8-MeO-ClEPAT and the non-selective 5-HT receptor antagonist methiothepin. (±)-Pindolol (Middlemiss et al., 1977) has been reported to antagonize the cardiovascular effect of flesinoxan in cats (Wouters et al., 1988b) and, although non selectively, the blood pressure and heart rate response to 8-OH-DPAT in rats (Fozard et al., 1987). In this study we also found the response to flesinoxan or 8-OH-DPAT to be attenuated in (±)-pindolol pretreated animals, but due to the effects of (±)-pindolol on the baseline values of mean blood pressure and heart rate per se, some involvement of a functional antagonism can not be excluded. In contrast, the results obtained with methiothepin, buspirone, spiroxatrine and 8-MeO-ClEPAT, provide strong evidence that the 5-HT_{1A} receptor is involved in the cardiovascular effects of flesinoxan as well as 8-OH-DPAT. Both buspirone and spiroxatrine, drugs with high affinity and good selectivity for the 5-HT_{1A} receptor (Peroutka et al., 1985; Nelson and Taylor, 1986) dose dependently blocked the cardiovascular response to flesinoxan and 8-OH-DPAT. Only at the highest dose, did buspirone as well as spiroxatrine affect the baseline values of blood pressure and heart rate. Previous studies with buspirone (Fozard et al., 1987) and spiroxatrine (Dabiré et

al., 1987) have yielded similar results regarding the blocking properties of both drugs for the cardiovascular effects of 8-OH-DPAT. The most suitable putative 5-HT_{1A} receptor antagonist appeared to be 8-MeO-ClEPAT, because it did not affect the baseline values of blood pressure and heart rate, which is in agreement with results of Fozard et al. (1987).

All the putative 5-HT_{1A} receptor antagonists that have been tested in this study block both the blood pressure and heart rate effects of flesinoxan as well as 8-OH-DPAT. Thus these observations are entirely consistent with the hypothesis that flesinoxan and 8-OH-DPAT exert their cardiovascular effects via a common mechanism in which the 5-HT_{1A} receptor plays the key role. Furthermore, other 5-HT receptors were excluded from being involved in the cardiovascular response to flesinoxan and 8-OH-DPAT. Both ritanserin a potent 5-HT_{1C} and 5-HT_2 receptor antagonist (Leysen et al., 1985) and GR 38032F, a selective 5-HT_3 receptor antagonist (Butler et al., 1988), failed to attenuate the blood pressure and heart rate effects of flesinoxan and 8-OH-DPAT.
In order to minimize the cardiovascular effects of the putative 5-HT_{1A} receptor antagonists per se, these drugs were administered via the cisterna magna. However, (±)-pindolol again turned out not to be useful because of its cardiovascular effects per se. 8-MeO-ClEPAT, in contrast with the i.v. administration did affect both blood pressure and heart rate, when administered i.c. I.c. administered spiroxatrine now caused moderate increases in blood pressure and heart rate. 8-MeO-ClEPAT and spiroxatrine only partially blocked the cardiovascular effects of flesinoxan and 8-OH-DPAT. This might again be due either to the fact that the cerebral sites via which flesinoxan and 8-OH-DPAT exert their effects are not readily accessible from the cisterna magna.

I.v. administered 8-MeO-ClEPAT, which appeared to be the most effective way to block the cardiovascular effects of flesinoxan and 8-OH-DPAT in the anaesthetized Wistar rat, was subsequently used in both anaesthetized and conscious SHR. However, in the anaesthetized SHR, next to an effect on blood pressure and heart rate per se, i.v. given 8-MeO-ClEPAT seemed less effective in blocking the cardiovascular response to flesinoxan or 8-OH-DPAT than in the anaesthetized Wistar rat. In the conscious SHR 8-MeO-ClEPAT itself affected blood pressure and heart rate even more than in the anaesthetized rat. Furthermore, in the conscious SHR 8-MeO-ClEPAT like metergoline and methiothepin (Gradin et al., 1985; Martin and Lis, 1985) failed to attenuate the blood pressure and heart rate effects of flesinoxan and 8-OH-DPAT.

In conclusion, the 5-HT_{1A} receptor agonists flesinoxan and 8-OH-DPAT lower blood pressure and heart rate in the anaesthetized and conscious rat via a central mechanism of action. In conscious SHR the bradycardia induced by flesinoxan is partially vagally mediated, whereas the bradycardia after 8-OH-DPAT seems to be largely caused by an increase of the vagal tone. Antagonist studies in anaesthetized Wistar rats confirm that the cardiovascular effects of flesinoxan as well as 8-OH-DPAT are due to 5-HT_{1A} receptor interaction.

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CHAPTER 6

CARDIOVASCULAR EFFECTS OF CENTRALLY ADMINISTERED 5-HT_{1A} RECEPTOR AGONISTS

Evidence has accumulated to suggest that the cardiovascular effects of 5-HT_{1A} receptor agonists are centrally mediated. However, the localization of the brain nuclei, involved in 5-HT_{1A} receptor mediated cardiovascular control, is still under investigation.

In rats, intracerebroventricular (i.c.v.) administration of 8-OH-DPAT produced hypotension but did not affect heart rate. In comparison with intraperitoneal (i.p.) (Martin & Lis, 1985) or intravenous (i.v.) (Gradin et al., 1985) administration of the drug, the time of onset for the fall in blood pressure was delayed. In addition, the i.c.v. doses required to produce the hypotension were not much different from the i.p. or i.v. doses necessary to gain significant effects. In another study, intracisternal (i.c.) administration of both 8-OH-DPAT and flesinoxan in rats also turned out to be less efficacious than the intravenous route (Dreteler et al., 1990). However, these results differed from those seen in cats. In this species i.c. administration of flesinoxan was evidently more potent than i.v. administration of this drug although the time of onset for the cardiovascular effects was prolonged (Wouters et al., 1988). In cats, administration of 8-OH-DPAT and flesinoxan and also the 5-HT_{1A} receptor agonists urapidil, DP-5-CT and R28935 into the vertebral artery, which selectively supplies the ponto medullary region of the brain (van Zwieten, 1975), has been shown to cause hypotension and bradycardia in doses lower than those which are systemically active (Doods et al., 1988; Wouters et al., 1988; Sanders et al., 1990). Furthermore, microinjection of 8-OH-DPAT into the IVth ventricle of anaesthetized cats produced sympathoinhibition and increases in vagal drive resulting in large reductions in blood pressure and heart rate (Shepheard et al., 1989). The results from these studies suggest that 5-HT_{1A} receptor agonists exert their cardiovascular effects at cerebral sites not readily accessible from the lateral cerebral ventricle or the cisterna magna. In the cat, brain nuclei surrounding the IVth ventricle might play an important role in mediating the cardiovascular effects of 5-HT_{1A} receptor agonists. Since the cisterna magna opens into the IVth ventricle, the delayed time of onset for cardiovascular effects to occur after i.c. administration might be explained by the transport time necessary for the drugs to enter the IVth ventricle.

The above described observations suggest that brain areas in the midbrain, pons and medulla could be involved in mediating the cardiovascular effects of 5-HT_{1A} receptor agonists. In addition, autoradiographic studies have demonstrated high densities of 5-HT_{1A} binding sites in several nuclei in these brain areas, that are involved in cardiovascular control (Pazos & Palacios, 1985; Vergé et al., 1986).

Dense labeling of 5-HT_{1A} binding sites was observed at the level of the midbrain dorsal and median raphe nuclei (Pazos & Palacios, 1985; Vergé et al., 1986). These nuclei provide a major source of central serotonergic pathways (Azmitia & Segal, 1987) and have been shown to play a role in blood pressure control (Kuhn et al., 1980). Connor & Higgins (1990) showed that microinjections of 8-OH-DPAT, flesinoxan and 5-CT into the dorsal raphe of conscious rats produced marked reductions in both blood pressure and heart rate. The putative 5-HT_{1A} receptor antagonist (-)pindolol attenuated the cardiovascular effects of 8-OH-DPAT, providing evidence for the effects to be 5-HT_{1A} receptor mediated. In contrast, injection of 8-OH-DPAT into the median raphe nucleus caused no cardiovascular changes.

Another area, that has been extensively investigated in several animal species, is the ventral medulla. Groups of cell bodies containing 5-HT are found in this brain region (Ciriello et al., 1988) and direct projections from this area to the intermediolateral cell column, the main site of origin of sympathetic preganglionic neurons, have been demonstrated (Loewy & McKellar, 1981). Laubie and colleagues (1988) induced hypotension, bradycardia and a decrease in renal sympathetic nerve activity by injecting 8-OH-DPAT into the ventrolateral pressor area (VLPA), an area located rostrally in the ventrolateral medulla, of anaesthetized dogs. These effects were prevented by microinjections into the same area of the non-selective 5-HT_1 receptor antagonist methiothepin, indicating that the VLPA is a major site for the 5-HT_{1A} receptor mediated sympathoinhibitory effects of 8-OH-DPAT. In cats, bilateral application of 8-OH-DPAT and another 5-HT_{1A} receptor agonist B695-40 onto the intermediate area of the ventral surface of the medulla (VSM) also causes marked reductions in blood pressure and heart rate at doses that did not affect these cardiovascular variables when given systemically (Gillis et al., 1989). The same results were obtained with application of urapidil onto this brain area (Mandal et al., 1989). The cardiovascular effects of urapidil could be counteracted by application of the 5-HT_1 receptor antagonist spiperone onto the same site but not by the 5-HT_2 receptor antagonist ketanserin. The importance of this intermediate area of the VSM was stressed by the results from successive experiments in

which topical application of spiperone to this area reversed the hypotension and bradycardia of i.v. administered urapidil (Mandal et al., 1989). The hypotensive effect of microinjection of 8-OH-DPAT into the subretrofacial nucleus and rostral part of the lateral reticular nucleus, both located within the intermediate area of the VSM, specified the nuclei responsible for the above described effects (Mandal et al., 1990).

There is immunocytochemical evidence that the nucleus paragigantocellularis lateralis (PGL), located in the rostral ventrolateral medulla, is rich in 5-HT-immunoreactive nerve terminals (Steinbusch, 1981). In rats, microinjections of 8-OH-DPAT and 5-CT into this nucleus produced large falls in blood pressure and heart rate (Lovick, 1989). Reductions in blood pressure and heart rate were also observed, when 8-OH-DPAT and 5-methylurapidil (a structural congener of urapidil) were injected into the medial parts of the area of the B_1/B_3 cell group, corresponding to the raphe pallidus and magnus nuclei within the ventral medulla (Valenta & Singer, 1990). In summary, the above data indicate an important role for the ventral medulla in 5-HT_{1A} receptor mediated cardiovascular control. Although the specific sites within the ventral medulla are still not totally clear, the above described observations suggest that at least the subretrofacial nucleus, the rostral part of the lateral reticular nucleus and the nuclei PGL, raphe pallidus and raphe magnus participate.

The nucleus tractus solitarius (NTS), located in the brainstem, plays an important role in the reflex regulation of cardiovascular variables (Miura & Reis, 1972). In rats, serotonin has been detected within the nerve terminals of the NTS (Steinbusch, 1981). Microinjection of 5-HT has variable effects on blood pressure (Wolf et al., 1981; Laguzzi et al., 1984). In addition, 5-HT_{1A} binding sites have been demonstrated to be present in the NTS of rats (B. Berkelmans, personal communication) and cats (Dashwood et al., 1988). However, microinjection of 8-OH-DPAT into the NTS of anaesthetized rats as well as dogs failed to alter either blood pressure or heart rate (Shvaloff & Laguzzi, 1986; Laubie et al., 1988), suggesting that this nucleus is not directly involved in the cardiovascular effects induced by 5-HT_{1A} receptor agonists.

The ability of 5-HT_{1A} receptor agonists to cause vagally mediated bradycardias (Ramage & Fozard, 1987; Wouters et al., 1988) has led to the suggestion that central 5-HT pathways are involved in the control of cardiac vagal motoneurons (Izzo et al., 1988). Recently, 5-HT-immunoreactive boutons have been shown to make synaptic contact with retrogradely labelled cardiac vagal motoneurons in the nucleus ambiguus of the cat (Izzo et al., 1988). Furthermore, 5-HT_{1A} binding sites can be identified within

both the dorsal vagal motor nucleus and the nucleus ambiguus (Dashwood et al., 1988). Microinjection of 8-OH-DPAT and flesinoxan into the dorsal vagal motor nucleus in anaesthetized rats caused a bradycardia (Sporton et al., 1989). The same heart rate effects were observed following injection of 8-OH-DPAT into the nucleus ambiguus of the anaesthetized cat (Izzo et al., 1988). These observations support the views that activation 5-HT_{1A} receptors in the DMVN and NA causes excitation of vagal motoneurons that results in a bradycardia.

The following two chapters will deal with the cardiovascular effects of central administration of 5-HT_{1A} receptor agonists into brain nuclei, that could be involved in 5-HT mediated cardiovascular control.

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CHAPTER 7

PRESSOR EFFECTS FOLLOWING MICROINJECTION OF 5-HT_{1A} RECEPTOR AGONISTS INTO THE RAPHE OBSCURUS OF THE ANAESTHETIZED RAT

Summary

The effects of electrical stimulation and microinjections (90 nl) of the 5-HT_{1A} receptor agonists, flesinoxan and 8-hydroxy-2(di-n-propylamino) tetralin (8-OH-DPAT), and glutamate into the raphe obscurus on blood pressure, heart rate and phrenic nerve activity (central inspiratory drive) were investigated in α-chloralose anaesthetized rats.

Electrical stimulation of the raphe obscurus caused a rise in blood pressure which was associated with bradycardia, while glutamate (2.7 nmol) caused only a rise in blood pressure. Flesinoxan (1.3 nmol) and 8-OH-DPAT (0.7 nmol) increased blood pressure by 9 ± 1 and 14 ± 2 mmHg, respectively and did not affect heart rate. For both agonists the effect on blood pressure was shown to be dose dependent; again no effect on the heart rate was observed over the dose ranges chosen. Microinjections of the non-selective 5-HT_{1A} receptor antagonists, (±)-pindolol (2.7 nmol) or methiothepin (5.2 nmol), into the raphe obscurus prevented the increase in blood pressure caused by microinjection of flesinoxan. However, (±)-pindolol caused a sustained rise in blood pressure of 15 ± 1 mmHg while methiothepin caused a transient rise in blood pressure. Neither drugs affected heart rate. The ability of methiothepin to attenuate the pressor effect of flesinoxan was found to be partially reversed after 30 min.

It is suggested that activation of 5-HT_{1A} receptors within the raphe obscurus can cause sympathoexcitation.

after: Dreteler, G.H., Wouters, W., Saxena, P.R., & Ramage A.G. (in press) Br. J. Pharmacol.

Introduction

The 5-HT_{1A} receptor agonists, flesinoxan and 8-OH-DPAT, have been shown to lower arterial blood pressure in rats (Gradin et al., 1985; Martin and Lis, 1985; Fozard et al., 1987; Dreteler et al., 1990), cats (McCall et al., 1987; Ramage & Fozard, 1987; Ramage et al., 1988; Wouters et al., 1988b) and dogs (Laubie et al., 1989) by a central action. In the rat, brain areas that contain 5-HT_{1A} binding sites are the hypothalamus, midbrain raphe nuclei, the nucleus tractus solitarius and medullary raphe nuclei (Pazos and Palacios, 1985; Vergé et al., 1986, Thor et al., 1990). All these areas are known to be involved in the central regulation of blood pressure (Loewy and Neil, 1981; Spyer, 1981). Serotonergic neurons from the nucleus raphe pallidus (B_1) and raphe obscurus (B_2) project directly to the intermediolateral cell column (Dahlström & Fuxe, 1965; Loewy, 1981; Loewy et al., 1981), the main site of origin of the sympathetic preganglionic neurons, which are the final site at which integration of cardiovascular control can occur within the central nervous system (Coote, 1988).

The present study was carried out to determine if the raphe obscurus is involved in the central hypotensive action of flesinoxan and 8-OH-DPAT by observing the effects on blood pressure and heart rate following microinjection of these 5-HT_{1A} receptor agonists into the raphe obscurus. Furthermore, as phrenic motoneurons in the cat have been shown to be surrounded by 5-HT immunoreactive fibres (Holtman et al., 1984) and chemical and electrical stimulation of the raphe obscurus in the cat causes excitation of phrenic motoneurons i.e. increases central inspiratory drive (Holtman et al., 1986), the effect of microinjection into the raphe obscurus of flesinoxan and 8-OH-DPAT was also examined on phrenic nerve activity in the rat.

A preliminary report of some of these observations has been made (Dreteler, 1990).

Materials and methods

Male Sprague-Dawley rats (250-375 g; Royal Free Hospital School of Medicine, London, U.K.) were anaesthetized initially with halothane to allow the implantation of cannulae (polythene) into the right femoral artery and vein. Subsequently, α-chloralose (70 $mg \cdot kg^{-1}$) was given intravenously (i.v.), tracheotomy was performed and the left femoral artery and vein were cannulated so that arterial blood gases could be measured

and an i.v. infusion could be given. The animals were then placed in a stereotaxic head frame. The dorsal aspect of the brainstem was exposed by retraction of the skin and muscles at the base of the skull followed by removal of the occipito-alantoid membrane and portions of the occipital bone. The animals were artificially ventilated (frequency 80 cycles per minute, tidal volume 3-4 ml) with room air enriched with oxygen following neuromuscular blockade with decamethonium iodide (1 mg per animal, i.v.). Arterial blood gases and pH were monitored throughout the experiment and maintained between 90-130 mmHg for P_O , 40-50 mmHg for P_{CO} and 7.3-7.4 for pH. Slow i.v. injections of sodium bicarbonate (1.0 M) or adjustments in respiration were made as necessary to maintain pH and blood gases within this range. The P_{CO2} was kept high in order to produce activity in the phrenic nerve. During the experiment an i.v. infusion (6 ml kg^{-1} hr^{-1}) of a solution of 50 ml gelofusine plasma substitute, 50 ml distilled water, 0.2 g glucose, 0.84 g sodium bicarbonate and 150 mg decamethonium iodide was given, to counteract the development of non-respiratory. acidosis and to maintain blood volume and neuromuscular blockade. Rectal temperature was monitored and maintained between 37 and 38 °C by means of a homeothermic blanket system. Blood pressure was recorded from the right femoral artery by means of a pressure transducer (Gould Statham P23XL) and heart rate was electronically derived from the blood pressure signal. Mean arterial blood pressure was calculated by adding one third of the pulse pressure to the diastolic pressure.

Phrenic nerve activity recordings

In animals in which phrenic nerve activity was recorded the nerve was exposed by deflecting the left scapula forward and dissecting the nerve free from the surrounding connective tissue. The nerve was cut and the central end was placed on bipolar silver hook electrodes for the recording of nerve activity. The number of events above the noise level was counted over a 5 s period using a spike processor (Digitimer D 130).

Microinjections into the raphe obscurus

Microinjections were made into the raphe obscurus using 5 barrel glass electrodes with a tip size of 25-35 μm. One barrel was filled with a 1:1 mixture of Wood's metal and Indium for electrical stimulation. Electrical stimuli were produced by a Digitimer DS2 stimulator (40Hz, 100μA, 0.5ms, 5s). The other four barrels were filled with the experimental drugs and saline. The pH of the saline solution was always adjusted to the

pH of the test solutions. The electrode was positioned 1.0-1.5 mm rostral to the obex along the midline and advanced 1.50-1.75 mm down from the brain surface according to Paxinos and Watson (1982). Once in position, 90 nl volumes of test solutions were applied by means of positive pressure injection. In all animals the site of injection was marked by application of Pontamine sky blue (2% in saline) via one of the barrels of the electrode. At the end of each experiment the animals were perfused with fixative (10% formalin; 1% glutaraldehyde solution) and the brain was removed and stored in the fixative until it was sectioned. Brains were sectioned on a freeze microtome (100 μm). The brain sections were mounted and stained with Neutral red for histological verification of the injection site.

Experimental protocols

Electrical and chemical stimulation and microinjection of single doses of flesinoxan or 8-OH-DPAT were performed in separate experiments.

In experiments used to construct dose response curves for the blood pressure effects of microinjection of flesinoxan or 8-OH-DPAT into the raphe obscurus, the time between drug injections was 15-30 min, depending on the time needed for blood pressure to return to its basal value. In the antagonist studies, a submaximal dose of flesinoxan (1.3 nmol) was microinjected initially and once the blood pressure had returned to its basal level either (±)-pindolol (2.7 nmol) or methiothepin (5.2 nmol) was microinjected, followed 5 min later by another microinjection of flesinoxan. Injections of flesinoxan were repeated at 10-15 min intervals. To investigate if the submaximal pressor response is repeatable, control experiments were performed. In these experiments only flesinoxan (1.3 nmol) was microinjected at time intervals as described for the antagonist studies.

Drugs

α-Chloralose (Sigma); L-glutamate, (Sigma); flesinoxan ((+)-(R)-N-[2-[4-(2,3-dihydro-2-hydroxymethyl-1,4-benzodioxin-5-yl)-1-piperazinyl]ethyl]-4-fluorobenzamide.HCl), Duphar B.V.; 8-OH-DPAT (8-hydroxy-2-(di-N-propylamino)tetralin.HBr), Research Biochemicals Inc. and methiothepin-monomethanesulfonate (Jilek et al., 1980), Pharmaceutical Biochem. Research Inst., Prague, Czechoslovakia were dissolved in saline. (±)-Pindolol (Sandoz) was dissolved in 0.01N HCl and saline and buffered to pH 7.4.

Statistical analysis

Data are expressed as mean ± s.e. mean. Paired Student's t-test was used to estimate the significance between mean values; $p<0.05$ was considered statistically significant.

Results

Effect of electrical and glutamate stimulation of the raphe obscurus

The baseline values for blood pressure and heart rate as well as the effects of the different treatments on blood pressure, heart rate and phrenic nerve activity are presented in Table 1.

Table 1: Effects of electrical stimulation (ES) and microinjection of glutamate, flesinoxan, 8-OH-DPAT or saline into the raphe obscurus of the anaesthetized rat on mean arterial blood pressure (MAP), heart rate (HR) and phrenic nerve activity (PNA).

Treatment	dose nmol	n	Baseline MAP(mmHg)	MAP mmHg	Baseline HR(bpm)	HR bpm	PNA %
ES		7	129±5	30±6*	404±14	-37±9*	ø
Glutamate	2.7	7	98±6	22±6*	410±14	ø	53±10*
Flesinoxan	1.3	9	101±6	9±1*	400±14	ø	ø
8-OH-DPAT	0.7	7	98±7	14±2*	407±19	ø	ø
Saline		6	104±4	ø	407±18	ø	ø

Data are expressed as mean±SEM. *, $p<0.05$ versus baseline values. ø: no effect.

Electrical stimulation of the raphe obscurus caused an immediate rise in mean arterial blood pressure (Fig. 1A). This rise in blood pressure (30±6 mmHg) was accompanied by a decrease in heart rate (37±9 beats per minute (bpm)). In two experiments there was a small increase in phrenic nerve activity (<15%), but in the other experiments there was no effect. To examine the effects of stimulating only the cell bodies in the raphe obscurus and avoiding stimulation of fibers of passage, the excitatory amino acid L-glutamate was microinjected into this nucleus. Injection of L-glutamate (2.7 nmol) into the raphe obscurus (Fig. 1B) caused an increase in blood pressure of 22±6 mmHg, had hardly any effect on heart rate and caused an increase in phrenic nerve activity

(53 ± 10%). The increase in phrenic nerve activity consisted of an increase in the number of bursts (Fig. 1B) and not of an increase in the number of events in each burst. The glutamate effects on blood pressure lasted 1-2 min. Electrical stimulation or chemical stimulation with glutamate outside the raphe obscurus (Fig. 1A and 1B) had no, or slight depressor effects. Injection of 90 nl of saline into the raphe obscurus never affected any of the variables measured.

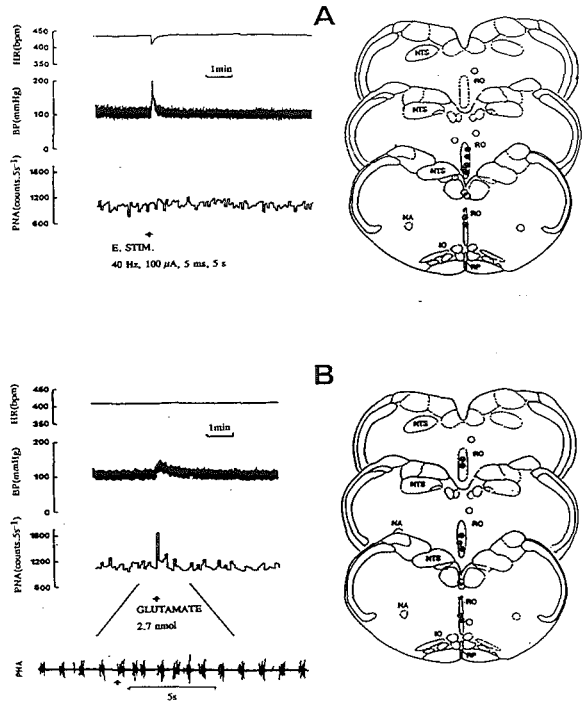

Fig. 1: Left: Effect of electrical stimulation (Fig. A) and chemical stimulation with glutamate (Fig. B) of the raphe obscurus on heart rate, blood pressure and phrenic nerve activity.
Right: Injection sites in rat brainstem at the level 1.0-2.0 mm rostral to the obex. RO, nucleus raphe obscurus; NA, nucleus ambiguus; NTS, nucleus tractus solitarius; RP, nucleus raphe pallidus; IO, inferior olive. ●: injection sites where positive effects were found; o: injection sites where no or different effects were found.

Effects of microinjection of flesinoxan or 8-OH-DPAT into the raphe obscurus

The baseline values for blood pressure and heart rate and the effects of microinjection of flesinoxan and 8-OH-DPAT into the raphe obscurus on blood pressure, heart rate and phrenic nerve activity are shown in Table 1.

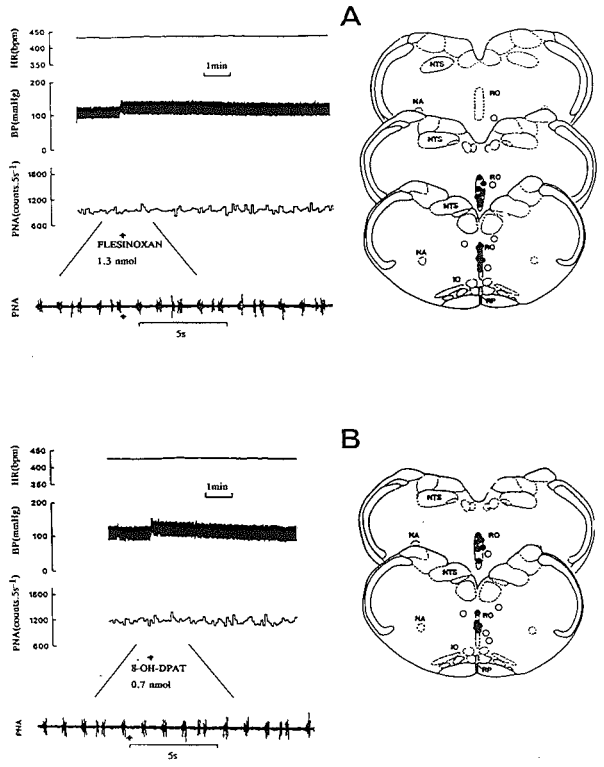

Fig. 2: Left: Effect of microinjection of flesinoxan (Fig. A) and 8-OH-DPAT (Fig. B) into the raphe obscurus on heart rate, blood pressure and phrenic nerve activity.
Right: injection sites in rat brainstem at the level 1.0-2.0 mm rostral to the obex. RO, nucleus raphe obscurus; NA, nucleus ambiguus; NTS, nucleus tractus solitarius; RP, nucleus raphe pallidus; IO, inferior olive. ●: injection sites where positive effects were found; o: injection sites where no effects were found.

Microinjection of flesinoxan (1.3 nmol; Fig. 2A) or 8-OH-DPAT (0.7 nmol; Fig. 2B) into the raphe obscurus caused an instantaneous significant increase in blood pressure of 9±1 mmHg and 14±2 mmHg, respectively which lasted between 5 and 10 min. However, the rise in blood pressure caused by these drugs was not associated with any changes in heart rate or phrenic nerve activity. Microinjection of either flesinoxan or 8-OH-DPAT outside the raphe obscurus nucleus (Fig. 2A and B) had no effects on blood pressure, heart rate or phrenic nerve activity.

In separate experiments dose response curves were constructed (Fig. 3) for the effects of flesinoxan and 8-OH-DPAT on blood pressure. Successive microinjections of increasing doses of flesinoxan (0.22-2.6 nmol) caused dose dependent increases in blood pressure (baseline value 93±4 mmHg) which at the highest dose used, reached 27±8 mmHg. Administration of 8-OH-DPAT (0.09-1.4 nmol) into the raphe obscurus also produced a dose-dependent rise in blood pressure (baseline value 91±8 mmHg) reaching a maximum of 21±6 mmHg at the highest dose given. Again, even at the highest doses administered, both flesinoxan and 8-OH-DPAT failed to have an effect on heart rate (baseline values 437±12 and 425±19 bpm, respectively) or phrenic nerve activity.

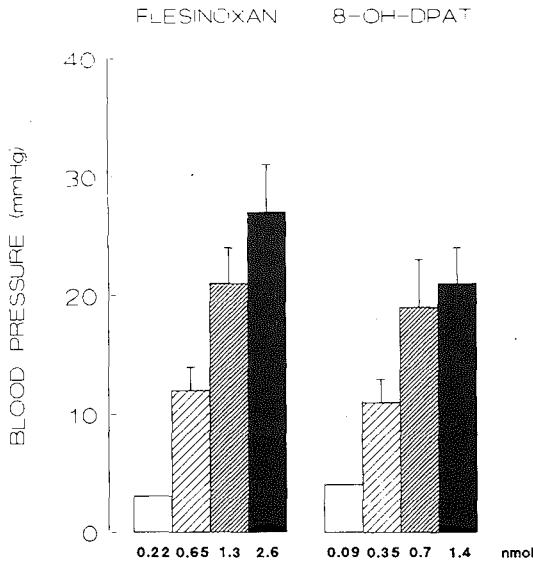

Fig. 3: Effect of microinjection of consecutive doses of flesinoxan (0.22, 0.65, 1.3 and 2.6 nmol) or 8-OH-DPAT (0.09, 0.35, 0.7 and 1.4 nmol) into the raphe obscurus of the anaesthetized rat on mean arterial blood pressure (MAP). Data are expressed as mean ± s.e. mean (n=5).

Effect of pretreatment with (±)-pindolol or methiothepin on repeated microinjections of submaximal pressor doses of flesinoxan into the raphe obscurus

In the control experiments the pressor responses, as caused by microinjection of successive doses of 1.3 nmol flesinoxan, were reproducible. Microinjection of (±)-pindolol (2.7 nmol; n=4) caused a maintained rise in blood pressure of 15±1 mmHg with no change in heart rate (baseline values 101±3 mmHg and 394±14 bpm, respectively). In these experiments the pressor response to flesinoxan was reduced from 10±2 mmHg before to 2±1 mmHg 5 min after pretreatment with (±)-pindolol. Microinjection of methiothepin (5.2 nmol; n=5) caused an increase in blood pressure of 7±2 mmHg (baseline value 104±4 mmHg) with no change in heart rate (baseline value 439±4 bpm). Blood pressure returned to near baseline value (108±9 mmHg) between 3-4 min after the injection of methiothepin. The increase in blood pressure produced by flesinoxan (13±2 mmHg) was abolished 5 min after the microinjection of methiothepin. However, in 3 experiments 30 min after the microinjection of methiothepin the pressor response to flesinoxan had returned to 6±1 mmHg.

Discussion

In the present study both electrical stimulation and chemical stimulation with glutamate and the selective 5-HT_{1A} receptor agonists, flesinoxan (Wouters et al., 1988a) and 8-OH-DPAT (Middlemiss & Fozard, 1983), consistently caused a pressor response when stimuli were made within, but not outside, the brain region known as the raphe obscurus. In the case of electrical stimulation the pressor effect was accompanied by a decrease in heart rate whereas the pressor effect caused by chemical stimulation failed to cause any change in heart rate. As stimulation with glutamate does not produce any changes in heart rate, the bradycardia caused by electrical stimulation may be due to stimulation of axons of passage. However, the rise in blood pressure caused by electrical stimulation of the raphe obscurus may have initiated a baroreceptor mediated reflex bradycardia while the pressor response after chemical stimulation with glutamate and also the 5-HT_{1A} receptor agonists may have caused a change in baroreceptor reflex preventing the expected bradycardia. The reason for the failure to observe reflex bradycardia to the pressor effect of chemical stimulation with glutamate or the 5-HT_{1A} receptor agonists of the raphe obscurus remains to be determined.

The effects of electrical stimulation on phrenic nerve activity varied from a small increase to no change in activity, whereas stimulation with glutamate caused an increase in phrenic nerve activity. However, microinjections of flesinoxan or 8-OH-DPAT had no effect on phrenic nerve activity. This difference between glutamate and flesinoxan and 8-OH-DPAT presumably reflects activation of different receptors. Flesinoxan and 8-OH-DPAT, although structurally unrelated, are selective agonists with a comparable high affinity for the 5-HT_{1A} receptor (Schoeffter & Hoyer, 1988; Hoyer, 1988, van Wijngaarden et al, 1990). Both drugs proved to be equipotent in eliciting a dose-dependent pressor response after microinjection into the raphe obscurus supporting the view that the 5-HT_{1A} receptor might mediate these blood pressure effects. Indeed, the non-selective 5-HT_1 receptor antagonist methiothepin (Schoeffter & Hoyer, 1988; Hoyer, 1988), which inhibits the cardiovascular effects of 8-OH-DPAT and flesinoxan in the anaesthetized rat (Fozard et al., 1987; Dreteler et al., 1990), appeared to reversibly block the action of flesinoxan microinjected into the raphe obscurus. Moreover, (±)-pindolol, which also displays an antagonist action at 5-HT_{1A} receptors (Schoeffter & Hoyer, 1988) and is known to block the cardiovascular effects of flesinoxan in the cat (Wouters et al., 1988b) and in the rat (although in this species some involvement of functional antagonism could not be excluded (Dreteler et al., 1990)), also attenuated the pressor effect of flesinoxan in the present study. Again, functional antagonism could not be excluded as (±)-pindolol caused a sustained rise in blood pressure similar to that observed for flesinoxan and 8-OH-DPAT. This increase in blood pressure observed with (±)-pindolol may reflect an agonist action at 5-HT_{1A} receptors (Hjorth & Carlsson, 1986). Methiothepin also caused a transient rise in pressure. However, an explanation for this action of methiothepin remains to be determined. Taken together, these observations suggest that the pressor effect elicited by flesinoxan and 8-OH-DPAT is mediated via 5-HT_{1A} receptor activation in the raphe obscurus. 5-HT_{1A} receptors do not seem to be involved in the increase in phrenic nerve activity caused by glutamate activation of the raphe obscurus. An increase in phrenic nerve activity has also been observed for injection of glutamate into the raphe obscurus in the cat (Holtman et al., 1986). But, the present results differ in that glutamate caused an increase in the number of bursts indicating an increase in frequency of respiration and not, as seen in the cat, an increase in the number of events in each burst indicating a greater depth of inspiration.

In the rat, stimulation of the midline region of the rostral medulla with glutamate has been reported to cause a small decrease in blood pressure (Minson et al., 1986). In the

present study, stimulation of neurons in the posterior part of the raphe obscurus by glutamate elicited a pressor response. This disparity in effects observed in the two studies may be due to the stimulation of different anatomical areas within the raphe obscurus. In this respect, Yusoff & Coote (1988) have shown that electrical stimulation of different areas of the raphe obscurus in the rat, though increasing skeletal muscle and skin sympathetic nerve activity, either increased or decreased renal sympathetic nerve activity. In the rat, microinjections of 5-HT_{1A} receptor agonists into other raphe nuclei e.g. raphe magnus and pallidus (Valenta & Singer, 1990) or the dorsal raphe nucleus (Connor & Higgins, 1990), in doses equivalent to the doses used in the present study, caused a decrease in blood pressure and heart rate, while microinjections into the median raphe had no effect (Connor & Higgins, 1990). The fall in blood pressure and heart rate observed after injection of the 5-HT_{1A} receptor agonists into the dorsal raphe was blocked by N-methylatropine, suggesting that these cardiovascular effects were vagally mediated (Connor & Higgins, 1990). These results, together with the results from the present study, suggest that the various raphe nuclei have complex effects on autonomic outflow. In this respect, electrical and chemical stimulation of the raphe obscurus in the rat has been reported to enhance gastric motility (McCann et al., 1989).

It is now well established that 5-HT_{1A} receptor agonists lower blood pressure via a reduction in sympathetic output (Ramage & Fozard, 1987; Ramage et al., 1988; Saxena & Villalon, 1990). Activation of central 5-HT_{1A} receptors causes inhibition (Colino & Halliwell, 1987; Sprouse & Aghajanian, 1987) of neuronal activity, which in the dorsal raphe is thought to be mediated via a somatodendritic autoreceptor (Vergé et al., 1985; Sprouse & Aghajanian, 1987). In contrast, the present results indicate that in the raphe obscurus activation of 5-HT_{1A} receptors, as well as neuronal excitation by glutamate or electrical stimulation, elicits sympathoexcitation. It is, however, not known whether the 5-HT_{1A} receptors in the raphe obscurus are located postsynaptically causing direct excitation of the neurons or presynaptically causing inhibition of an inhibitory input to these cells.

In conclusion, microinjection of the 5-HT_{1A} receptor agonists flesinoxan and 8-OH-DPAT into the raphe obscurus of the anaesthetized rat dose-dependently increases blood pressure without any change in heart rate. Since these drugs lower blood pressure following systemic administration, the consequences of the activation of raphe obscurus activation are apparently overruled by the sympathoinhibition resulting from the activation of the other sites, such as the dorsal raphe nucleus and raphe magnus and

pallidus. However, the pathways that underlie the blood pressure effects caused by microinjection of the 5-HT_{1A} agonists into the raphe obscurus and the way in which this nucleus might be involved in central cardiovascular regulation will need further investigation.

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CHAPTER 8

CARDIOVASCULAR EFFECTS OF MICROINJECTION OF 5-HT, 8-OH-DPAT AND FLESINOXAN INTO THE HYPOTHALAMUS OF THE RAT

Summary

The cardiovascular effects of injection of 5-HT and the 5-HT_{1A} receptor agonists, 8-OH-DPAT and flesinoxan, into the hypothalamus of anaesthetized spontaneously hypertensive rats (SHR) was investigated. Injection of 5-HT (10 μg) into the anterior hypothalamic (AH) region increased blood pressure with 16±2%, but did not change heart rate. Injection of 8-OH-DPAT (5 μg) into this area did not affect blood pressure or heart rate. Pressor responses (16±3%) were also observed following 5-HT (10 μg) administration directly into the ventromedial hypothalamic (VMH) area. In contrast, however, injection of 8-OH-DPAT (1.25, 2.5 and 5 μg) into the VMH caused dose-dependent reductions in both blood pressure and heart rate (22±4 and 15±3%, respectively at the highest dose). But surprisingly, injection of the 5-HT_{1A} receptor agonist flesinoxan (10 μg) into the VMH did not affect blood pressure or heart rate. The results, therefore, suggest that 5-HT_{1A} receptors are not involved in the cardiovascular effects elicited via the AH and VMH nuclei of the hypothalamus and that 8-OH-DPAT in the VMH seems to possess properties other than 5-HT_{1A} receptor agonism.

after: Dreteler, G.H., Wouters, W. & Saxena, P.R. (submitted) Eur. J. Pharmacol.

Introduction

It has been shown that central 5-hydroxytryptamine (5-HT) neurones are important in cardiovascular regulation (Kuhn et al., 1980). However, despite extensive investigations, the exact role of central 5-HT in cardiovascular control is still not clear. The cardiovascular effects of central administration of 5-HT are variable and depend on the dose, animal species and site of drug administration. In rats, intracerebroventricular (i.c.v.) injections of 5-HT mainly produced pressor responses that were greatest when the drug was given into the third cerebral ventricle (Lambert et al., 1975; Lambert et al., 1978). Since, after i.c.v. administration, drugs are widely distributed throughout the brain, the exact central sites responsible for the cardiovascular effects of i.c.v. administered 5-HT are difficult to determine. However, Smits & Struyker-Boudier (1976) found that injection of 5-HT directly into the anterior hypothalamic/preoptic (AH/PO) nucleus, which is located in the diencephalon near the third ventricle, resulted in pressor responses that could be blocked by i.c.v. administration of methysergide. The importance of the hypothalamus in 5-HT mediated cardiovascular control was further stressed by the detection of rather high levels of 5-HT in the nucleus (Saavedra et al., 1974). Furthermore, the hypothalamus receives 5-HT neurones ascending from the midbrain raphe nuclei (Azmitia & Segal., 1978; Moore et al., 1978) and electrical stimulation of these raphe nuclei produced pressor responses as a result of 5-HT release in the AH/PO nucleus (Smits et al., 1978).

8-OH-DPAT and flesinoxan are potent, centrally acting agents, that lower blood pressure and heart rate in a variety of animal models (Gradin et al., 1985; Martin & Lis, 1985; Fozard et al., 1987; Ramage & Fozard, 1987; Laubie et al., 1988; Wouters et al., 1988a,b; Dreteler et al., 1990). The cardiovascular effects are mediated by stimulation of central 5-HT_{1A} receptors (Fozard et al., 1987; Wouters et al., 1988b; Saxena & Villalòn, 1990), since they can be blocked by the 5-HT receptor antagonist methiothepin (Fozard et al., 1987; Dreteler et al., 1990) and the putative 5-HT_{1A} and 5-HT_{1B} receptor antagonist pindolol (Wouters et al., 1988; Connor & Higgins, 1990). In rats, several brain nuclei have now been shown to be involved in 5-HT_{1A} receptor mediated cardiovascular control. Activation of 5-HT_{1A} receptors in the dorsal raphe by microinjection of 8-OH-DPAT or flesinoxan into this nucleus induced hypotension and bradycardia in conscious rats (Connor & Higgins, 1990). The same blood pressure and heart rate effects were observed following injection of the 5-HT_{1A} receptor agonists 5-

methylurapidil and 8-OH-DPAT into the raphe magnus and pallidus of the anaesthetized rat (Valenta & Singer, 1990). In contrast, activation of 5-HT_{1A} receptors in raphe obscurus induced pressor responses (Dreteler et al., in press).

Autoradiographic studies have revealed that 5-HT_{1A} binding sites are also present in the hypothalamus (Vergé et al., 1986). In the present study, a possible involvement of the hypothalamus in 5-HT_{1A} receptor mediated cardiovascular control has been investigated by using 8-OH-DPAT and flesinoxan, which were injected directly into the hypothalamus of anaesthetized rats.

Material and methods

General procedures

Male spontaneously hypertensive rats (275-325 g; Charles River, Sulzfeld, F.R.G.) were anaesthetized with pentobarbital sodium (70 mg kg^{-1}; intraperitoneal). The left femoral artery was cannulated (PP10 tubing, outer diameter 0.61 mm and inner diameter 0.28 mm, connected to a length of medical PVC tubing) in order to allow blood pressure and heart rate measurements during the experiment. Subsequently, the animal was placed in a stereotaxic apparatus (David Kopf Instrument) and the skull of the animal was exposed. A stainless steel guide cannula (outer diameter: 0.8 mm; inner diameter 0.5 mm) with a length of 9.45 mm was inserted unilaterally into the brain at the stereotaxic coördinates according to Paxinos & Watson (1982): A -1.3, L +0.6, V -4.0 for the anterior hypothalamic region (AH) and A -2.8, L +1.0, V -4.0 for the ventromedial hypothalamus (VMH). Bregma was used as a point of reference for the determination of the anterior-posterior coördinate and the dorso-ventral coördinate was found by using the surface of the skull as zero. The cannula tip terminated 4.8 mm above the hypothalamus region to be injected. During the surgery and the experiment the body temperature of the animal was kept at 37 °C by means of a homeothermic blanket. Blood pressure was measured by connecting the femoral arterial cannula to a pressure transducer (Statham) and heart rate was derived from the pulse pressure.

Experimental procedures

After stabilisation of the blood pressure and heart rate, a needle (outer diameter: 0.47 mm; inner diameter: 0.15 mm) with a length of either 14.25 mm (AH) or 14.75

mm (VMH) attached to a 1 μl Hamilton syringe was inserted into the brain so that the tip of the needle was aimed at either the AH or the VMH region. The insertion of the needle consistently caused a drop in blood pressure and heart rate, which lasted approximately two minutes. When blood pressure and heart rate had returned to baseline values, 0.5 μl of a saline (0.9%) or drug solution was injected over a period of 1 minute. The effects on the cardiovascular variables were measured until 30 minutes after the hypothalamus injection. At the end of the experiment 0.5 μl of a Evans blue solution (5%) was injected into the hypothalamus region and the rat was perfused with a 10% formaldehyde solution. The brain was removed and transverse sections of a 100 μm were cut by means of a freeze microtome. Dia positive photographs were taken of the brain slices stained with Evans Blue and the injection sites were identified using the atlas of Paxinos & Watson (1982); see Fig. 1.

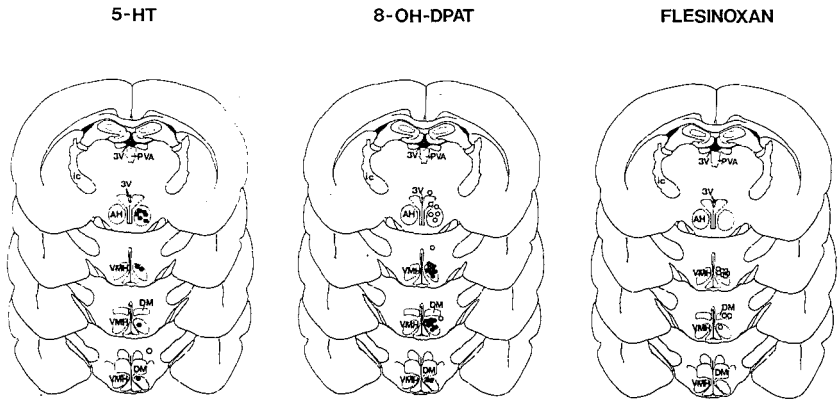

Fig. 1: Cross-sections of rat brain at the level 1.8-3.3 mm caudal from bregma (adapted from Paxinos & Watson, 1982) showing the histologically verified injections sites of 5-HT, 8-OH-DPAT and flesinoxan. ●: injection sites where positive effects were found; o: injection sites where no effects were found; □: injection sites where flesinoxan did not induce blood pressure and heart rate effects but 8-OH-DPAT did. 3V, third ventricle; PVA, anterior paraventricular thalamic nucleus; ic, internal capsule; AH, anterior hypothalamic nucleus; DM, dorsomedial hypothalamic nucleus; VMH, ventromedial hypothalamic nucleus.

Drugs

The following drugs were used: 5-HT (5-hydroxytryptamine creatinine sulfate; Sigma, St. Louis), 8-OH-DPAT (8-hydroxy-2-(di-n-propylamino)tetralin.HBr; Research Biochemicals Inc., Natick), flesinoxan ((+)-R-N-[2-[4-(2,3-dihydro-2-hydroxymethyl-1,4-benzodioxin-5-yl)-1-piperazinyl]ethyl]-4-fluorobenzamide.HCl; Duphar B.V., Weesp). All drugs were dissolved in physiological saline (0.9%) and adjusted to pH 6.0-7.0.

Statistics

All values are given as mean ± S.E.M. Statistical differences between drug and vehicle treated animals were assessed using Student's t-test (unpaired). $P<0.05$ was considered statistically significant.

Results

Effects of microinjection of 5-HT and 8-OH-DPAT into the AH nucleus

Baseline values for mean arterial blood pressure and heart rate for the various groups of animals are presented in Table 1.

Table 1: Baseline values for mean arterial blood pressure (MAP) and heart rate (HR) in the various groups of rats that were microinjected with vehicle (0.5 μl) or drug into the AH nucleus. Values are mean ± S.E.M. from n observations.

Treatment	Dose μg	Baseline value MAP	Baseline value HR	n
vehicle		156±6	351±12	6
5-HT	10	149±4	317±11	6
8-OH-DPAT	5	142±2	321±20	4

Microinjection of saline into the AH nucleus did not affect blood pressure and heart rate, but injection of 5-HT (10 μg) into the AH (Fig. 1) caused a significant increase in blood pressure (16±2%) but hardly affected heart rate (Fig. 2). The effects were immediate in onset and maximum changes were reached about 1-5 minutes after drug injection. 8-OH-

DPAT in a dose of 5 μg, injected directly into the AH, did not alter either blood pressure or heart rate (Fig. 2).

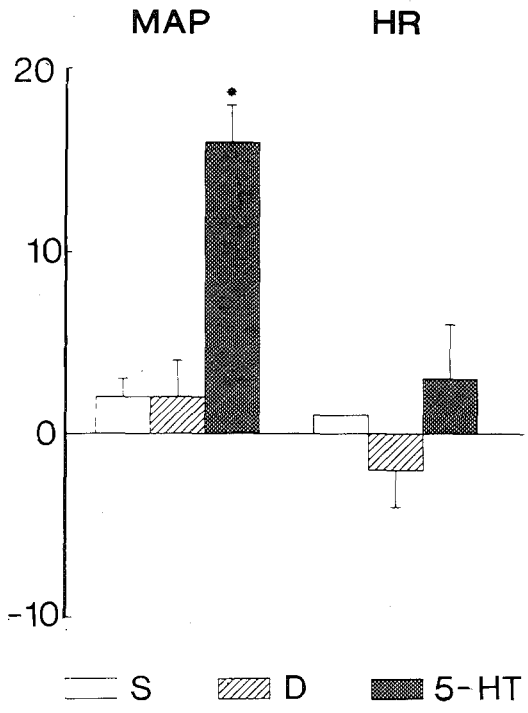

Fig. 2: The effects on blood pressure and heart rate of microinjection of 0.5 μl saline (S), 5-HT (10 μg) or 8-OH-DPAT (D; 5 μg) into the anterior hypothalamic (AH) nucleus of the anaesthetized SHR. *, $p<0.05$ versus vehicle treated animals.

Effects of microinjection of 5-HT, 8-OH-DPAT and flesinoxan into the VMH nucleus

Baseline values for the groups of rats microinjected with drug or vehicle directly into the VMH nucleus are presented in Table 2.

Injection of vehicle (0.5 μl) into the VMH did not cause any change in either blood pressure or heart rate (Fig. 3). Injection of 5-HT (10 μg) into the VMH (Fig. 1) caused an immediate significant increase in blood pressure reaching a maximum of $16\pm3\%$ (Fig. 3) after approximately 1-5 minutes. 5-HT also increased heart rate, although the sizes of the increments varied considerably (Fig. 3).

Table 2: Baseline values for mean arterial blood pressure (MAP) and heart rate (HR) in the various groups of rats that were microinjected with vehicle (0.5 μl) or drug into the VMH nucleus. Values are mean ± S.E.M. from n observations.

Treatment	Dose μg	Baseline value MAP	Baseline value HR	n
vehicle		144±3	336± 7	4
5-HT	10	139±3	330± 4	4
8-OH-DPAT	1.25	161±5	377± 8	6
8-OH-DPAT	2.5	152±5	369±11	6
8-OH-DPAT	5	146±5	347±10	6
flesinoxan	10	170±9	349± 9	8

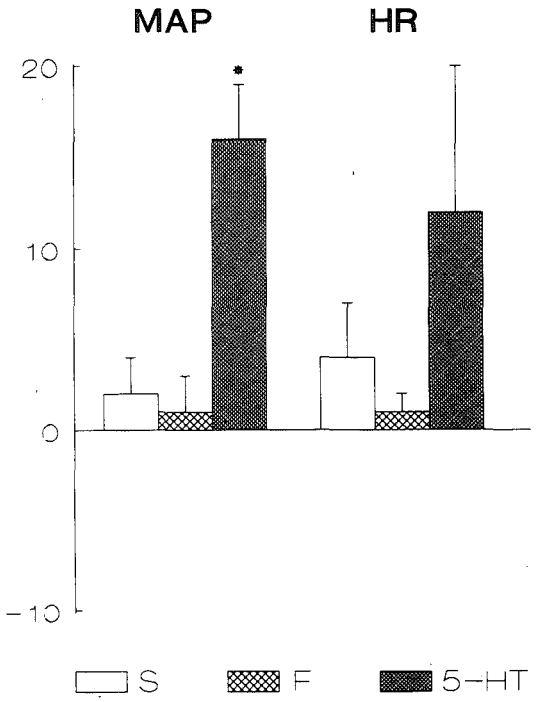

Fig. 3: The effects on blood pressure and heart rate of microinjection of 0.5 μl saline (S), flesinoxan (F; 10 μg) or 5-HT (10 μg) into the ventromedial hypothalamic (VMH) nucleus of the anaesthetized SHR. *, $p<0.05$ versus vehicle treated animals.

Microinjection of 8-OH-DPAT (1.25, 2.5 and 5 μg) into the VMH (Fig. 1) caused dose-dependent decreases in blood pressure and heart rate (Fig. 4) with the largest reductions (22$\pm$4% for blood pressure and 15$\pm$3% for heart rate) observed at the highest dose given. The effects were rapid in onset and peak changes were obtained 10-15 minutes after the injection.

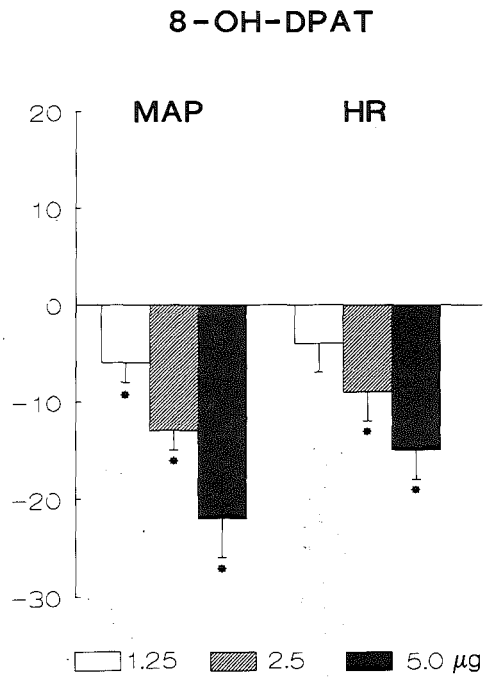

Fig. 4: The effects on blood pressure and heart rate of microinjection of 8-OH-DPAT (1.25, 2.5 and 5 μg) into the ventromedial hypothalamic (VMH) nucleus of the anaesthetized SHR. *, $p<0.05$ versus vehicle treated animals.

Microinjection of flesinoxan (10 μg) into the VMH (Fig. 1) did not modify either blood pressure or heart rate (Fig. 3). In five animals, 8-OH-DPAT (5 μg) was also microinjected into the same spot following the injection of flesinoxan (Fig. 1). In all these five experiments, 8-OH-DPAT, in contrast with flesinoxan, did cause a marked decrease in both blood pressure and heart rate.

Discussion

The aim of the present study was to investigate if the hypothalamus plays a role in 5-HT_{1A} receptor mediated cardiovascular regulation. Thus, the 5-HT_{1A} receptor agonists, 8-OH-DPAT and flesinoxan, were injected directly into two distinct areas within the hypothalamus i.e. the anterior hypothalamic (AH) and the ventromedial hypothalamic (VMH) region of anaesthetized rats. The effects on blood pressure and heart rate, following the drug injections, were studied.

In the present study, direct injection of 5-HT into the AH region caused increases in blood pressure and did not affect heart rate. These results are in agreement with the studies of Smits & Struyker-Boudier (1976), who first demonstrated that injection of 5-HT into the AH/PO region induced a pressor response, which was antagonized by i.c.v. injection of the unselective 5-HT receptor antagonist methysergide. In contrast with 5-HT, 8-OH-DPAT neither affected blood pressure nor heart rate when injected into the AH region. These results suggest that the AH nucleus, although integrated in central 5-HT induced cardiovascular effects, does not play a role in 5-HT_{1A} receptor mediated cardiovascular control.

Electrical stimulation of the VMH nucleus of anaesthetized rats evokes either pressor or depressor responses, depending on the frequency of the stimulus (Faiers et al., 1976). Furthermore, 5-HT_{1A} receptors have been localized within the VMH (Vergé et al., 1986) and are suggested to be involved in the 5-HT induced hyperpolarization of VMH neurones (Newberry, 1989). The data from the present study show that, like in the AH region, microinjection of 5-HT into the VMH region caused pressor effects. Direct administration of 8-OH-DPAT into the VMH induced dose-dependent decreases in both blood pressure and heart rate. Peak changes in these cardiovascular effects were not reached until 10-15 min following the injection, whereas peak changes in blood pressure and heart rate after intravenous administration of 8-OH-DPAT take about 5 min to occur (Dreteler et al., 1990). Furthermore, in order to gain blood pressure and heart rate effects that are quantitatively comparable with the effects of intravenous administration (Dreteler et al., 1990), relatively high doses of 8-OH-DPAT were needed, when this drug was injected directly into the VMH. This in contrast with injections of 5-HT_{1A} receptor agonists into other brain nuclei like the dorsal raphe (Connor & Higgins, 1990) and raphe pallidus and magnus (Valenta & Singer, 1990), where very low doses of 8-OH-DPAT induced marked cardiovascular effects. Moreover, no changes in either blood

pressure or heart rate were monitored following microinjection of another 5-HT_{1A} receptor agonist flesinoxan (10 μg) into the VMH region, whereas microinjection of 5 μg of flesinoxan into the dorsal raphe of conscious SHR caused marked reductions in both blood pressure and heart rate (Connor & Higgins, 1990). These results indicate that the 5-HT_{1A} receptor in the VMH is not directly involved in mediating the cardiovascular effects of 8-OH-DPAT or flesinoxan.

The discrepancy between the cardiovascular effects of 8-OH-DPAT and flesinoxan following injection into the VMH is not likely to be due to different pharmacokinetic properties of these drugs. However, recently, in several experimental models 8-OH-DPAT has been described to act on a 5-HT receptor subtype different from the 5-HT_{1A} receptor. 8-OH-DPAT contracts the dog isolated saphenous vein and increases the carotid arterial resistance in anaesthetized dogs by activation of a 5-HT_1-like receptor that is not of the 5-HT_{1A} or 5-HT_{1D} receptor subtype (Perren et al., in press). In rabbit isolated saphenous vein 8-OH-DPAT mimicks the 5-HT induced contractions by acting on a 5-HT_1-like receptor, which does not seem to correlate with any of the known 5-HT_1 binding sites (van Heuven-Nolsen et al., in press). Furthermore, Bom and colleagues (1989a) have shown that 8-OH-DPAT constricts arteriovenous anastomoses in anaesthetized pigs, which can be antagonized by methiothepin. However, in the same model, the 5-HT_{1A} receptor agonist ipsapirone appeared to be inactive with regard to the effect on arteriovenous anastomotic flow (Bom et al., 1988). In addition, the increase in arteriovenous anastomotic resistance by the 5-HT_{1A} and 5-HT_{1B} receptor agonist RU 24969, antagonizable with methiothepin, was resistant to blockade by pindolol, a putative antagonist of 5-HT_{1A} and 5-HT_{1B} receptors (Bom et al., 1989). These results indicate that in anaesthetized pigs, 8-OH-DPAT and RU 24969 constrict arteriovenous anastomoses by acting at a 5-HT_1-like receptor, that does not seem to correspond to the 5-HT_{1A} or 5-HT_{1B} receptor subtype. Therefore it is possible that the cardiovascular effects of 8-OH-DPAT administered into the VMH are, particularly in view of the lack of effectiveness of flesinoxan, mediated via a 5-HT receptor dissimilar to the 5-HT_{1A} receptor.

In conclusion, it has been demonstrated that, although the AH and VMH nucleus do play a role in 5-HT-mediated cardiovascular control, the 5-HT_{1A} receptors are not involved. Furthermore, it seems that 8-OH-DPAT may reduce blood pressure via non-5-HT_{1A} receptors in the VMH.

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CHAPTER 9

GENERAL DISCUSSION

9.1 The cardiovascular effects of 5-HT_{1A} receptor agonists.

The selective 5-HT_{1A} receptor agonists 8-OH-DPAT and flesinoxan lower blood pressure and heart rate in several animal species (Wouters et al., 1988a). In anaesthetized cats, the reduction in heart rate is always observed at doses higher than needed to reduce blood pressure (Ramage & Fozard, 1987; Wouters et al., 1988b; chapter 3). In conscious spontaneously hypertensive rats (SHR), the effects of flesinoxan differ from those observed in anaesthetized rats in that the fall in blood pressure is accompanied by only a small reduction in heart rate (chapter 5). 8-OH-DPAT, on the other hand, induces moderate decreases in blood pressure associated with large falls in heart rate in conscious SHR, whereas in anaesthetized rats large falls in blood pressure are accompanied by moderate decreases in heart rate (chapter 5).

The cardiovascular effects of both flesinoxan and 8-OH-DPAT are centrally mediated. In the anaesthetized cat, administraton of either 8-OH-DPAT or flesinoxan via the vertebral artery increases the drug's hypotensive potency compared with the intravenous route (Doods et al., 1988; Wouters et al., 1988b). Further evidence supporting a central site of action was provided by the inability of both 8-OH-DPAT and flesinoxan to lower blood pressure in the vasopressin-blood pressure supported pithed rat (Fozard et al., 1987; chapter 5).

Both 8-OH-DPAT and flesinoxan induce hypotension and bradycardia associated with organ-dependent sympatho-inhibition and an increase in vagal tone (Ramage & Wikinson, 1989). In anaesthetized cats, the bradycardia observed after both flesinoxan and 8-OH-DPAT can be abolished by vagotomy (Ramage et al., 1987; 1988), indicating that the bradycardia was mainly due to an increase in vagal tone. In conscious SHR, atropine completely reversed the bradycardia induced by 8-OH-DPAT, but only partly the bradycardia as produced by flesinoxan. These results suggest that the bradycardia after flesinoxan is not exclusively caused by an increase in vagal activity (chapter 5). Thus, 8-OH-DPAT and flesinoxan lower blood pressure as a result of sympathoinhibition. The bradycardia is mainly a consequence of vagal stimulation.

In anaesthetized cats, flesinoxan decreases blood pressure by an increase in total peripheral vascular conductance, whereas in the case of 8-OH-DPAT a decrease in cardiac output also contributes (chapter 3). In conscious (SHR), an increase in peripheral vascular conductance is the sole cause for the decrease in blood pressure induced by flesinoxan as well as 8-OH-DPAT (chapter 4). Studies in the open-chest rabbit (Hof & Fozard, 1989), open-chest cat (chapter 3) and conscious SHR (chapter 4) emphasize the similarity in the regional haemodynamic changes produced by 8-OH-DPAT and flesinoxan. The decrease in blood pressure is mainly caused by an increment in peripheral vascular conductance, which is not uniformly distributed over the various organs and tissues. Vasodilatation is predominantly observed in the splanchnic area and the skeletal muscles.

Receptor binding studies show that both flesinoxan and 8-OH-DPAT are potent and selective 5-HT_{1A} ligands (table 5, chapter 1). Although selective 5-HT_{1A} receptor antagonists are still lacking, studies in which antagonists like (±)-pindolol, buspirone, methiothepin, spiroxatrine and 8-MeO-ClEPAT were used, validate the hypothesis that the cardiovascular effects of flesinoxan and 8-OH-DPAT are mediated via the 5-HT_{1A} receptor (Dabiré et al., 1987; Fozard et al., 1987; Doods et al., 1988; Wouters et al., 1988b; chapter 5). Only very recently, 8-OH-DPAT has been described to also act on a 5-HT_1-like receptor subtype different from the 5-HT_{1A} receptor. 8-OH-DPAT contracts dog (Perren et al., in press) and rabbit (van Heuven-Nolsen et al., in press a) isolated vein by activating a non-defined 5-HT_1-like receptor. Moreover, the reduction in arteriovenous anastomotic blood flow in the anaesthetized pig as induced by 8-OH-DPAT is not mediated via one of the characterized 5-HT_1-like receptor subtypes (Bom et al., 1989; van Heuven-Nolsen, in press b).

Of the many other drugs that display 5-HT_{1A} receptor agonist activity, the anxiolytic drug ipsapirone displays qualitatively similar cardiovascular effects to 8-OH-DPAT in the anaesthetized cat, albeit less potent (Ramage & Fozard, 1987). Ipsapirone also partially blocked the cardiovascular effects of flesinoxan in the rat (unpublished observations). Buspirone lowers blood pressure and heart rate in anaesthetized cats (W. Wouters, personal communication). In addition, buspirone blocked the blood pressure and heart rate effects of 8-OH-DPAT and flesinoxan in anaesthetized rats (Fozard et al., 1987; chapter 5). Urapidil induces hypotension and, depending on the dose and route of administration, bradycardia in anaesthetized cats (Gillis et al., 1988; Mandal et al., 1989). However, the blood pressure lowering activity of this drug is only partially

mediated by central 5-HT_{1A} receptors. Blockade of peripheral α_1 receptors substantially contributes to the hypotensive action of urapidil (Gillis et al., 1988; van Zwieten, 1989). N,N-Dipropyl-5-carboxamidotryptamine (DP-5-CT) is known as a very potent 5-HT_{1A} receptor agonist (Hagenbach et al., 1986). The cardiovascular effects of DP-5-CT in the anaesthetized cat are commensurate with those of 8-OH-DPAT, although its potency to lower blood pressure is ten fold less (Doods et al., 1988). However, DP-5-CT is less lipophilic as compared with 8-OH-DPAT, which probably accounts for penetration of DP-5-CT into the brain to a lesser extent than 8-OH-DPAT. The cardiovascular effects of DP-5-CT in the rat differ from those observed in the cat, and also from those of 8-OH-DPAT and flesinoxan. Intravenous administration of DP-5-CT causes hypotension associated with reflex tachycardia in conscious rats and hypotension but no change in heart rate in pithed rats (Mir et al., 1987). In this species, the vasodepressor response of DP-5-CT, like 5-CT, has been reported to be mediated through a direct vasodilator action at a 5-HT_1-like receptor. In conclusion, compared with the above described 5-HT_{1A} receptor ligands, 8-OH-DPAT and flesinoxan, being selective full agonists for the 5-HT_{1A} receptor, hold a very interesting antihypertensive profile. These compounds induce a pronounced reduction in blood pressure, mainly via an increase in peripheral vascular conductance. The decrease in blood pressure is accompanied by a bradycardia, whereas a tachycardia is never observed.

9.2 The central mechanism of 5-HT_{1A} receptor agonist induced cardiovascular changes

The cardiovascular effects of flesinoxan and 8-OH-DPAT are mediated via central 5-HT_{1A} receptors. 5-HT_{1A} receptors are known to be located presynaptically on serotonergic cell bodies and/or dendrites and postsynaptically in major terminal areas of the serotonergic neurons (Vergé et al., 1986).

The observed increase in hypotensive potency of 5-HT_{1A} receptor agonists after administration via the vertebral artery compared with the intravenous route (Doods et al., 1988; Wouters et al., 1988) suggests the hindbrain to be an important area in 5-HT_{1A} receptor mediated cardiovascular control. By now, using a variety of techniques including: immuno- and fluorohistochemistry, autoradiography and local application of drugs by microinjection, many brain nuclei have been identified that seem to be involved in the 5-HT_{1A} receptor agonist induced cardiovascular effects.

Pretreatment of rats with PCPA, in order to deplete the brain of 5-HT, did not abolish the cardiovascular effects of 8-OH-DPAT (Fozard et al., 1987). However, specific lesions of central 5-HT neurones by intracerebroventricular administration of the neurotoxin 5,7-DHT did attenuate the cardiovascular response to 8-OH-DPAT (Mir & Fozard, 1987). These results suggest that depletion of central 5-HT neurones does not per se affect the cardiovascular response to 8-OH-DPAT, whereas the integrity of central 5-HT neurones appears to be important (Mir & Fozard, 1987). The above described studies with PCPA (Fozard et al., 1987) suggest that the cardiovascular effects of 8-OH-DPAT are mediated by postsynaptically located 5-HT_{1A} receptors. Over the last few years, evidence has also accumulated for the involvement of presynaptic 5-HT_{1A} autoreceptors in the cardiovascular effects of 5-HT_{1A} receptor agonists.

It has been shown in several species that groups of 5-HT containing neurons in the brainstem project directly to the intermediolateral cell column (IML) (Fig. 1), the main site of origin of preganglionic sympathetic neurons, which are involved in cardiovascular control (Coote, 1988). Anatomical studies in the rat indicate that the raphe pallidus, obscurus and magnus project to the IML (Loewy, 1981; Loewy & McKellar, 1981). The effects, which these 5-HT neurons have on preganglionic sympathetic activity are controversial (Coote, 1990). For electrical stimulation of the brainstem raphe nuclei both inhibitory and excitatory actions on preganglionic sympathetic neurons (Cabot et al., 1979; Coote & MacLeod, 1974) and thus variable effects on blood pressure (Adair et al., 1977) have been described. Microiontophoresis of 5-HT directly onto the preganglionic neurons mainly results in sympatho-excitation (de Groat & Ryall, 1967; Coote et al., 1981).

Microinjecton of the 5-HT_{1A} receptor agonists 8-OH-DPAT and 5-methylurapidil into the raphe pallidus and magnus (B_1/B_3 area) (Fig. 1) yielded only depressor responses associated with bradycardia (Valenta & Singer, 1990). These effects might have been caused by activation of somatodendritic 5-HT_{1A} autoreceptors in the raphe nuclei, leading to a decreased 5-HT release onto the preganglionic neurons, which then results in sympatho-inhibition. In contrast, direct application of 8-OH-DPAT and flesinoxan into the raphe obscurus produced pressor responses as did the excitatory amino acid L-glutamate (chapter 7). The mechanism underlying these 5-HT_{1A} receptor mediated pressor effects is still obscure and might consist of direct activation of postsynaptic receptors located on a excitatory neuron or presynaptic inhibition of an inhibitory input via autoreceptors. Very recently however, Bacon and colleagues (1990) showed

monosynaptic pathways between cells in the raphe pallidus and magnus, but not raphe obscurus, and preganglionic sympathetic neurons. This observation suggests that the raphe obscurus projection to the IML might be more complex than previously indicated (Loewy, 1981).

In recent years, the ventrolateral medulla has received considerable attention as an important area for the control of the circulation (Ciriello et al., 1986). Located in the rostral ventrolateral medulla, the nucleus paragigantocellularis lateralis (PGL) (Fig. 1) contains 5-HT-immunoreactive nerve terminals (Steinbusch, 1981) and provides a major excitatory drive to preganglionic sympathetic neurons in the IML (Lovick, 1987). The ventrolateral pressor area, which is located adjacent to the PGL, is also considered to be a major source of sympathetic vasomotor drive via an excitatory bulbospinal pathway to preganglionic sympathetic neurons in the IML. The injection of 8-OH-DPAT into the PGL of the rat (Lovick, 1989) and also into the ventrolateral pressor area of the dog (Laubie et al., 1989) induced depressor responses. Thus, activation of 5-HT_{1A} receptors in these regions leads to inhibition of the vasomotor drive, which results in a depressor response. It is not clear yet, whether the cardiovascular effects of 8-OH-DPAT following administration into these specific areas of the ventrolateral medulla are due to activation of 5-HT_{1A} autoreceptors (Laubie et al., 1989) or are mediated via postsynaptically located 5-HT_{1A} receptors.

In the anaesthetized cat, microinjection of 8-OH-DPAT into the subretrofacial nucleus (SRF) (Fig. 1), which is also located in the ventrolateral medulla, produced hypotension (Mandal et al., 1990). Neurones in the region of the SRF project directly to the IML and appear to comprise a major descending vasomotor pathway (Mandal et al., 1990). So, 8-OH-DPAT might be acting at 5-HT_{1A} receptors in the SRF inhibiting this descending vasomotor pathway to the preganglionic sympathetic neurones in the IML. Furthermore, Ciriello and colleagues (1988) have demonstrated the presence of 5-HT immunoreactive neurones in the ventrolateral medulla of the cat and the presence of perikarya containing 5-HT immunoreactivity in the area of the SRF. If the serotonergic neurones in the region of the SRF project directly to the IML, then microinjection of 8-OH-DPAT might activate 5-HT_{1A} autoreceptors in the SRF leading to inhibition of serotonergic neuronal firing, which then results in hypotension (Mandal et al., 1990).

In the midbrain, the dorsal raphe nucleus (Fig. 1) contains a high density of 5-HT_{1A} receptors and provides a major source of central 5-HT neuronal pathways. Evidence exists that the 5-HT_{1A} receptors in the dorsal raphe nucleus are presynaptic autoreceptors

located on serotonergic cell bodies and/or dendrites: Intracerebral administration of 5,7-DHT reduced the number of 5-HT_{1A} receptors in the dorsal raphe nucleus (Vergé et al., 1986; Weissmann-Nanopoulos et al., 1985) and 8-OH-DPAT and ipsapirone reduced the firing rate of dorsal raphe 5-HT neurones (Sprouse & Aghajanian, 1986; 1987). Microinjection of flesinoxan and 8-OH-DPAT into the dorsal raphe induced hypotension and bradycardia (Connor & Higgins, 1990). These observations suggest that the cardiovascular effects of flesinoxan and 8-OH-DPAT are mediated via somatodendritic 5-HT_{1A} autoreceptors in the dorsal raphe. The neuronal pathways, that underlie the cardiovascular effects induced by injection of the 5-HT_{1A} receptor agonists into the dorsal raphe, have not been identified as yet, but observations in different studies (Kuhn et al., 1980; Robinson et al., 1985) allow some speculation about a possible indirect role for the hypothalamus. Specific lesions of 5-HT containing fibers, that ramificate from the dorsal raphe nucleus, drastically reduced the 5-HT level in the hypothalamus and reduced the pressor response to electrical stimulation of the dorsal raphe (Robinson et al., 1985). Furthermore, 8-OH-DPAT and other 5-HT_{1A} receptor agonists inhibit the firing rate of dorsal raphe neurons via activation of somatodendritic autoreceptors (Sprouse & Aghajanian, 1986; Hjorth & Magnusson, 1988), which leads to a reduction in 5-HT function in the hypothalamus as measured by the 5-HIAA:5-HT ratio in this terminal area (Higgins et al., 1988). Lesion of the dorsal raphe nucleus does not affect the 5-HT level in the spinal cord (Palkovits et al., 1977; Robinson et al., 1985), although there is one report of a serotonergic projection to the spinal cord (Bowker et al., 1981). Lack of a reduction of 5-HT in the spinal cord, after lesioning dorsal raphe 5-HT neurones, indicates that the raphe pallidus, obscurus and magnus, which project directly to the spinal cord, remain intact, thus receiving no direct serotonergic input from the dorsal raphe nucleus. Thus, a speculative pathway in the central mechanism mediating the cardiovascular effects of 5-HT_{1A} receptor agonists might be as follows: Activation of somatodendritic 5-HT_{1A} autoreceptors within the dorsal raphe nucleus results in a decreased 5-HT release in the hypothalamus, which leads to inhibition of central sympathetic outflow. The neuronal pathway from the hypothalamus to the IML is yet unknown.

A direct role for the hypothalamus, at least for the anterior and ventromedial nuclei, in mediating the 5-HT_{1A} receptor mediated hypotension could not be demonstrated (chapter 8).

The bradycardia induced by intravenous administration of either 8-OH-DPAT or flesinoxan is due to an increase in vagal activity (Wouters et al., 1988b; chapter 5). Vagal preganglionic neurones innervating the heart are located both in the dorsal vagal motor nucleus and/or in the nucleus ambiguus (Fig. 1). 5-HT terminals surround the perikarya of neurones in both nuclei (Steinbusch, 1981) and 5-HT immunoreactive boutons have been observed in close apposition to cardiac vagal motoneurones (Izzo et al., 1988). Furthermore, in the rat 5-HT_{1A} receptors have been demonstrated to be present within the nucleus ambiguus (Pazos & Palacios, 1985). The action of 5-HT_{1A} receptor agonists on the cardiac vagal neurones is likely to be excitatory. In recent studies, microinjection of 8-OH-DPAT and flesinoxan (Sporton et al., 1989) into the dorsal vagal motor nucleus in the rat or into the nucleus ambiguus in the cat (Izzo et al., 1988) produced bradycardia. These observations suggest that activation of 5-HT_{1A} receptors within the dorsal vagal motor nucleus and/or nucleus ambiguus leads to excitation of cardiac vagal motoneurons, which results in a bradycardia.

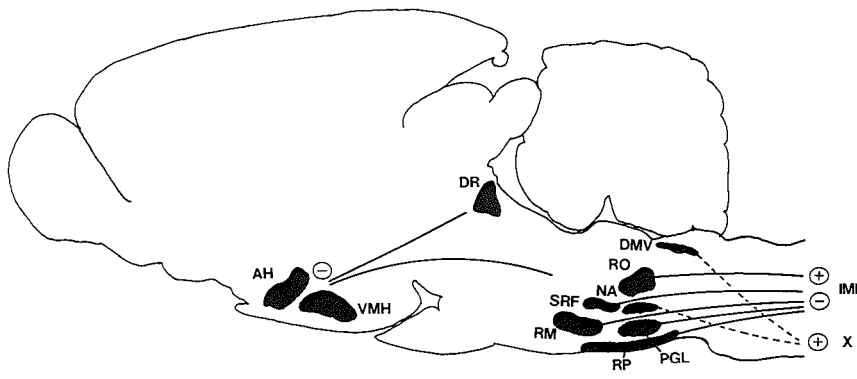

Fig. 1: Parasagittal brain section showing areas that are likely involved in the cardiovascular effects of 5-HT_{1A} receptor agonists. -: inhibitory action, +: excitatory action.
AH: anterior hypothalamus, VMH: ventromedial hypothalamus, DR: dorsal raphe nucleus, RO: raphe obscurus, RM: raphe magnus, RP: raphe pallidus, PGL: nucleus paragigantocellularis lateralis, SRF: subretrofacial nucleus, DMV: dorsal vagal motor nucleus, NA: nucleus ambiguus, IML: intermediolateral cellcolumn, X: vagus nerve.

In conclusion, the effects of 5-HT_{1A} receptor agonists are probably mediated via presynaptically located somatodendritic autoreceptors as well as via postsynaptically located receptors, depending on the brain nuclei that are integrated into the neuronal pathways neccessary for the physiological response. Studies in which separate brain nuclei are lesioned electrically or by 5,7-DHT (leaving the rest of the brain intact), followed by systemic administration of a 5-HT_{1A} receptor agonist, might provide better insight in the importance of these nuclei in 5-HT_{1A} receptor mediated cardiovascular control. Microinjection of 5-HT_{1A} receptor agonists into separate brain nuclei of PCPA pretreated animals, could reveal the location of the 5-HT_{1A} receptor (i.e. pre- or postsynaptic) in that nucleus.

9.3 Clinical significance of 5-HT_{1A} receptor activation in antihypertensive treatment

Activation of 5-HT_{1A} receptors may be considered a novel approach to antihypertensive therapy. In spite of its blood pressure lowering propreties, 8-OH-DPAT is not a suitable compound to be developed as a new antihypertensive drug, since the compound is hardly orally active. Flesinoxan has a high bioavailability and has been developed further, based on its attractive antihypertensive profile. In animals, flesinoxan induces a pronounced, long lasting decrease in blood pressure (chapter 5), which is mainly a consequence of an organ-dependent increase in peripheral vascular conductance. The decrease in blood pressure is always accompanied by a bradycardia. In contrast with peripheral vasodilator drugs, a reflex tachycardia has never been observed after administration of flesinoxan. The blood pressure lowering activity is also accompanied by a mild diuresis. Furthermore, Wouters and colleagues (1988) have shown that in spontaneously hypertensive rats, no tolerance develops to the antihypertensive effect of flesinoxan during subchronic dosing.

In a preliminary clinical study, single doses of flesinoxan (0.6-1.6 mg) caused a significant reduction in blood pressure in patients with mild essential hypertension. The effects on heart rate were less marked and, as in experimental animals, reflex tachycardia was never observed (de Voogd & Prager, 1990). Only very recently, the results of a larger, double blind, placebo controlled clinical trial (139 on treatment versus 36 on placebo) with repeated dosing of flesinoxan in essential hypertensives have come out. The design of the study consisted of a 4 week placebo run-in period followed by 10 weeks treatment with flesinoxan or placebo. Dosing started at 0.5 mg twice a day and could be increased at 2, 4 and 6 weeks to a maximum of 4 mg twice a day. Criteria for

increasing the dose were based on the diastolic blood pressure (>90 mm Hg) and tolerance. Flesinoxan appeared to be quite well tolerated. Side effects occurred mainly during the first couple of days following the installment of a higher dose. At later times during treatment the incidence of side effects diminished largely. Blood pressure was measured at the end of each 2 weeks period. At no dose-level a significant difference with respect to placebo treatment was observed.

These results show that in humans, tolerance develops to the antihypertensive effect of flesinoxan.

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SUMMARY

The first part of chapter 1 presents a review of the literature concerning the classification of the 5-HT receptors and the functional responses to activation of the different 5-HT receptor subtypes. Based on extensive studies using selective receptor agonists and antagonists, the 5-HT receptors have been categorized into four main classes; i.e. the 5-HT_1, 5-HT_2, 5-HT_3 and the 5-HT_4 receptor. Based on receptor binding studies and functional responses, the 5-HT_1 class has been further subdivided into the 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} and 5-HT_{1D} receptor subtypes. Recently, some additional 5-HT_1-like receptors have been described using functional assays. Activation of these different 5-HT receptor types yields a variety of responses. The second part of chapter 1 describes the effects of 5-HT on both peripheral and central cardiovascular regulation. Centrally administered 5-HT has complex effects on cardiovascular function. Increases, decreases and biphasic effects on autonomic outflow, blood pressure and heart rate have been described depending on the dose, site of administration and species used. A brief overview has been given of the brain nuclei possibly involved in the cardiovascular effects of centrally administered 5-HT. The complexity in cardiovascular effects of centrally administered 5-HT also reflects the presence of multiple 5-HT receptor subtypes in the central nervous system. The inhibitory and excitatory effects of 5-HT on sympathetic nervous outflow are mediated by different receptor subtypes (section 1.4.3). The 5-HT_2 receptor plays a role in the 5-HT induced sympathoexcitatory effects, whereas the 5-HT_{1A} receptor mediates the sympathoinhibitory effects. This thesis has been set up in order to further elucidate the role of the 5-HT_{1A} receptor in central cardiovascular control. To accomplish this, use has been made of the selective 5-HT_{1A} receptor agonists, 8-OH-DPAT and flesinoxan.

8-OH-DPAT and flesinoxan decrease blood pressure and heart rate in several animal species. The hypotensive action of these 5-HT_{1A} receptor agonists is mainly due to organ-dependent sympathoinhibition, which results in an increase in total peripheral conductance. In order to provide better insight into which vascular beds are responsible for this increase in vascular conductance, the haemodynamic profiles of 8-OH-DPAT and flesinoxan have been studied in both anaesthetized cats (chapter 3) and conscious spontaneously hypertensive rats (SHR) (chapter 4). In the anaesthetized cat, 8-OH-DPAT and flesinoxan both decreased blood pressure and heart rate. In the case of flesinoxan

this decrease in blood pressure was due to an increase in peripheral vascular conductance, whereas in the case of 8-OH-DPAT a reduction in cardiac output also contributed. The regional haemodynamic profiles of 8-OH-DPAT and flesinoxan are qualitatively similar. Both drugs decreased tissue perfusion in the heart, lungs, gastrointestinal tract, eyes and skin, whereas renal and cerebral blood flows were preserved as a consequence of increased vascular conductances in these organs. Especially 8-OH-DPAT increased vascular conductance in the skeletal muscles. In the conscious SHR, 8-OH-DPAT and flesinoxan both decreased blood pressure and heart rate. In this model, for both 5-HT_{1A} receptor agonists, the decrease in blood pressure was due to an increase in peripheral vascular conductance. Cardiac output was not affected, which in the case of 8-OH-DPAT might have been due to the drug induced behavioural effects. The regional haemodynamic profiles of 8-OH-DPAT and flesinoxan were qualitatively similar. Like in the anaesthetized cat, vascular conductance in the skeletal muscles was increased. It was concluded that the 5-HT_{1A} receptor agonists 8-OH-DPAT and flesinoxan decrease blood pressure mainly by an increment in peripheral vascular conductance. Vasodilatation in the skeletal muscles contributes largely to this increase in vascular conductance.

Chapter 5 deals with the pharmacology of the cardiovascular effects of flesinoxan and 8-OH-DPAT in the rat. In conscious SHR, the bradycardia induced by flesinoxan was partially and that induced by 8-OH-DPAT completely reversed by atropine. These results indicated that in contrast with 8-OH-DPAT, the heart rate effects of flesinoxan in conscious SHR are not entirely due to an increase in vagal tone. Evidence for a central mechanism of action of flesinoxan and 8-OH-DPAT was provided by the observation that both drugs did not decrease blood pressure in the vasopressin-blood pressure supported pithed rat. The cardiovascular responses to flesinoxan and 8-OH-DPAT in the anaesthetized Wistar rat were reduced by the non-selective 5-HT receptor antagonist methiothepin and the putative 5-HT_{1A} receptor antagonists (±)-pindolol, buspirone, spiroxatrine and 8-MeO-ClEPAT but resistant to the 5-HT_{1C}, 5-HT_2 receptor antagonist ritanserin and the 5-HT_3 receptor antagonist GR 38032F. It was concluded that central 5-HT_{1A} receptors are involved in the cardiovascular effects of flesinoxan and 8-OH-DPAT.

Chapter 6 deals with the different brain nuclei that might be involved in the cardiovascular effects of 5-HT_{1A} receptor agonists. Brain nuclei that seem to be directly integrated into the central mechanism of action of 5-HT_{1A} receptor agonists are the

dorsal raphe nucleus in the midbrain and several nuclei in the ventral medulla i.e. the ventrolateral pressor area, subretrofacial nucleus, rostral part of the lateral reticular nucleus, nucleus paragigantocellularis, raphe pallidus and magnus. The nucleus ambiguus and the dorsal vagal motor nucleus seem to be important in the vagally mediated bradycardias induced by 5-HT_{1A} receptor agonists.

Chapter 7 and 8 deal with the cardiovascular effects of microinjection of flesinoxan and 8-OH-DPAT in yet two other brain nuclei that might be important in 5-HT_{1A} receptor agonist induced cardiovascular effects. In chapter 7, the effects of microinjection of flesinoxan and 8-OH-DPAT into the raphe obscurus of the anaesthetized rat were investigated. 5-HT_{1A} receptors have been demonstrated in the raphe obscurus and 5-HT neurons from this nucleus project directly to the intermediolateral cell column. Microinjection of flesinoxan and 8-OH-DPAT into the raphe obscurus did not affect heart rate but did induce pressor responses as did electrical stimulation and microinjection of glutamate. The pressor responses produced by flesinoxan could be attenuated by methiothepin. It was concluded that activation of 5-HT_{1A} receptors within the raphe obscurus can cause sympathoexcitation, which results in a pressor response. In chapter 8, microinjection of 5-HT, 8-OH-DPAT and flesinoxan into the hypothalamus of the anaesthetized SHR was examined. Evidence had accumulated to state that the hypothalamus is involved in 5-HT mediated cardiovascular control. Furthermore, autoradiographic studies had revealed that 5-HT_{1A} receptors are present in the hypothalamus. Injection of 5-HT into the anterior hypothalamic region caused pressor responses but did not affect heart rate. 8-OH-DPAT injected into this region did not induce any changes in both blood pressure and heart rate. Injection of 5-HT into the ventromedial hypothalamic area also produced pressor responses, whereas injection of relatively high doses of 8-OH-DPAT into this area induced dose-dependent decreases in blood pressure and heart rate. Flesinoxan, injected into this nucleus, did not affect blood pressure or heart rate. It was concluded that neither the anterior nor the ventromedial region in the hypothalamic nucleus is directly involved in the cardiovascular effects of systemically administered 5-HT_{1A} receptor agonists.

In the first part of chapter 9, the cardiovascular effects of flesinoxan and 8-OH-DPAT in relation to their haemodynamic profiles are discussed. In this chapter, also the cardiovascular effects of some other drugs (ipsapirone, buspirone, urapidil and DP-5-CT) with affinity for the 5-HT_{1A} receptor are considered. It was concluded that flesinoxan and 8-OH-DPAT, being selective full agonists for the 5-HT_{1A} receptor hold a very interesting

blood pressure lowering profile: a pronounced reduction in blood pressure, which is mainly caused by an increase in peripheral vascular conductance. This decrease in blood pressure is accompanied by a bradycardia, whereas a tachycardia is never observed.
The second part of chapter 9 deals with the central mechanism of 5-HT_{1A} receptor agonist induced cardiovascular changes. The dorsal raphe nucleus in the midbrain and several nuclei in the medulla seem to be involved in the cardiovascular effects of 5-HT_{1A} receptor agonists. For these nuclei the neuronal pathways and the possible involvement of pre- and postsynaptic 5-HT_{1A} receptors in mediating the 5-HT_{1A} receptor agonist induced cardiovascular effects are discussed.
The last part of chapter 9 considers the clinical significance of 5-HT_{1A} receptor activation in antihypertensive treatment. Single doses of flesinoxan effectively lower blood pressure in patients with mild hypertension, whereas after chronic dosing no significant differences with respect to placebo treatment were observed. These results suggest that in humans tolerance develops to the antihypertensive effect of flesinoxan.

SAMENVATTING

Het eertste deel van hoofdstuk 1 geeft een overzicht van de literatuur betreffende de classificatie van de verschillende 5-HT receptor subtypes en de fysiologische responsen wanneer deze 5-HT receptor subtypes geactiveerd worden. Uitgebreide studies, waarin gebruik werd gemaakt van selectieve agonisten en antagonisten, hebben aangetoond dat de 5-HT receptoren kunnen worden ingedeeld in vier hoofdklassen te weten de 5-HT_1, 5-HT_2, 5-HT_3 en de 5-HT_4 receptor. Op basis van receptor binding studies en functionele studies werd de 5-HT_1 klasse verder onderverdeeld in de 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} en de 5-HT_{1D} receptor subtypes. Recentelijk zijn met behulp van functionele studies nog enige andere 5-HT_1-achtige receptoren aangetoond. Activatie van de verschillende 5-HT receptoren induceert een verscheidenheid aan responsen. Het tweede deel van hoofdstuk 1 beschrijft de effecten van 5-HT op zowel de perifere als de centrale cardiovasculaire regulatie. Wanneer 5-HT centraal wordt toegediend heeft dit verschillende effecten op het cardiovasculaire systeem. Stijgingen, dalingen en bifasische effecten op de activiteit van het autonome zenuwstelsel, op de bloeddruk en de hartfrequentie zijn beschreven, afhankelijk van de dosering, de locatie van de toediening en de diersoort. De hersenkernen, welke eventueel van belang kunnen zijn voor de cardiovasculaire effecten van centraal toegediend 5-HT een kort overzicht gegeven van. De complexiteit van de cardiovasculaire effecten van centraal toegediend 5-HT suggereert de aanwezigheid van verschillende 5-HT receptor subtypes in het centrale zenuwstelsel. De inhibitoire en excitatoire effecten van 5-HT op het sympatische zenuwstelsel kunnen worden gemedieerd via verschillende 5-HT receptor subtypes. Sympatho-excitatoire effecten van 5-HT komen tot stand via activatie van 5-HT_2 receptoren terwijl sympatho-inhibitoire effecten het gevolg zijn van activatie van 5-HT_{1A} receptoren. Het doel van dit proefschrift is de rol van de 5-HT_{1A} receptor in de centrale cardiovasculaire regulatie te verduidelijken. Hiertoe zijn studies uitgevoerd, waarin gebruik werd gemaakt van de selectieve 5-HT_{1A} receptor agonisten 8-OH-DPAT en flesinoxan.

8-OH-DPAT en flesinoxan verlagen de bloeddruk en hartfrequentie in verschillende diersoorten. De bloeddruk verlagende werking van deze 5-HT_{1A} receptor agonisten is voornamelijk het gevolg van een orgaan afhankelijke remming van het sympathische zenuwstelsel, welke resulteert in een stijging van de perifere vasculaire conductantie. Om beter inzicht te krijgen in de betrokkenheid van de diverse vaatbedden bij deze stijging in

vasculaire conductantie, zijn de haemodynamische profielen van 8-OH-DPAT en flesinoxan onderzocht in genarcotiseerde katten (hoofdstuk 3) en wakkere spontaan hypertensieve ratten (SHR) (hoofdstuk 4). 8-OH-DPAT en flesinoxan verlaagden de bloeddruk en hartfrequentie in de genarcotiseerde kat. De bloeddrukdaling na flesinoxan was het gevolg van een stijging in vasculaire conductantie, terwijl de bloeddrukdaling na 8-OH-DPAT te wijten was aan een stijging in vasculaire conductantie en een daling in het hart minuut volume. De haemodynamische profielen van 8-OH-DPAT en flesinoxan in de regionale vaatbedden zijn kwalitatief gelijk. Beide stoffen verlaagden de weefseldoorbloeding van het hart, de longen, het gastro-intestinale stelsel, de ogen en de huid. De doorbloeding van de nieren en de hersenen bleef gelijk ten gevolge van een gestegen vasculaire conductantie in deze organen. Met name 8-OH-DPAT verhoogde de vasculairë conductantie in de skelet spieren. 8-OH-DPAT en flesinoxan verlaagden beide de bloeddruk en de hartfrequentie in de wakkere SHR. In dit diermodel was de verlaging in bloeddruk voor zowel 8-OH-DPAT als flesinoxan het gevolg van een stijging in vasculaire conductantie. Het hart minuut volume bleef gelijk. In het geval van 8-OH-DPAT kan dit te wijten zijn aan gedragsactivering, welke door deze stof geinduceerd wordt. De regionale haemodynamische profielen van 8-OH-DPAT en flesinoxan zijn kwalitatief gelijk. Ook in de wakkere SHR was de vasculaire conductantie in de skeletspieren verhoogd. Uit deze resultaten wordt geconcludeerd dat de 5-HT_{1A} receptor agonisten, 8-OH-DPAT en flesinoxan, de bloeddruk voornamelijk verlagen ten gevolge van een stijging in perifere vasculaire conductantie. Deze stijging wordt voor een belangrijk deel veroorzaakt door vaatverwijding in de skeletspieren.

Hoofdstuk 5 behandelt de farmacologie, die ten grondslag ligt aan de cardiovasculaire effecten van flesinoxan en 8-OH-DPAT in de rat. De bradycardie, welke geinduceerd was door flesinoxan in de wakkere SHR kon gedeeltelijk worden opgeheven door toediening van atropine, terwijl de bradycardie, geinduceerd door 8-OH-DPAT, geheel werd te niet gedaan door atropine. Deze resultaten suggereren dat, in tegenstelling tot 8-OH-DPAT, de bradycardie na flesinoxan slechts gedeeltelijk het gevolg is van een verhoogde vagale activiteit. Zowel 8-OH-DPAT als flesinoxan hadden geen effect op de bloeddruk in ratten waarbij het centrale zenuwstelsel uitgeschakeld was, hetgeen het bewijs vormt voor een centraal werkings mechanisme van deze stoffen. De non-selectieve 5-HT receptor antagonist methiothepine en de vermeende 5-HT_{1A} receptor antagonisten (±)-pindolol, buspiron, spiroxatrine en 8-MeO-ClEPAT reduceerden de cardiovasculaire effecten van flesinoxan en 8-OH-DPAT in de genarcotiseerde Wistar rat. De 5-HT_{1C}/5-HT_2 receptor

antagonist ritanserine en de 5-HT_3 receptor antagonist GR 38032F hadden geen effect. Uit de hierboven beschreven resultaten wordt geconcludeerd dat 5-HT_{1A} receptoren in het centrale zenuw stelsel een rol spelen in de cardiovasculaire effecten van flesinoxan en 8-OH-DPAT.

In hoofdstuk 6 wordt een kort literatuur overzicht gegeven van de hersenkernen die betrokken kunnen zijn bij de cardiovasculaire effecten van 5-HT_{1A} receptor agonisten. De dorsale raphe in het mesencephalon en verschillende kernen/nuclei in de ventrale medulla te weten de ventrolaterale pressor regio, de nucleus subretrofacialis, het rostrale gedeelte van de laterale reticulaire nucleus, de nucleus paragigantocellularis en de raphe pallidus en magnus zijn waarschijnlijk direct betrokken bij het centrale werkingsmechanisme van 5-HT_{1A} receptor agonisten. The nucleus ambiguus en de dorsale vagale motor nucleus lijken van belang te zijn bij de bradycardie, die het gevolg is van activatie van de nervus vagus.

In de hoofdstukken 7 en 8 worden de cardiovasculaire effecten beschreven van flesinoxan en 8-OH-DPAT wanneer deze stoffen, via microinjectie, lokaal worden toegediend aan twee hersenkernen, die ook van belang kunnen zijn bij de cardiovasculaire effecten van 5-HT_{1A} receptor agonisten. In hoofdstuk 7 worden de effecten van microinjectie van flesinoxan en 8-OH-DPAT in de raphe obscurus van de genarcotiseerde rat beschreven. 5-HT_{1A} receptoren zijn aangetoond in the raphe obscurus en 5-HT neuronen in de raphe obscurus projecteren direct op de intermediolaterale celkolom in het ruggemerg. Microinjectie van zowel flesinoxan als 8-OH-DPAT in de raphe obscurus had geen effect op de hartfrequentie en induceerde kortdurende (5-10 minuten) bloeddrukstijgingen, welke ook werden waargenomen na electrische stimulatie en chemische stimulatie door middel van microinjectie van glutamaat. Microinjectie van methiothepine in de raphe kern verminderde de bloeddrukstijging na flesinoxan. Hieruit wordt geconcludeerd dat activatie van 5-HT_{1A} receptoren in de raphe obscurus sympatho-excitatie kan veroorzaken hetgeen leidt tot een bloeddrukstijging. Hoofdstuk 8 behandelt de cardiovasculaire effecten van microinjecties van 5-HT, 8-OH-DPAT en flesinoxan in de hypothalamus van de genarcotiseerde SHR. Er zijn aanwijzingen dat de hypothalamus een rol speelt bij de cardiovasculaire effecten, die worden geinduceerd door 5-HT. Tevens is bekend uit autoradiografische studies dat de 5-HT_{1A} receptor ook gelokaliseerd is in de hypothalamus. Injectie van 5-HT in de anterior hypothalamische regio induceerde bloeddrukstijgingen maar had geen effect op de hartfrequentie. Injectie van 8-OH-DPAT had echter geen effect op zowel de bloeddruk als de hartfrequentie. 5-HT.

toegediend in het ventromediale gedeelte van de hypothalamus, had een bloeddruk- en hartfrequentiestijging tot gevolg, terwijl relatief hoge doseringen van 8-OH-DPAT een dosis-afhankelijke daling in zowel bloeddruk als hartfrequentie veroorzaakten. Microinjectie van flesinoxan in de ventromediale hypothalamus had geen effect op de bloeddruk en de hartfrequentie. De conclusie uit deze resultaten is dat zowel het anterior als het ventromediale gedeelte van de hypothalamus geen directe rol spelen in de cardiovasculaire effecten van 5-HT_{1A} receptor agonisten.

In het eerste deel van hoofdstuk 9 worden de cardiovasculaire effecten van flesinoxan en 8-OH-DPAT in relatie tot het haemodynamische profiel van deze stoffen bediscussieerd. Tevens worden de cardiovasculaire effecten van enige andere stoffen, die affiniteit voor de 5-HT_{1A} receptor vertonen zoals ipsapiron, buspiron, urapidil en DP-5-CT besproken. Er wordt geconcludeerd, dat de selectieve, volle 5-HT_{1A} receptor agonisten, flesinoxan en 8-OH-DPAT, een interessant bloeddruk verlagend profiel vertonen. Deze stoffen induceren een bloeddrukdaling, welke voornamelijk het gevolg is van een stijging in perifere vasculaire conductantie. De hypotensie is geassocieerd met een bradycardie.

Het tweede gedeelte van hoofdstuk 9 behandelt het centrale werkingsmechanisme dat ten grondslag ligt aan de cardiovasculaire effecten van 5-HT_{1A} receptor agonisten. De dorsale raphe nucleus in het mesencephalon en verschillende nuclei in de medulla lijken direct geïntegreerd te zijn in de cardiovasculaire effecten van 5-HT_{1A} receptor agonisten. De neuronale projecties en de mogelijke rol van pre- en postsynaptische 5-HT_{1A} receptoren worden bediscussieerd.

Het laatste gedeelte van hoofdstuk 9 behandelt de mogelijke klinische betekenis van 5-HT_{1A} receptor activatie met betrekking tot de antihypertensie therapie. Een eenmalige dosering van flesinoxan veroorzaakte een significante bloeddrukdaling in hypertensieve patienten, terwijl chronische toediening van flesinoxan, in vergelijking met de placebo behandelde groep, geen significante verschillen te zien gaf. Deze resultaten suggereren dat in de mens tolerantie wordt ontwikkeld tegen het antihypertensieve effect van flesinoxan.

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CURRICULUM VITAE

De schrijfster van dit proefschrift werd op 11 juli 1961 geboren te Delden. Na het doorlopen van het gymnasium, werd in 1979 het VWO-B diploma behaald aan de Bataafse Kamp te Hengelo. In 1979 werd begonnen met de studie biologie aan de Rijksuniversiteit Groningen. Het doctoraalexamen werd in 1986 (cum laude) behaald. De hoofdvakken tijdens de doctoraalfase waren neurofysiologie (Dr. A.B. Steffens) en vegetatieve fysiologie (Dr. J.R. Brunsting). Tevens werd de bevoegdheid tot het geven van onderwijs in de biologie verkregen. Na haar studie is zij van 1986-1987 werkzaam geweest als assistent in opleiding op de afdeling Biochemie II van de Faculteit der Geneeskunde van de Erasmus Universiteit te Rotterdam (Dr. F.F.G. Rommerts). Vanaf april 1987 tot november 1990 was zij werkzaam als research assistant op de afdeling Farmacologie van Duphar B.V. te Weesp (Dr. W. Wouters), alwaar het in dit proefschrift beschreven werk gedeeltelijk werd verricht. Tevens werd een gedeelte van het in dit proefschrift beschreven onderzoek verricht op het Instituut Farmacologie van de Faculteit der Geneeskunde van de Erasmus Universiteit te Rotterdam (Prof.Dr. P.R. Saxena) en op "The Department of Pharmacology" van het Royal Free Hospital te Londen (Dr. A.G. Ramage). Sinds december 1990 is zij in dienst bij Duphar B.V. op de afdeling Farmacologie als groepschef Circulatoire Farmacologie.

APPENDIX

CHEMICAL NAMES

5-HT	5-hydroxytryptamine; serotonin
5-HTP	5-hydroxytryptophan
5,6-DHT	5,6-dihydroxytryptamine
5,7-DHT	5,7-dihydroxytryptamine
PCPA	para-chlorophenylalanine
5-CT	5-carboxamidotryptamine
LSD	lysergic acid diethylamide
8-OH-DPAT	8-hydroxy-2-(di-n-propylamino)tetralin
B695-40	1,3-dimethyl-4-(γ-[4-(0-methoxyphenyl)-piperazinyl-(1)]-propyl-amino)-5-formyl-uracil
8-MeO-ClEPAT	8-methoxy-2-(N-2-chloroethyl-N-n-propylamino)tetralin
DP-5-CT	N,N-dipropyl-5-carboxamidotryptamine
R 28935	erythro-1-{1-[2(1,4-benzodioxan-2-yl)-2-hydroxyethyl]-4-piperidyl}-2-benzimidazolinone
RU 24969	5-methoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl)1H-indole succinate
DOI	1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane
LY 53857	4-isopropyl-7-methyl-9-(2-hydroxy-1-methylpropoxycarbonyl)4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-fg]quinoline maleate
MDL 72222	1αH,3α,5αH-tropan-3-yl-3,5-dichlorobenzoate
ICS 205-930	(3α-tropanyl)-1H-indole-3-carboxylic acid ester
GR 67330	(±)1,2,3,9-tetrahydro-9-methyl-3-[(5-methyl-1-H-imidazol-4-yl)methyl]-4H-carbazol-4-one
GR 38032F	(1,2,3,9-tetrahydro-3-[methylimidazol-1-yl)methyl]-9-methyl-4H-carbazol-4-one