CARDIAC AND CAROTID VASCULAR EFFECTS OF 5-HYDROXYTRYPTAMINE-RELATED DRUGS IN THE PIG

CARLOS MIGUEL VILLALÓN HERRERA

CARDIAC AND CAROTID VASCULAR EFFECTS OF 5-HYDROXYTRYPTAMINE-RELATED DRUGS IN THE PIG

CARDIAC AND CAROTID VASCULAR EFFECTS OF 5-HYDROXYTRYPTAMINE-RELATED DRUGS IN THE PIG

CARDIALE EN CAROTID VASCULAIRE EFFECTEN VAN 5-HYDROXYTRYPTAMINE-GERELATEERDE FARMACA IN DE VARKEN

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR
AAN DE ERASMUS UNIVERSITEIT ROTTERDAM
OP GEZAG VAN DE RECTOR MAGNIFICUS
PROF.DR. C.J. RIJNVOS
EN VOLGENS BESLUIT VAN HET COLLEGE VAN DEKANEN.

DE OPENBAAR VERDEDIGING ZAL PLAATSVINDEN OP
WOENSDAG 18 SEPTEMBER 1991 OM 13:45 UUR

door

CARLOS MIGUEL VILLALóN HERRERA
GEBOREN TE MEXICO STAD

universiteits drukkerij
1991

PROMOTIECOMMISSIE

PROMOTOR:	PROF.DR. P.R. SAXENA
OVERIGE LEDEN:	PROF.DR. A.J. MAN IN'T VELD
	PROF.DR. P.D. VERDOUW
	PROF.DR. P.A. VAN ZWIETEN

1991

Dedicatoria:

La presente tesis está completamente dedicada a mi madre, Sra. María Herrera Carmona, por su sublime dedicación y apoyo durante cada etapa de mi vida, y a la memoria de mi padre, Sr. Miguel Villalón Alemán.

CONTENTS

PART I: LITERATURE SURVEY AND AIMS OF THE THESIS

PART I

CHAPTER 1

GENERAL INTRODUCTION

CHAPTER 1

GENERAL INTRODUCTION

1.1 ANTECEDENTS

Serotonin, chemically known as 5-hydroxytryptamine (5-HT), is a biogenic monoamine with a molecular weight of 176 dalton. In mammals, 5-HT is mainly found in the platelets, enterochromaffin cells and in the central nervous system (CNS), where it plays an important role as a neurotransmitter (Page, 1958).

Thanks to the seminal discovery of selective 5-HT receptor agonists and antagonists, many unsuspected functions of 5-HT in the CNS and the periphery have been elucidated. Accordingly, these findings originated a new era in the development of drugs with therapeutic usefulness in the treatment of pathologies such as anxiety, schizophrenia, hypertension, migraine, peripheral vascular diseases, etc.

1.1.1 Serotonin in the 19th century

Long before the identification of serotonin as 5-hydroxytryptamine (5-HT), pharmacologists and physiologists already knew that a substance active on blood vessels and heart appears in serum when blood is allowed to clot. The first set of publications describing objectively that blood serum is by no means an innocuous substance dates back to the 19th century, when researchers such as Creite (1869), Rummo and Bordoni (1889) and Weiss (1896) reported that a diminution in the amount of urine, albuminuria, an increase in both respiratory frequency and heart rate, and an increase in blood pressure followed by a sudden decrease (and eventually death) were induced when serum was injected subcutaneously or intravenously into an animal.

Of particular interest are the cardiovascular effects observed by Weiss (1896), namely that "if serum was continuously but slowly injected into a rabbit, cat or dog until it caused death, the heart rate was increased and at first the beat was stronger but gradually failed, though it persisted until the respiration had ceased; in addition, an

increase in the peristalsis of the small intestines and death due to paralysis of the respiratory and vasomotor centres was noticed" (Weiss, 1896).

1.1.2 Serotonin (5-hydroxytryptamine; 5-HT) in the 20th century

Subsequently, Brodie (1900) described in an extensive study that the intravenous injection of blood serum from any source into the cat produced a vagally-mediated reflex resulting in a reversible bradycardia, hypotension and arrest of the respiration, whereas injection of either blood plasma or boiled serum did not show this effect.

Simultaneously, the vasoconstrictor properties of blood serum on perfused organs and excised arterial strips were being described and investigated as to its nature and origin. With respect to the latter, Janeway et al. (1918) reported some findings of particular relevance, namely: i) that either the ox uncoagulated blood or the citrated plasma has no constrictor action on the excised strip of the ox carotid, in contrast to the marked constrictor action exerted by serum to which citrate had been subsequently added; ii) that the extract of platelets from either dog, ox or pig shows marked vasoconstrictor properties, but not that of erythrocytes or leucocytes; and iii) that either the "platelet substance(s)" or the blood serum causes a constriction of the ox coronary artery, whereas adrenaline causes relaxation. Therefore, Janeway et al. (1918) concluded that the vasoconstrictor substance of clotted blood is derived from the disintegration of platelets, and that it is not adrenaline. Based on this conclusion, Hirose (1918) confirmed and extended these findings by establishing for the first time a direct correlation between the platelet count of human blood and its vasoconstrictor action after clotting in the ox carotid artery. From all the above findings it is quite clear that the basic cardiovascular effects of blood serum could be characterized before the chemical nature of the substance involved could have been elucidated; there was no further substantial advance in connection with the latter until the late 1940s, when Page, who was searching for humoral pressor agents that might explain arterial hypertension, in collaboration with Green and Rapport could isolate this vasoconstrictor substance as a crystalline complex and named it serotonin, as it could indeed increase the vascular tone (Rapport et al., 1948a,b); shortly thereafter, Rapport (1949) deduced that the active moiety of this complex (for which he retained the name serotonin) was 5-hydroxytryptamine (5-HT). This compound, when prepared synthetically by Hamlin and Fischer (1951) and others, proved to have all the properties of natural serotonin, including those in the gastrointestinal system (Erspamer and Asero, 1952). It is

therefore not surprising that the introduction of synthetic 5-HT in 1951 (see Hamlin and Fischer, 1951) touched off an explosion of research.

1.2 DISTRIBUTION AND METABOLIC PATHWAYS OF 5-HT

When serotonin was identified as 5-hydroxytryptamine (5-HT), an extensive mass of evidence showed that it is widely distributed in nature as it had been found not only in the animal kingdom (vertebrates and invertebrates), but also in the vegetable kingdom (plants, fruits, nuts, etc.; see Erspamer, 1966).

1.2.1 Localization

In mammals 5-HT can be found in the central nervous system, where it plays an important role as a neurotransmitter (Page, 1958) and in several peripheral structures such as the enterochromaffin cells of the gastrointestinal mucosa, platelets, certain nerves of the blood vessels, the blood vessels wall, lungs, and heart (amongst others).

1.2.2 Biosynthesis

Perhaps with the exception of 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. 5-HT is synthesized de novo from the essential amino acid L-tryptophan by two enzymatic reactions: in the first reaction 5-hydroxytryptophan is formed, catalyzed by the cytoplasmic enzyme tryptophan 5-hydroxylase, the activity of which is rate limiting (Gershon et al., 1977). Subsequently, 5-hydroxytryptophan is converted into 5-hydroxytryptamine (5-HT; serotonin) catalyzed by the enzyme aromatic L-amino acid decarboxylase, which is also involved in the biosynthesis of catecholamines (Douglas, 1985).

1.2.3 Biodegradation

The predominant metabolic pathway of 5-HT is oxidative deamination by MAO-A, to yield the unstable intermediate 5-hydroxyindole-acetaldehyde, which is subsequently oxidized to 5-hydroxyindole acetic acid (5-HIAA) by the enzyme aldehyde-dehydroxygenase. 5-HIAA is finally excreted in the urine.

1.2.4 Fate

The bulk of 5-HT is synthesized in the enterochromaffin cells of the gastrointestinal tract (Douglas, 1985). 5-HT released from these cells into the portal venous circulation is rapidly removed by hepatic metabolism, and by uptake in platelets and pulmonary endothelial cells (Vane, 1969). It must be highlighted that the uptake process in the endothelial cells of the pulmonary circulation is a very rapid and effective mechanism, susceptible to inhibition by either imipramine or cocaine (Catravas and Gillis, 1983). After endothelial uptake, 5-HT is metabolized by the isoenzyme A of the monoamine oxidase system (MAO-A; Roth and Gillis, 1975); however, it has been shown that inhibition of MAO-A does not influence the clearance of 5-HT by the lungs, suggesting that not the metabolism of 5-HT but rather its uptake by the endothelial cells is the rate limiting step in the inactivation of 5-HT (Vane, 1969; Wiersma and Roth, 1980). Conversely, in the liver the rate-limiting is not the endothelial uptake but the deamination of 5-HT by MAO-A, which can be explained by the hepatic architecture, since the endothelial lining of the liver sinusoids is discontinuous (Ham, 1974).

Compared with the pulmonary endothelial cells, the uptake of 5-HT by platelets is a relatively slow process, which does not contribute to a major degree to the rapid removal of 5-HT from the portal venous plasma (Vane, 1969). However, since platelets take up 5-HT during their entire life time, they do play an important role in the overall removal of 5-HT from the circulation (Vane, 1969).

Because of the effective clearance of 5-HT from the portal plasma, the plasma level of 5-HT in the systemic circulation remains extremely low (about 2 nM), and it is therefore highly improbable that circulating 5-HT influences the cardiovascular system under physiological conditions (Houston and Vanhoutte, 1986).

1.3 FUNCTION OF 5-HT

The precise physiological role of 5-HT, other than as a neurotransmitter in the central and, perhaps, the enteric nervous system (Gershon et al., 1990), is, at best, debatable, although some progress has been made in the understanding of the role of 5-HT in certain pathophysiological mechanisms. 5-HT exerts a complex variety of effects in the body. Since the substance is unable 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 the contraction of smooth muscle cells, aggregation of blood platelets and presynaptic modulation (stimulation or inhibition) of transmission in the autonomic neurons. In the central nervous system 5-HT serves as a neurotransmitter and seems to play an important role in the regulation of -amongst others- memory, appetite, anxiety, sleep, depression, body temperature, sexual behaviour and the cardiovascular system.

CHAPTER 2

CLASSIFICATION OF 5-HT RECEPTORS

CHAPTER 2

CLASSIFICATION OF 5-HT RECEPTORS

2.1 INTRODUCTION

At present, it seems to be a general rule that there exist multiple types and subtypes of a receptor for each of the several neurotransmitters, neuromodulators and autocoids occurring in both the central nervous system and the periphery. As far as 5-HT is concerned, this is particularly complex, as 5-HT is considered to act on four different receptor populations designated the 5-HT_1-like, 5-HT_2, 5-HT_3 and the recently discovered 5-HT_4 receptor (for references, see Göthert and Schlicker, 1990; Saxena and Villalón, 1990a, 1991). It is noteworthy that 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 (see below). Not until more selective agonists and antagonists became available, could these 5-HT receptor types 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 (see Saxena and Villalón, 1990a). However, this current knowledge did not originate overnight.

2.1.1 Historical remarks

As far back as 1957, Gaddum and Picarelli (1957) suggested the existence of two types of 5-HT receptors based on the studies of 5-HT-induced contractions of the isolated guinea-pig ileum. These contractions could be partially blocked by morphine (M) or dibenzyline (D), but were completely antagonized by the combined use of both compounds. In the dibenzyline-pretreated ileum, atropine and cocaine were also able to block the effect of 5-HT, whereas in the morphine-pretreated ileum, lysergic acid diethylamide (LSD), 2-bromolysergic acid diethylamide and dihydroergotamine antagonized the effect to 5-HT. They concluded that 5-HT must act by two different mechanisms and receptors: a M-receptor located on the parasympathetic nerve endings mediating the release of acetylcholine, and a D-receptor, located on the smooth muscles. Nevertheless, neither morphine nor dibenzyline are specific 5-HT receptor antagonists, but the distinction made between these two types of receptors is still valid.

In the following two decades, progress in the classification of 5-HT receptors was rather limited though, from time to time, it was pointed out that the 'M' and 'D' receptor classification of Gaddum and Picarelli (1957) did not always hold true (for example, see Saxena et al., 1971; Saxena and De Vlaam-Schluter, 1974). In 1974, Bennett and Aghajanian reported the first successful radioligand binding study of 5-HT receptors using d-[^{3}H]-LSD. [^{3}H]-5-HT also showed a high affinity, but because of discrepancies between the binding of [^{3}H]-LSD and [^{3}H]-5-HT, a recognition site with two different states was suggested (Bennett and Snyder, 1976; Fillion et al., 1978). Nonetheless, studies with [^{3}H]-spiperone and [^{3}H]-5-HT revealed that at least two distinct 5-HT recognition sites exist in the cerebral membranes: a 5-HT$_1$ recognition site with high affinity for [^{3}H]-5-HT and low affinity for spiperone; and a 5-HT$_2$ recognition site with high affinity for [^{3}H]-spiperone (Peroutka and Snyder, 1979).

2.1.2 Discovery of "selective" 5-HT receptor antagonists

The development of ketanserin, a quinazolindione derivative with high affinity for 5-HT$_2$ recognition sites (Leysen et al., 1981), made it possible to correlate 5-HT$_2$ recognition sites with functional D-receptors, which were renamed as 5-HT$_2$ receptors. Shortly afterwards, ritanserin was shown to have higher affinity and selectivity for 5-HT$_2$ receptors (Leysen, 1985). The availability of these and other antagonists (see Saxena and Villalón, 1990a) made it possible to clearly characterize the 5-HT$_2$ binding site, and correlate it with the Gaddum and Picarelli's D-receptor. It is of interest to note that many compounds which are thought to be selective antagonists of the 5-HT$_2$ receptor show a similar degree of affinity for 5-HT$_{1C}$ receptors, such as cyproheptadine, ritanserin and LY 53857 (Hoyer, 1988a). However, the main difference between 5-HT$_2$ and 5-HT$_{1C}$ receptor appears to be the affinity of both ketanserin and spiperone for these receptors: both compounds have appreciable higher affinity for 5-HT$_2$ receptors (Hoyer, 1988a,b). Also, in general, the affinity constants of the drugs for the 5-HT$_{1C}$ "receptor" is apparently closer to those for the 5-HT$_2$ receptor than for the other 5-HT$_1$ binding site subtypes (Hoyer, 1988a,b).

On the other hand, the M-receptor described by Gaddum and Picarelli (1957) does not fall into either the 5-HT$_1$ or the 5-HT$_2$ category as this site cannot be blocked by classical 5-HT receptor antagonists (methiothepin, mianserin, cyproheptadine; see below), but can be blocked by selective antagonists, like MDL 72222 (Fozard, 1984a,b) and ICS 205-930 (Richardson et al., 1985). Since both compounds did not label 5-HT recognition sites in the brain, these receptors were called 5-HT$_3$ receptors.

Notwithstanding, shortly afterwards, 5-HT_3 recognition sites were described in the brain by Kilpatrick et al. (1987). Therefore, it is not surprising that by the use of selective antagonists, the 5-HT_2 and 5-HT_3 receptors have been well characterized.

2.2 PROPOSALS FOR THE CLASSIFICATION OF FUNCTIONAL 5-HT RECEPTORS

By definition, 5-HT has a higher affinity for 5-HT_1 than for 5-HT_2 recognition sites, and some effects of 5-HT cannot be blocked by 5-HT_2 or 5-HT_3 receptor antagonists; hence an international committee proposed the existence of "5-HT_1-like" receptors (Bradley et al., 1986). According to their criteria 5-HT_1-like receptors are not blocked by selective antagonists at 5-HT_2 (ketanserin, cyproheptadine) or 5-HT_3 (MDL 72222 or ICS 205-930) receptors, but can be selectively stimulated by 5-carboxamidotryptamine (5-CT). Furthermore, methiothepin, a compound with high affinity for both 5-HT_1 and 5-HT_2 recognition sites, must block the effects induced by 5-HT and 5-CT.

2.2.1 Heterogeneity of the 5-HT_1 binding site

Since the discovery of 5-HT_1 binding sites by Peroutka and Snyder (1979), and further subclassification into 5-HT_{1A} and 5-HT_{1B} by Pedigo et al. (1981), it was already quite clear that the 5-HT_{1B} binding site population was a heterogeneous entity. By definition, ^{3}H-5-HT labels all 5-HT_1 recognition sites, and at present, it is admitted that the 5-HT_1 recognition site entity is highly heterogeneous in nature, as five 5-HT_1 binding site subtypes (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, 5-HT_{1D} and 5-HT_{1E}) have been currently recognized (Pedigo et al., 1981; Peroutka, 1986; Heuring and Peroutka, 1987; Hoyer, 1988a; Waeber et el., 1988; Leonhardt et al., 1989; Göthert and Schlicker, 1990; Saxena and Villalón, 1990a; Lowther et al., 1991), and even these binding site subtypes do not seem to correspond with some 5-HT_1-like receptor-mediated functional responses (see Table 1; Saxena and Villalón, 1990a).

2.2.2 Functional 5-HT_1-like receptors unrelated to the known 5-HT_1 binding sites

Two additional 5-HT_1-like receptor subtypes have been described by Saxena and Villalón (1990a). Although these two receptor subtypes are still unnamed, they will be defined

here, in accordance with Saxena and Villalón (1990a) as the 5-HT_{1x} and 5-HT_{1y} receptor (Table 1).

The 5-HT_{1x} receptor-mediated responses can be characterized by the 5-HT_1 receptor agonists 5-CT (Bradley et al., 1986) and sumatriptan (GR 43175; Humphrey et al., 1988), 8-OH-DPAT, RU 24969 and indorenate (Saxena and Villalón, 1990a), which are antagonized by 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_{1x} receptor appears to be present in certain cephalic arteries (basilar, pial), on the rabbit and dog saphenous vein, and on the arteriovenous anastomoses in the carotid region. Activation of the 5-HT_{1x} receptor results in constriction of these blood vessels (Bom et al., 1988b, 1989a,b; Connor et al., 1989; van Heuven-Nolsen et al., 1990; 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-mediated responses can be characterized by the 5-HT_1 receptor agonist 5-CT and the unselective 5-HT receptor antagonists methiothepin and methysergide (Saxena and Villalón, 1990a). The 5-HT_{1y} receptor is involved in 5-HT-induced smooth muscle relaxation in several blood vessels including the cat isolated saphenous vein (Feniuk et al., 1983) and porcine arterioles (Saxena and Verdouw, 1982, 1985a,b). The 5-HT_1-like receptor that mediates tachycardia in the cat (Saxena et al., 1985b; Saxena, 1988) also seems to be related to the 5-HT_{1y} receptor. Therefore, both 5-HT_{1x} and 5-HT_{1y} receptor types share certain similarities with the 5-HT_1 binding site, but are not identical to any of the known subtypes described above. As a consequence, the discovery of selective 5-HT_1 receptor antagonists in the future may result in the adaptation of the 5-HT_1-like receptor classification.

2.2.3 Evidence supporting the existence of a new 5-HT receptor type

In connection with the nature of 5-HT receptors, it should be noticed that the 5-HT receptor classification scheme proposed by Bradley et al. (1986) would seem currently outdated, as a novel 5-HT receptor type positively linked to the adenylate cyclase system of mouse embryo colliculi neurons has been described (Dumuis et al., 1988, 1989). At this receptor, 5-HT, 5-methoxytryptamine, α-methyl-5-HT and benzamide derivatives such as renzapride, zacopride and cisapride, act as agonists, and only high doses of the 5-HT_3 receptor antagonist ICS 205-930 acts as an antagonist (Dumuis et al., 1988, 1989). In addition, the potency of 5-methoxytryptamine and the ineffectiveness of

2-methyl-5-HT clearly distinguished the site from a 5-HT_3 receptor and removed the possibility that the low affinity for ICS 205-930 merely represents the disclosure of yet another 5-HT_3 receptor subtype. Significantly, in this context, the site is resistant to other 5-HT_3 receptor antagonists such as MDL 72222, granisetron or ondansetron, or to 5-HT_1-like and/or 5-HT_2 receptor antagonists. Therefore, this novel receptor type was designated as the putative 5-HT_4 receptor (Dumuis et al., 1988, 1989; Clarke et al., 1989; Saxena and Villalón, 1990a).

2.2.4 Reconsideration of new proposals for the classification of 5-HT receptors

Taking into consideration the current evidence (Bradley et al., 1986; Dumuis et al., 1988, 1989; Clarke et al., 1989; Humphrey and Feniuk, 1989; Saxena, 1989; Saxena and Ferrari, 1989; Feniuk and Humphrey, 1990; Fozard, 1990; Mylecharane, 1990; Saxena and Villalón, 1990a, 1991; Saxena et al., 1986a,b), 5-HT receptors can be identified and delineated as follows:

5-HT_1-like receptors. Selective agonist: 5-carboxamidotryptamine (5-CT); antagonists: methiothepin (metitepine) or methysergide, both of which display even higher affinity for 5-HT_2 binding sites.

5-HT_2 receptors. Agonists: α-methyl-5-HT or 1-(2,5-dimethoxy-4-iodophenyl)-aminopropane (DOI); antagonists: ketanserin, ritanserin or 4-isopropyl-7-methyl-9-(2-hydroxy-1-methylpropoxycarbonyl)-4,6,6a,7,8,9,10,10a-octa-hydroindolol[4,3-fg] quinoline maleate (LY 53857).

5-HT_3 receptors. Agonists: 2-methyl-5-HT or 1-phenyl-biguanide; 5-methoxytryptamine must be inactive; antagonists: 1αH,3α,5αH-trophan-3yl-3,5-dichlorobenzoate (MDL 72222), (3α-tropanyl)-1H-indole-3-carboxilic acid ester (ICS 205-930), granisetron or ondansetron.

Putative 5-HT_4 receptors. Agonists: 5-methoxytryptamine, α-methyl-5-HT as well as certain benzamide derivatives like renzapride, zacopride, cisapride, etc.; 2-methyl-5-HT must be inactive; antagonist: high concentrations of ICS 205-930.

2.3 ARE THE KNOWN 5-HT RECEPTOR TYPES HOMOGENEOUS ENTITIES ?

Nowadays, it is recognized that only the 5-HT_1 binding site population (and the 5-HT_1-like receptor) is highly heterogeneous in nature (see above). Apparently, only 5-HT_{1A} receptor-mediated responses can be selectively discriminated by using ligands such as 8-OH-DPAT, flesinoxan, ipsapirone or urapidil (Hoyer, 1988a; Waeber et al., 1988; Feniuk and Humphrey, 1990).

With respect to the other 5-HT receptor types, heterogeneity cannot be ruled out for the 5-HT_2, 5-HT_3 and 5-HT_4 receptors, although good evidence is still to be offered. Notwithstanding, the current evidence for such heterogeneity is considered to be insufficient (Bradley et al., 1986; Dumuis et al., 1988, 1989; Clarke and Craig, 1989; Craig and Clarke, 1990; Saxena and Villalón, 1990a, 1991; present thesis). Thus, the ongoing development of drugs with different pharmacological properties may lead to the extension of the present 5-HT receptor family (see, for example, Cadogan and Humphrey, 1991).

2.4 FUNCTIONAL RESPONSES MEDIATED BY 5-HT RECEPTOR ACTIVATION

2.4.1 Introduction

5-HT and related agonist drugs exert a variety of functional responses, via the stimulation of several receptors in the central nervous system (CNS), on the autonomic nerve endings and on various smooth muscle containing tissues (see Table 1). The multiplicity of 5-HT binding sites raises the question as to whether these sites do indeed correspond to receptors, i.e. whether each subtype is coupled to an effector system. During the last few years, progress has been made to define the second messenger systems that are linked to the putative 5-HT receptors (Table 1; Saxena and Villalón, 1990a).

2.4.2 Responses mediated by 5-HT_1 receptors

Except for the 5-HT_{1C} and 5-HT_{1E} receptors, the 5-HT_1 receptor subtypes have been coupled to the modulation of adenylate cyclase (see below). 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, though a more recently developed 5-HT_{1A} receptor agonist is flesinoxan (Ramage et al., 1988; Wouters et al., 1988a,b). So far, the 5-HT_{1A} receptor appears to have been identified only in the CNS. However, in the sympathetic neurons of the dog external carotid artery, the 5-HT_{1A} receptor subtype may be involved, as indicated by the agonist activity of 5-HT and indorenate (a 5-HT_{1A} receptor ligand; Dompert et al, 1985) and the antagonism by methiothepin and (±)-pindolol (Hong and Villalón, 1988), but not by ritanserin (Villalón and Hong, 1988). Basically, 5-HT_{1A} receptors are involved in central cardiovascular regulation as well as in neurogenic, behavioral, metabolic and endocrine mechanisms (see Table 1).

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}, 5-HT_{1D} or 5-HT_{1E} receptor types. Consequently, the characterization of functional responses mediated by these receptor types has mainly been carried out by the exclusion of the other 5-HT receptor mechanisms. Activation of the 5-HT_{1B} receptor, which appears to be entirely confined to rodents (see Hoyer, 1986a, 1988a) mediates 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 delineated as 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 similarities, and they may be equivalent in the different species. For example, like the 5-HT_{1B} receptor in rat substantia nigra (Bouhelal et al., 1988), the 5-HT_{1D} receptor in calf substantia nigra 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 (Galzin et al., 1988; Schipper and Tulp, 1988; Schlicker et al., 1989). In cats, the 5-HT_{1D} site has been implicated in RU 24969-induced emesis (Lucot, 1990); and very recently, the 5-HT_{1D} receptor has also been localized peripherally in the pig, subserving endothelium-dependent relaxation of the coronary arteries (Schoeffter and Hoyer, 1990).

Table 1. Classification of and some functional responses mediated by 5-HT Receptors.

Receptor subtype	"Selective" agonists	"Selective" antagonists	Binding site	Second messenger	Some functional responses
5-HT_1-like					
5-HT_{1A}	5-CT 8-OH-DPAT RU 24969 5-CH_3O-T Sumatriptan	Cyanopindolol Methysergide Methiothepin	5-HT_{1A}	Negative or positive coupling to adenylate cyclase, K^+ channel	Behavioral changes, centrally evoked hypotensive response, Some endocrinological effects.
5-HT_{1B}	RU 24969 5-CT 5-CH_3O-T Sumatriptan	Cyanopindolol Methiothepin	5-HT_{1B}	Negative coupling to adenylate cyclase	Autoreceptor in the rat brain, Inhibition of acetylcholine and noradrenaline release in rats.
5-HT_{1C}[a]		Mesulergine Methiothepin Methysergide	5-HT_{1C}	Phosphoinositide-specific phospholipase-C	Not yet convincingly demonstrated due to unselective drugs.
5-HT_{1D}	5-CT RU 24969 Sumatriptan	Metergoline Methiothepin	5-HT_{1D}	Negative coupling to adenylate cyclase	Not yet convincingly demonstrated due to unselective drugs.
5-HT_{1E}	5-HT	GTP-gamma-S	5-HT_{1E}	Interaction with a GTP-binding protein	Not yet convincingly demonstrated due to unselective drugs.
5-HT_{1x}[b]	5-CT AH25086[c] Sumatriptan 8-OH-DPAT RU 24969	Methiothepin Methysergide[d]	Not yet found[e]	Not yet known	Contraction of cephalic arteries (basilar, pial) and arteriovenous anastomoses in the carotid region, decrease of neuronal noradrenaline release.
5-HT_{1y}[b]	5-CT	Methiothepin Methysergide	Not yet found[e]	Not yet known	Vascular smooth muscle relaxation, hypotension, tachycardia in the cat.
5-HT_2	α-CH_3-5-HT	Ketanserin Cyproheptadine Methysergide Methiothepin	5-HT_2	Phosphoinositide-specific phospholipase-C	Contraction of various vascular, gastrointestinal and bronchial smooth muscles, platelet aggregation, Head twitch and convulsion.
5-HT_3	5-HT 2-CH_3-5-HT	MDL 72222 ICS 205-930 Granisetron Ondansetron	5-HT_3	Cation channels	Membrane depolarization, dermal pain and flare response.
5-HT_4	5-HT Renzapride 5-CH_3O-T α-CH_3-5-HT	ICS 205-930	5-HT_4?	Positive coupling to adenylcyclase	Gastrokinetic action, cholinergically mediated guinea-pig ileum contraction, Myocardial stimulation in pigs and humans.

[a], Shows little difference from the 5-HT_2 receptor; [b], The receptor subtype is as yet unnamed and this name has been put for convenience in this manuscript to distinguish between the two unnamed 5-HT_1-like receptors; [c], Ligand binding profile is not yet reported; [d], Partial agonist; [e], Does not correlate with 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} or 5-HT_{1D} binding sites. AH25086, 3-aminoethyl-N-methyl-1H-indole-5-methane carboxamide; 5-CH_3O-T, 5-methoxytryptamine; α-CH_3-5-HT, α-methyl-5-HT; GTP-gamma-S, guanosine 5'-O-(3-thiotriphosphate. Modified from Saxena and Villalón (1990a).

As far as the 5-HT_{1C} receptor is concerned, this receptor has been identified in either rat, pig or human choroid plexus (Pazos et al., 1985; Yagaloff and Hartig, 1985; Hoyer et al., 1986b) and shows remarkable similarities with the 5-HT_2 receptor regarding molecular, pharmacological and biochemical properties (Schmidt and Peroutka, 1989). As a consequence, it is difficult to identify contributions of the 5-HT_{1C} receptor subtype to functional responses. Like the 5-HT_2 receptor, the 5-HT_{1C} receptor has been linked to phosphoinositide turnover (Conn et al., 1986). So far, the 5-HT_{1C} receptor appears to be involved in the modulation of the production of cerebrospinal fluid.

A 5-HT_{1E} binding site has also been described (Leonhardt et al., 1989). As to the binding profile, both 5-HT_{1D} and 5-HT_{1E} binding sites are located in human frontal cortex, but unlike the 5-HT_{1D} binding site, the 5-HT_{1E} binding site displays higher affinity for 5-HT and low affinity for 5-CT and ergotamine, and remarkably, interacts with a GTP-binding protein, which makes it functionally different from the other 5-HT_1 binding sites (see above). In this respect, the non-hydrolysable derivatives of GTP such as guanosine 5'-O-(3-thiotriphosphate) (GTP-gamma-S) and 5'-guanylylimidodiphosphate [Gpp(NH)p] potently inhibited the binding of [^{3}H]-5-HT to the 5-HT_{1E} binding site, while adenosine 5'-O-(3-thiotriphosphate) and 5'-adenylylimidodiphosphate were without effect (Leonhardt et al., 1989). As far as we know, no functional response mediated by the 5-HT_{1E} receptor has been delineated in the periphery, whereas second messenger systems have not yet been defined for the 5-$HT_{1\alpha}$ and the 5-$HT_{1\gamma}$ receptor subtypes (Saxena and Villalón, 1990a).

2.4.3 Responses mediated by 5-HT_2 receptors

The 5-HT_2 receptor has been linked to the modulation of phosphoinositide (PI) turnover (Conn and Sanders-Bush, 1986), and exists both in the periphery and in the CNS. Peripherally, 5-HT_2 receptors have been involved in vascular smooth muscle contraction, e.g. canine basilar arteries (Müller-Schweinitzer and Engel, 1983); contraction of uterine, bronchial, gastro-intestinal and bladder smooth muscle (Saxena and Lawang, 1985; Saxena et al., 1985a; Bradley et al., 1986), platelet aggregation (de Clerk et al., 1984), etc. In the CNS 5-HT_2 receptors mediate responses such as 5-HT-induced excitation of rat brain stem neurons (Davies et al., 1988), 5-HT-induced depolarization of guinea-pig cortical pyramidal neurons (Davies et al., 1987), some behavioral responses in rodents such as head twitch and wet-dog shakes (Lucki et al., 1984) and the hallucinogenic action induced by certain drugs (Glennon, 1990). Furthermore, 5-HT_2 receptors seem to be involved in some neuroendocrine functions such as the secretion

of ß-endorphin and corticosterone in the rat (Koenig et al., 1987) as well as the release of prolactin in rhesus monkeys (Heninger et al., 1987).

2.4.4 Responses mediated by 5-HT_3 receptors

In contrast with the other 5-HT receptor families, the 5-HT_3 receptors are directly coupled to a cation channel (Derkach et al., 1989). Functional 5-HT_3 receptors have been identified in both the peripheral and central nervous system. 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, 1984a,b) and cat urinary bladder (Saxena et al., 1985a), noradrenaline from sympathetic neurons in e.g. the rabbit heart (Fozard and Mwaluko, 1976) and the superior cervical ganglion (Wallis and North, 1978), and substance P from enteric neurons in e.g. the guinea pig ileum (Bradley et al., 1986). 5-HT_3 receptors mediate also 5-HT-induced pain sensation in human skin, the cardiovascular von Bezold-Jarisch reflex and depolarization of C-fibres in the nodose ganglion and vagus nerve (Richardson et al., 1985). In the CNS, 5-HT_3 receptors are involved in the anxiogenic effects of some drugs in rats, mice and marmosets (Tyers et al., 1987), and the cytotoxic drug-induced emesis (Higgins et al., 1989).

2.4.5 Responses mediated by the putative 5-HT_4 receptor

The putative 5-HT_4 receptor has been positively linked to adenylate cyclase in both the mouse and guinea-pig CNS (Dumuis et al., 1988, 1989; Bockaert et al., 1990), and has been involved in the increase in electroencephalographic energy produced by zacopride and renzapride when applied i.c.v. in the rat (Boddeke and Kalkman, 1990). In the periphery, the 5-HT_4 receptor is involved in e.g. the activation of cholinergically mediated contractions in the guinea-pig ileum (Clarke et al., 1989; Craig and Clarke, 1990).

From the foregoing information, it is obvious that the actions of 5-HT in the organism are extremely complex (see for example Bradley et al., 1986; Saxena and Villalón, 1990a, 1991). There are still a lot of unanswered questions concerning the nature of the mechanisms underlying the central and peripheral effects of 5-HT, for example, in the cardiovascular system of several species including man; accordingly, a review of the cardiovascular effects of 5-HT (and related drugs) will be detailed.

CHAPTER 3

5-HT AND THE CARDIOVASCULAR SYSTEM

CHAPTER 3

5-HT AND THE CARDIOVASCULAR SYSTEM

3.1 INTRODUCTION

In general terms, the primary role of the cardiovascular system is to ensure at every moment the survival of all cells of the body. These cells must receive nutrients and eliminate their waste substances so that there must be a continuous stream of nutrients from the outside world to the extracellular fluid, and a continuous stream of waste substances from the cells to the outside world. Therefore, the organism needs specialized tissues for exchange (lungs, gut and kidneys) and a transport (cardiovascular) system (blood, blood vessels and heart) to link these tissues with the individual cells.

3.2 PHYSIOLOGICAL REGULATION OF THE CARDIOVASCULAR SYSTEM

The mechanisms involved in the control of the functions of the cardiovascular system are under nervous, humoral and/or local regulation. All of them together adjust the 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. Thus, the central nervous system (CNS) unifies the information gathered from several sensors within the cardiovascular system and modulates the function of its components by means of the autonomic nervous system (sympathetic and parasympathetic; see Kuntz, 1953) and certain endocrine glands. Summarizing, the regulation of the cardiovascular system consists of:

3.2.1 Nervous Regulation

There exist numerous peripheral receptors within the circulation, which sense either changes in blood pressure (mechanoreceptors) or variations in the chemical composition of the blood (chemoreceptors). The degree of activity of these receptors is conveyed by afferent nerves to centres in the brain stem which control the function of the heart

and blood vessels by altering the activity of the noradrenergic and cholinergic nerves. When the activity of the former is increased, the neurotransmitter noradrenaline is released, which stimulates the heart (ß-adrenoceptors) and constricts the blood vessels (α-adrenoceptors). When the cholinergic nerves are activated, the released acetylcholine depresses the heart (muscarinic cholinoceptors). Certain cardiovascular changes may initiate compensatory reflex adjustments, such as the baroreceptor reflex, cardiopulmonary reflex, chemoreflexes (e.g. the von Bezold-Jarisch reflex) and spinal reflexes. Activation of each of these reflexes results in changes in sympathetic and/or parasympathetic outflow in order to modify the cardiovascular function.

3.2.2 Humoral Regulation

The activity of both heart and blood vessels is also modulated by substances released into the circulation from endocrine glands, e.g. adrenaline from the adrenal medulla, which, amongst other effects: 1. increases both rate and contraction force of the heart; 2. constricts most systemic resistance vessels; 3. dilates resistance vessels of skeletal muscles, etc.

3.2.3 Local Regulation

In the tissues, products of cellular metabolism (H^+, HCO_3^-, ADP, adenosine, etc.) regulate the local blood flow in accordance with their needs. In addition, the autacoids (prostaglandins, kinins, angiotensin, histamine, 5-HT) can induce vasoconstriction and/or vasodilatation by themselves (see for example Vanhoutte et al., 1981), and may modify cardiac function. Likewise, it must be highlighted that certain metabolites and the autocoids (notably 5-HT) can also modulate the activity of central, sympathetic and/or parasympathetic nerves either stimulating or inhibiting the release of their respective neurotransmitters (for references, see Langer, 1980), and consequently, will modify the cardiovascular function. It is interesting to note that 5-HT can interact in all components of the cardiovascular system in a variety of ways, acting on practically all types of 5-HT receptors delineated so far (Saxena, 1986; Saxena and Villalón, 1990a, 1991).

3.3 CARDIOVASCULAR EFFECTS OF 5-HT AND RELATED DRUGS

It is well known that 5-HT elicits complex changes in the cardiovascular system comprising bradycardia or tachycardia, hypotension or hypertension and vasodilatation or vasoconstriction (Saxena and Villalón, 1990a,b, 1991). The eventual response depends — amongst other factors — upon the species, the basal vascular tone, the vascular bed under study, dose employed and, most importantly, the nature of the 5-HT receptors involved. With the recent discovery of a variety of drugs acting selectively at 5-HT receptors (see Chapter 1), it is nowadays clear that the cardiovascular and, indeed, other effects of 5-HT seem to be mediated by the previously described four types of 5-HT receptors.

5-HT and related agonist drugs exert a variety of functional responses via stimulation of the different 5-HT receptors in the central nervous system, on the autonomic nerve endings, on various smooth muscle containing tissues and on the cardiac tissue (see Table 1; Saxena and Villalón, 1990a,b, 1991).

3.3.1 Cardiovascular reflex responses to 5-HT and related drugs

5-HT has long been known to elicit a variety of cardiovascular reflexes by acting mainly on the carotid body arterial chemoreceptors, cardiopulmonary receptors and pulmonary J (deflation) receptors (McQueen, 1990). These reflex responses will obviously contribute to the all-over effect of 5-HT as follows:

Carotid body arterial chemoreceptors. Stimulation of carotid body chemoreceptors, quite marked in dogs but weak in cats (Black et al., 1952), consists of bradycardia and hypotension followed by tachycardia, hypertension and hyperventilation (Ginzel, 1958; Skinner and Whelan, 1962; McQueen and Ungar, 1971). Because both 1-phenylbiguanide and 2-methyl-5-HT mimic 5-HT, and MDL 72222 and ICS 205-930 each antagonize these reflex responses, the receptor involved is of the 5-HT_3 type (McQueen, 1990).

Cardiopulmonary receptors. Stimulation by 5-HT of sensory receptors in the heart and lungs elicits reflex responses leading to bradycardia, hypotension, tachypnoea and nausea. Usually, one such reflex (von Bezold-Jarisch-like reflex), evoked mainly from the cardiac receptors (left and right ventricular pressure, type A and B left atrial and type A right atrial and epicardial receptors) on C-fibre afferents, results in pronounced

bradycardia, hypotension and apnoea (Thoren, 1979). This reflex is mediated mainly by the vagus nerve and, in case of 5-HT and related drugs, involves 5-HT_3 receptors (McQueen, 1990).

Pulmonary J receptors. 5-HT and 1-phenylbiguanide can increase the activity of the type J vagal afferent fibres (Paintal, 1977). Injection of these drugs into the right atrium (Armstrong et al., 1986a) and pulmonary embolism (Armstrong et al., 1986b) in anaesthetized rabbits results in bradycardia, hypotension and rapid shallow breathing, typical of J receptor stimulation, via 5-HT_3 receptors.

3.3.2 Blood pressure responses to 5-HT and related drugs

5-HT has a triphasic effect on arterial blood pressure (Table 2), comprising an initial intense, but brief, hypotension followed by a moderate hypertension and, finally, a longer-lasting hypotension (Page and McCubbin, 1953; Kalkman et al., 1984). Contrary to the connotation conveyed by the word "serotonin", 5-HT is much more potent in eliciting the late longer-lasting hypotensive phase than in evoking the initial hypotensive phase or the pressor response (Saxena and Lawang, 1985) and, therefore, more readily deserves the name "serodilin".

The hypertensive phase varies quantitatively depending upon the species and the experimental conditions; for instance, rabbits (Schneider and Yonkman, 1954; Bolt and Saxena, 1985; Wright and Angus, 1989), cats (Schneider and Yonkman, 1954; Saxena et al., 1985b) and pigs (Saxena and Verdouw, 1982, 1985b; Saxena et al., 1986a) exhibit a poor hypertensive phase, while it is prominent in the dog (Schneider and Yonkman, 1954) particularly after ganglion blockade (Feniuk et al., 1981). As delineated below, these differences may be due to the type of receptors and their distribution in the different species.

Initial transient depressor response. The initial hypotensive response to 5-HT is the result of an abrupt bradycardia (and the consequent decrease in cardiac output) following stimulation of 5-HT_3 receptors on afferent cardiac vagal nerve endings (Table 2). However, there is some evidence that a short-lasting vasodilatation may be obtained via 5-HT_3 receptors, which probably invokes an axon-like reflex (Blauw et al., 1988).

Table 2: 5-HT receptors mediating effects on arterial blood pressure in different species.

Species	Condition	Receptor	Main mechanism
Initial depressor response			
Rat	Intact	5-HT_3	von Bezold-Jarisch reflex
Cat	Intact	5-HT_3	von Bezold-Jarisch reflex
Pressor response			
Rat	Intact	5-HT_2	Vascular effect
Rat	Pithed	5-HT_2	Vascular effect
Cat	ß-Adrenoceptor block	5-HT_2	Vascular effect
Cat	Spinal	5-HT_2	Vascular effect
Dog	Ganglion-blockade	5-HT_2	Adrenal catecholamine release
Late depressor response[a]			
Rat	Intact	5-HT_1-like	Vascular effect
Rat	Pithed	5-HT_1-like	Vascular effect
Rat	Atropinized	5-HT_1-like	Vascular effect
Cat	ß-Adrenoceptor block	5-HT_1-like	Vascular effect
Pig	Intact	5-HT_1-like	Vascular effect

[a], Most sensitive response.

Taken from Saxena and Villalón (1990a).

Table 3: 5-HT receptors mediating vascular contractions in different species.

Species	Vessel	Condition	Receptor
Rat	Basilar artery	Isolated	5-HT_2
Rat	Jugular vein	Isolated	5-HT_2
Rat	Portal vein	Isolated	5-HT_2
Rat	Aorta	Isolated	5-HT_2
Rat	Caudal artery	Perfused	5-HT_2
Rabbit	Aorta	Isolated	5-HT_2
Rabbit	Common carotid artery	Isolated	5-HT_2
Rabbit	Femoral artery	Isolated	5-HT_2
Cat	Middle cerebral artery	Isolated	5-HT_2
Cat	Pial arteries	In situ	5-HT_2
Cat	Saphenous vein	Isolated	5-HT_2
Dog	Basilar artery	Isolated	5-HT_2[a]
Dog	Middle cerebral artery	Isolated	5-HT_2
Dog	External carotid artery	Isolated	5-HT_2
Dog	Coronary artery	Isolated	5-HT_2
Dog	Femoral artery	Isolated	5-HT_2
Dog	Femoral artery	In vivo	5-HT_2
Pig	Common carotid artery	In vivo	5-HT_2
Calf	Coronary artery	Isolated	5-HT_2
Calf	Pulmonary artery	Isolated	5-HT_2
Monkey	Basilar artery	Isolated	5-HT_2[a]
Human	Umbilical artery	Isolated	5-HT_2
Human	Femoral artery	Isolated	5-HT_2
Guinea-pig	Basilar artery	Isolated	5-HT_1-like
Cat	Cranial AVAs	In vivo	5-HT_1-like
Dog	Saphenous vein	Isolated	5-HT_1-like
Dog	Basilar artery	Isolated	5-HT_1-like[a]
Dog	Auricular artery	Isolated	5-HT_1-like
Pig	Cranial AVAs	In vivo	5-HT_1-like
Monkey	Basilar artery	Isolated	5-HT_1-like[a]
Human	Basilar artery	Isolated	5-HT_1-like
Rabbit	Ear artery	Isolated	α-adrenoceptor
Rabbit	External carotid artery	Isolated	α-adrenoceptor

[a], both 5-HT_1-like and 5-HT_2 receptors seem to mediate contraction.
AVAs, arteriovenous anastomoses.
Taken from Saxena and Villalón (1990a).

Pressor response. The pressor component, amenable to blockade by ketanserin-like drugs, is due to activation of 5-HT_2 receptors (Table 2), mainly on the blood vessels in, for example, the rat (Kalkman et al., 1983, 1984; Saxena and Lawang, 1985; Dalton et

al., 1986; Dabiré et al., 1989a,b) and cat (Saxena et al., 1985b; Connor et al., 1986; Saxena, 1988) or, as in the dog, on the adrenal medulla to release catecholamines (Feniuk et al., 1981; Kimura and Sato, 1983).

Accordingly, the contractile effects on both arteries and veins are generally mediated by 5-HT_2 receptors (Table 3; Apperley et al., 1980; Black et al., 1981; Leysen, 1981; Cohen et al., 1981; Cohen et al., 1983; Feniuk et al., 1983; Müller-Schweinitzer and Engel, 1983; Van Nueten, 1983; Frenken and Kaumann, 1984; Glusa and Markwardt, 1984; Lemberger et al., 1984; Saxena and Verdouw, 1984; Thompson et al., 1984; Van Nueten et al., 1984; Verdouw et al., 1984; Blackshear et al., 1985; Clancy and Maayani, 1985; McGrath et al., 1985; Young et al., 1986; Chang, 1987; Chang and Owman, 1989a,b); drugs such as ketanserin, cyproheptadine, pizotifen and methysergide are competitive antagonists with pA_2 being similar to their pK_D values determined in 5-HT_2 binding assay (Leysen, 1981; Bradley et al., 1986). However, in cranial blood vessels of different species, including humans, as well as the dog saphenous vein, 5-HT_1-like receptors mediate vasoconstrictor responses (Table 3; Engel et al., 1983; Peroutka et al., 1983; Peroutka and Kuhar, 1984; Verdouw et al., 1985; Taylor et al., 1986; Humphrey et al., 1988; Chang and Owman, 1989a,b; Connor et al., 1989; Parsons et al., 1989; Perren et al., 1989). In some cases, both 5-HT_1-like and 5-HT_2 receptors seem to mediate contractile effects in the same vessel, for example the dog (Müller-Schweinitzer and Engel, 1983; Peroutka et al., 1983; Peroutka and Kuhar, 1984; Van Nueten et al., 1984; Taylor et al., 1986; Connor et al., 1989) and monkey (Peroutka and Kuhar, 1984; Chang, 1987; Connor et al., 1989; Parsons et al., 1989) basilar arteries and, perhaps to a lesser extent, the pig carotid arteriovenous anastomoses (Saxena and Verdouw, 1982, 1984; Verdouw et al., 1984; Saxena et al., 1986a). In rarer instances, 5-HT may act directly on the α-adrenoceptors in rabbit ear and external carotid arteries (Apperley et al., 1976; Black et al., 1981, Van Nueten, 1983) (Table 3). In addition, there is some limited evidence that in the rat coronary arteries, 5-HT may also release a contractile substance from the endothelium. The nature of this substance is not known (Vanhoutte et al., 1990).

It may also be pointed out here that 5-HT has, in addition to a central vasodepressor effect (see below), an excitatory effect on preganglionic sympathetic activity via ponto-medullary serotonergic pathways and 5-HT_2 receptors (see Mir and Fozard, 1990). These nerves, however, do not seem to exert a tonic influence and, moreover, spinal 5-HT_2 receptors can also increase sympathetic nervous activity (Mir and Fozard, 1990).

The 5-HT-induced increase in the sympathetic nerve activity may also involve a 5-HT_1-like receptor; such an effect is mimicked by intrathecal administration of 5-CT

(but not 8-OH-DPAT), and remains unaffected by ketanserin, MDL 72222 or ICS 205 930 (Yusof and Coote, 1988).

Late depressor response: The late depressor effect of 5-HT is not affected by selective 5-HT_2 or 5-HT_3 receptor antagonists, but is antagonized by methysergide or methiothepin (Kalkman et al., 1984; Saxena and Lawang, 1985; Dalton et al., 1986; Saxena, 1988). A similar depressor response can be evoked by 5-CT and this effect is also blocked by methysergide or methiothepin (Saxena and Lawang, 1985; Saxena et al., 1985a; Saxena, 1988). Finally, there is a good correlation between the hypotensive activity of tryptamine derivatives and their affinity for the 5-HT_1 binding site (Kalkman et al., 1983). Therefore, the late hypotensive phase is mediated by 5-HT_1-like receptors (Table 2), but several mechanisms probably contribute to different degrees in different experimental situations and species. These 5-HT_1-like receptor-mediated mechanisms are:

Central vasomotor inhibition: Injection of 5-HT into the central nervous system has been reported to cause depressor, pressor or biphasic responses (Bhargava and Tangri, 1959; Smit et al., 1978; Krstić, 1985; Dalton et al., 1986; Yusof and Coote, 1988; Mir and Fozard, 1990). The magnitude and the nature of the response (pressor, depressor or biphasic) to centrally administered 5-HT largely depends upon the exact site of application, the dose employed or whether conscious or anaesthetized and normotensive or hypertensive animals are used (Sukamoto et al., 1984; Krstić, 1985; Wolf et al., 1985; Dalton et al., 1986; Mir and Fozard, 1990). This discrepancy may be because 5-HT neurones in different brain areas have divergent effects; that is, dorsal and median raphe, anterior hypothalamus, and ventrolateral medullary raphe areas seem to be associated with pressor effects, whereas midline medullary raphe nuclei produce either pressor or depressor effects (Wolf et al., 1985; Ramage et al., 1988). These central pressor and depressor effects of 5-HT seem to be mediated via different 5-HT receptors in the central nervous system (Mir and Fozard, 1990).

Recently, several agonists at 5-HT_{1A} receptors — 8-OH-DPAT (Main et al., 1984; Gradin et al., 1985a,b; Martin and Lis, 1985; Fozard et al., 1987; Ramage and Fozard, 1987; Doods et al., 1988; Mir and Fozard, 1990), flesinoxan (Wouters et al., 1988a,b; Dreteler et al., 1989; Coote, 1990), N,N-dipropyl-5-CT (Doods et al., 1988; Mir and Fozard, 1990), urapidil (Doods et al., 1988; Gillis et al., 1988; Sanders et al., 1988; Kolassa et al., 1989; Mir and Fozard, 1990) — have been found to decrease arterial blood pressure and heart rate (see above) in different animal species (Mir and Fozard, 1990). Though urapidil also possesses α_1-adrenoceptor antagonist properties, the 5-HT_{1A}

agonist activity does contribute to the action of the drug (Sanders et al., 1988; Kolassa et al., 1989).

The involvement of the central nervous system in the cardiovascular responses to putative 5-HT_{1A} receptor agonists is supported by several observations: Administration of 8-OH-DPAT, flesinoxan and urapidil into the central nervous system (via either the vertebral arteries, the atlanto-occipital membrane or topically to the ventral surface of the medulla) decreases heart rate and blood pressure, and their potency considerably increases as compared to the intravenous administration in cats (Doods et al., 1988; Gillis et al., 1988; Wouters et al., 1988a,b; Kolassa et al., 1989; Mir and Fozard, 1990). The cardiovascular responses to these drugs are effectively blocked by central administration of a putative 5-HT_{1A} receptor antagonist, 8-MeO-ClEPAT, spiroxatrine, methiothepin and/or (-)-pindolol (Dabiré et al., 1987; Fozard et al., 1987; Doods et al., 1988; Sanders et al., 1988; Mir and Fozard, 1990). Furthermore, 8-OH-DPAT, flesinoxan and/or urapidil reduce sympathetic neural activity (Ramage, 1986; McCall et al., 1987; Ramage and Fozard, 1987; Coote, 1990; Mir and Fozard, 1990), particularly of the renal nerves (Ramage and Wilkinson, 1988) which leads to renal vasodilatation (Dreteler et al., 1989; Hof and Fozard, 1989). Hypotension, along with bradycardia and a decrease in the renal nerve activity, has also been shown to occur with the administration of 5-HT into the fourth ventricle in the cat (Wolf et al., 1985), as has the centrally-induced increase in the vagal tone in the rat (Wolf et al., 1985; Dalton et al., 1986).

5-HT also seems to act at the spinal level. 5-HT_2 receptors may be involved in the inhibitory effect on the sympathetic nerve activity, because such an effect is mimicked by intrathecal administration of α-methyl-5-HT and antagonized by ketanserin, but unaffected by prazosin, MDL 72222 or ICS 205 930 (Yusof and Coote, 1988).

Inhibition of noradrenergic neurones: 5-HT has a dual action on postganglionic sympathetic neurons in the dog isolated saphenous vein; lower concentrations inhibit the release of noradrenaline in response to transmural stimulation, and high concentrations provoke catecholamine release by a tyramine-like action (McGrath, 1977). The inhibitory action of 5-HT and related drugs on the transmitter release from postganglionic sympathetic nerves has now been confirmed in many organs of different species (Göthert et al., 1990), such as the canine and human saphenous vein (McGrath, 1977; Göthert et al., 1990), canine coronary (Cohen, 1985) and carotid (Hong and Villalón, 1988; Mylecharane and Phillips, 1989) arteries, and rat kidney (Charlton et al., 1986), vena cava (Molderings et al., 1987) and heart (Göthert et al., 1986) and porcine coronary artery (Molderings et al., 1989). The involvement of 5-HT_1-like

receptors (Göthert et al., 1990), except in the pig coronary sympathetic neurones where a 'novel' receptor may exist (Molderings et al., 1989; Göthert et al., 1990), is favoured by the high agonist potency of 5-CT and 5-HT (Cohen, 1985; Charlton et al., 1986; Molderings et al., 1987) and the antagonism by methiothepin, metergoline and methysergide (Lorenz and Vanhoutte, 1985; Charlton et al., 1986; Göthert et al., 1986), but not ketanserin, LY 53857 (Cohen, 1985; Charlton et al., 1986; Göthert et al., 1986) or metoclopramide (Charlton et al., 1986).

Obviously, the interference with the sympathetic neurotransmission by 5-HT will modify its direct cardiovascular effects; namely, 5-HT has been reported to cause vasodilatation in the external carotid vascular bed of the dog when the sympathetic vascular tone is high, and vasoconstriction when it is low (Mena and Vidrio, 1979; Hong and Villalón, 1988).

Vascular smooth muscle responses: The vascular relaxation elicited by 5-HT is exclusively mediated via 5-HT_1-like receptors in several vessels, for example, *in vitro* in the cat saphenous vein (Feniuk et al., 1983), dog coronary artery (Cohen et al., 1983; Van Nueten et al., 1984) and pig vena cava (Trevethik et al., 1984) and *in vivo* in the dog femoral (Blackshear et al., 1985) and pig carotid arterioles (Saxena and Verdouw, 1982, 1984, 1985a,b; Verdouw et al., 1984; Verdouw et al., 1985; Saxena et al., 1986a,b); both 5-HT and 5-CT are potent agonists and methysergide and methiothepin are antagonists, whereas ketanserin is ineffective.

In the blood vessels, where both 5-HT_1-like and 5-HT_2 receptors are present and elicit opposing effects (relaxation by 5-HT_1-like receptors and contraction by 5-HT_2 receptors), the ultimate response to 5-HT depends upon the pre-existing vascular tone, the dose employed, and the proportions in which the two receptors types are distributed. *In vivo*, studies in our laboratory (Saxena and Verdouw, 1982, 1984; Verdouw et al., 1984) and elsewhere (Blackshear et al., 1985) indicate that the number of 5-HT_1-like and 5-HT_2 receptors varies in different segments of a vascular bed. The vasodilator 5-HT_1-like receptor is located primarily on resistance vessels (arterioles), the vasoconstrictor 5-HT_1-like receptor on non-nutrient vessels (arteriovenous anastomoses), while the vasoconstrictor 5-HT_2 receptor is mainly present on large conducting vessels. Therefore, 5-HT redistributes arterial blood flow in such a way that, despite a decrease in the total blood flow, the arteriolar component, particularly in the skin, increases (Saxena and Verdouw, 1982, 1984, 1985b; Verdouw et al., 1984). The segmental distribution of 5-HT receptors is also, at least partly, responsible for the fact that *in vitro* studies, performed mainly with large conducting vessels, generally show a 5-HT_2 receptor-mediated contractile response. *In vivo*, where presynaptic (see above) and

reflex effects of 5-HT may modify the vascular responses, the amine causes vasodilatation in some vascular beds and vasoconstriction in others (Takacs and Vajda, 1963; Saxena et al., 1978; Zinner et al., 1983).

Release of endothelium-derived relaxing factor (EDRF): In the absence of endothelium the relaxant effect of 5-HT is attenuated while the contractions are exaggerated in isolated coronary and other vessels of the pig, dog, chick and rabbit (Cocks and Angus, 1983; Cohen et al., 1983; Imaizumi et al., 1984; Leff et al., 1987; Martin et al., 1987; Houston and Vanhoutte, 1988). These findings indicate that 5-HT can release EDRF and this effect is mediated by 5-HT_1-like receptors (Cocks and Angus, 1983; Cohen et al., 1983; Martin et al., 1987; Houston and Vanhoutte, 1988). *In vivo*, the 5-HT-induced endothelium-dependent vasodilatation has thus far been studied indirectly by showing that the contractile responses to 5-HT, but not to angiotensin II or phenylephrine, in the canine left anterior descending artery, are enhanced after damage to the endothelium (Lamping et al., 1985).

CHAPTER 4

CARDIAC RESPONSES TO 5-HT AND RELATED DRUGS

CHAPTER 4

CARDIAC RESPONSES TO 5-HT AND RELATED DRUGS

4.1 INTRODUCTION

As previously stated, 5-HT can elicit either bradycardia or tachycardia. The nature of the 5-HT receptors and the underlying mechanisms involved in the heart rate responses to 5-HT have been generally well worked out (Saxena, 1986, 1989; Saxena and Villalón, 1990a, 1991; see Table 4).

4.2 BRADYCARDIAC ACTION

The overwhelming effect of 5-HT in different species with intact central nervous system and vagi is an intense, but transient, bradycardia, which is abolished by ganglion blockade, vagotomy or spinal section (Saxena, 1986, 1989; Saxena and Villalón, 1990a, 1991) and is, therefore, due to a von Bezold-Jarisch-like reflex originating from depolarization of afferent cardiac neurons (see above; McQueen, 1990). However, bradycardia can be obtained via a central or a presynaptic action on sympathetic (inhibition) and/or cholinergic (stimulation) neurons.

4.2.1 von Bezold-Jarisch-like reflex

In the anaesthetized rats, intravenous bolus injections of 5-HT, 1-phenylbiguanide and 2-methyl-5-HT (Richardson et al., 1985; Dalton et al., 1986) elicit a short-lasting bradycardia that is effectively antagonized by metoclopramide (Fozard, 1984a), MDL 72222 (Fozard, 1984a,b), ICS 205-930 (Richardson et al., 1985) and a variety of other 5-HT_3 receptor antagonists (Cohen, 1990; Fozard, 1990). A similar agonist-antagonist action is also observed with respect to the depolarization of the rat cervical vagus nerve fibres (Ireland and Tyers, 1987).

Table 4: Heart rate responses to 5-HT and related drugs and the mechanisms involved in different species.

Species	Condition	Receptor	Main mechanism of action
Bradycardia			
Rat	Intact[a]	5-HT_3	Afferent neuronal stimulation[b]
Rabbit	Intact	5-HT_3	Afferent neuronal stimulation[b]
Cat	Intact	5-HT_3	Afferent neuronal stimulation[b]
Dog	Intact	Unknown	Afferent neuronal stimulation[c]
Rat	Intact	5-HT_{1A}	Central autonomic changes[d]
Cat	Intact	5-HT_{1A}	Central autonomic changes[d]
Rat	Intact	Unknown	Inhibition of sympathetic neurones
Dog	Intact	5-HT_1-like?	Inhibition of sympathetic neurones
Rabbit	Isolated heart[e]	5-HT_3	Cholinergic stimulation
Tachycardia			
Guinea-pig	Spinal	-	Tyramine-like action[f]
Dog	Isolated atria	-	Tyramine-like action[f]
Dog	Intact	-	Tyramine-like action[f]
Cat	Spinal	5-HT_1-like	Myocardial effect
Cat	ß-Adr. block[g]	5-HT_1-like	Myocardial effect
Rat	Ganglion-block	5-HT_2	Myocardial effect
Rat	Intact/pithed	5-HT_2[h]	Myocardial effect
Dog	Ganglion-block	5-HT_2	Adrenal catecholamine release
Rabbit	Isolated heart	5-HT_3	Neuronal catecholamine release
Dog	Conscious	5-HT_3	Not via autonomic nervous system
Pig	Intact	5-HT_4 ?	Myocardial effect
Mollusc	Isolated heart	Unknown	Unknown
Hamster	Intact	Unknown	Unknown
Man[i]	Intact	Unknown	Unknown

[a], Intact means anaesthetized or conscious animal; [b], von-Bezold-jarisch-like reflex; [c], cardiogenic hypertensive reflex; [d], inhibition of sympathetic neural activity plus augmentation of vagal tone; [e], prior reserpinization; [f], high concentrations are needed; [g], ß-adrenoceptor blockade; [h], receptor may differ from the usual 5-HT_2 receptor and high concentration may release catecholamines; [i], 5-HT_4 receptors may mediate inotropic effects in isolated human atria (Kaumann et al., 1990a,b). Taken from Saxena and Villalón (1990a).

5-HT also elicits bradycardia in the rabbit (Dawes and Mott, 1950; Bolt and Saxena, 1985; Wright and Angus, 1989). This effect is mimicked by 1-phenylbiguanide (Dawes and Mott, 1950; Wright and Angus, 1989) and 5-CT (probably by releasing platelet 5-HT; Wright and Angus, 1989), and antagonized by methylscopolamine (Wright and Angus, 1989), MDL 72222 (Wright and Angus, 1989), 2-naphtyl-guanidine and bufotenine (Fastier et al., 1959), but not by ketanserin (Wright and Angus, 1989).

Rapid bolus injections of 5-HT in the cat evoke bradycardiac effects that can be blocked by bilateral vagotomy, procaine, atropine, 2-methyl,3-ethyl,5-amino indole and MDL 72222 (Comroe et al., 1953; Saxena et al., 1985b). Injections of 5-HT into the left ventricle (Kottegoda and Mott, 1955), left coronary artery (Zakusov, 1962) or pericardial sac (Mohr et al., 1987), but not into the ascending aorta (Kottegoda and Mott, 1955), also induce reflex bradycardia. MDL 72222 blocks the effects of 5-HT without attenuating that of nicotine (Mohr et al., 1987).

The above findings clearly show that bradycardia due to 5-HT mainly results from an effect on cardiac sensory receptors (von Bezold-Jarisch-like reflex) belonging to the 5-HT_3 type.

4.2.2 Cardiogenic hypertensive chemoreflex

In the dog 5-HT induces a cardiogenic hypertensive chemoreflex which increases efferent vagal and sympathetic nerve activities, leading to an initial transient decrease in heart rate, followed by tachycardia (James, 1964; James et al., 1975). Curiously, cyproheptadine abolished the cardiogenic hypertensive reflex in atropine-pretreated dogs, but methysergide was reported to be ineffective (Hageman et al., 1977, 1980). A possible role of 5-HT_3 receptors in the cardiogenic hypertensive chemoreflex, because of antagonism by ICS 205-930, has also been described (Berthold et al., 1989).

4.2.3 Central 5-HT_{1A} mechanism

8-OH-DPAT, a 5-HT_{1A} receptor ligand (Table 1) reduces heart rate in both normotensive and two-kidney Goldblatt, DOCA-salt or spontaneously hypertensive rats; this response was not blocked by LY 53857, metergoline, methysergide, methiothepin, cinanserin or pirenperone, but was antagonized by pimozide (partly) or atropine + propranolol (completely) (Main et al., 1984; Gradin et al., 1985a,b). In contrast, Martin and Lis (1985) found that methiothepin (but not cyproheptadine or metergoline) blocked

the bradycardiac effects of 8-OH-DPAT in spontaneous hypertensive rats. More recently, a detailed analysis by Fozard et al. (1987) showed that the bradycardiac and hypotensive responses to 8-OH-DPAT in spontaneous hypertensive rats are centrally-mediated, and is not affected by p-chloro-phenylalanine, prazosin, ketanserin (0.1 mg/kg, s.c.) or MDL 72222, but are antagonized by 8-methoxy-2-(N-2-chloroethyl-N-n propyl) aminotetralin (8-MeO-ClEPAT), atropine, metergoline, methiothepin, (±)-pindolol, (±)-cyanopindolol, buspirone, yohimbine, idazoxan and WY 26392. Employing higher doses of metergoline and methiothepin than the earlier investigators (Gradin et al., 1985a,b; Martin and Lis, 1985), Fozard could conclude that the central effect of 8-OH-DPAT is apparently mediated via 5-HT_{1A} receptors on neurons that synapse distally via α_2-adrenoceptors (Fozard et al., 1987). Methysergide, but not cyproheptadine, also lowers heart rate in spontaneous hypertensive rats by a central action (Antonaccio and Cote, 1976).

In cats several agonist compounds having a high affinity for 5-HT_{1A} recognition sites, like 8-OH-DPAT, N,N-dipropyl-5-CT, flesinoxan, ipsapirone, p-aminophenyl-ethyl-m-trifluoromethylphenyl piperazine, 1-(2-methoxyphenyl) piperazine, lower heart rate whereas compounds with a higher affinity for 5-HT_{1B} recognition sites — 1-(3-trifluoromethyl) piperazine (TFMPP), 5-HT_{1C} recognition sites [(1-(3-chlorophenyl) piperazine (mCPP)] or 5-HT_2 recognition sites [1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) and piperazinyl-6-chloropyrazine (MK 212)] are ineffective (Ramage, 1985a,b; McCall et al., 1987; Ramage and Fozard, 1987; Doods et al., 1988; Wouters et al., 1988a,b).

The above results indicate that activation of central 5-HT_{1A} receptors increase vagal but decrease sympathetic tone to elicit bradycardia and hypotension; this pharmacological approach is of particular interest for the development of novel drugs with potential therapeutic usefulness in the treatment of hypertension (see Saxena and Villalón, 1990b).

4.2.4 Presynaptic inhibition of sympathetic activity

In rat (vagotomized, pithed or pretreated with atropine, captopril and ketanserin), preganglionic stimulation of the sympathetic nerves results in an increase in heart rate, which can be reduced by 5-methoxytryptamine (Göthert et al., 1986). The exact nature of the receptor involved has to be established.

In dogs 5-HT can attenuate the tachycardia induced by electrical stimulation of the ansa subclavia (Kimura and Satoh, 1983). This effect cannot be blocked by pretreatment by cyproheptadine suggesting the involvement of non-5-HT_2 receptors.

4.2.5 Stimulation of cholinergic neurones

In isolated perfused heart, obtained from reserpine-treated rabbits, 5-HT elicits a MDL 72222-susceptible bradycardia, which has been ascribed to the presence of 5-HT_3 receptors on postganglionic cholinergic nerve endings (Fozard, 1984a,b). However, it is likely that these 5-HT_3 receptors are located on cardiac vagal ganglia, since 5-HT_3 receptors have been found on parasympathetic ganglia (Saxena et al., 1985a; Bradley et al., 1986).

4.3 TACHYCARDIAC ACTION

The tachycardiac effect of 5-HT is notoriously species-dependent and the responses are mediated by a variety of mechanisms, namely:

4.3.1 Tyramine-like action

In the spinal guinea pig intravenous administration of 5-HT elicits tachycardia, and this response is not modified by methiothepin, ketanserin or MDL 72222, but is antagonized by ß-adrenoceptor antagonists (propranolol and atenolol) or by the 5-HT-uptake inhibitor indalpine. Pretreatment with reserpine did not affect the first challenge with 5-HT but the responses to the subsequent doses showed rapid tachyphylaxis (Dhasmana et al., 1988). In the guinea-pig isolated atrium, results similar to those in spinal animals, were reported except that reserpine was not able to influence the responses to 5-HT (Eglen and Whiting, 1989). However, it is not known if these authors administered 5-HT repeatedly in reserpinized atria as was done in an earlier study (Dhasmana et al., 1988). At this stage it would appear that the main action of 5-HT on the guinea-pig heart is via a release of catecholamines by a mechanism similar, but not identical, to that of tyramine. In addition, it is possible that 5-HT may release calcitonin gene-related peptide, which is present in the guinea pig sinus node and has a positive chronotropic action (Saito et al., 1986).

In atria obtained from the dog, the chronotropic response to 5-HT is attenuated by both desipramine and ß-adrenoceptor antagonists, suggesting an indirect sympathomimetic action (Chiba, 1977; Chiba et al., 1978). Similar findings have been obtained with high doses of 5-HT *in vivo* (Fillion et al., 1971).

4.3.2 5-HT_1-like receptor stimulation

In an excellent investigation Trendelenburg (1960) reported that the 5-HT-induced cardiostimulation in the cat isolated atria, being antagonized by lysergide but not by reserpine, dichloroisoprenaline or cocaine, involves "D" 5-HT receptors (Gaddum and Picarelli, 1957), which may belong to either the 5-HT_1-like or the 5-HT_2 type (Saxena, 1989; Saxena and Ferrari, 1989). Accordingly, the responses to 5-HT in the reserpine-pretreated kitten atria can be blocked by phenoxybenzamine or methysergide, but not by ketanserin, MDL 72222 or yohimbine (Kaumann, 1985).

In a detailed analysis in spinal cats (Saxena et al., 1985b), it was established that the 5-HT-induced tachycardia is not or only slightly susceptible to blockade by guanethidine, propranolol, burimamide, 5-HT_2 receptor antagonists (cyproheptadine, ketanserin, ritanserin, pizotifen and mianserin) or bilateral adrenalectomy, despite the fact that 5-HT may sometimes release catecholamines from the adrenal medulla (Reid, 1952). This response is effectively blocked by mixed 5-HT_1-like and 5-HT_2 receptor antagonists (methiothepin, methysergide, mesulergine) and mimicked by 5-CT, to establish that 5-HT_1-like receptors mediate tachycardia in the cat (Saxena et al., 1985b; Connor et al., 1986; Saxena, 1988)

4.3.3 5-HT_2 receptor stimulation

In pithed rats 5-HT (100 μg/kg) increases heart rate and blood pressure and these responses are effectively antagonized by cyproheptadine but, in contrast, another 5-HT_2 receptor antagonist 3-{2-[4-(4-fluorobenzoyl)-1-piperidinyl]ethyl}-2-methyl-4H-pyrido[1,2-a] pyrimidin-4-one (R 50656) suppressed the pressor response but not the tachycardia (Krstic' and Katusic, 1982). Subsequently, Saxena and Lawang (1985) showed that 5-CT, in contrast to 5-HT, does not increase heart rate in ganglion-blocked rats and the tachycardia elicited by 5-HT (6.25-200 μg/kg) is blocked by ketanserin (0.5 mg/kg) or cyproheptadine (0.5 mg/kg), suggesting the involvement of 5-HT_2 receptors. Accordingly, in pithed normotensive and/or spontaneously hypertensive rats,

5-methoxytryptamine and 5-HT, but not 5-CT, 8-OH-DPAT, ipsapirone, RU 24969, 5-methoxy-N,N-dimethyltryptamine, TFMPP or DOI, cause tachycardia which is susceptible to blockade by ketanserin, LY 53857, methysergide or methiothepin, but not by MDL 72222, propranolol or desipramine (Göthert et al., 1986; Docherty, 1988; Dabiré et al., 1989a,b). It is interesting to note that DOI, which exhibited a partial agonist action at 5-HT_2 receptors mediating pressor responses, neither elicited tachycardia nor antagonized the tachycardiac action of 5-HT (Dabiré et al., 1989a, b). The ineffectiveness of DOI (Dabiré et al., 1989a,b) and R 50656 (Krstic' and Katusic, 1982) suggests that the 5-HT_2 receptors mediating tachycardia in the rat are 'atypical' in nature. However, at high doses (100 μg/kg and above) 5-HT can also increase heart rate probably by a tyramine-like action, which can be reduced by desipramine or propranolol (Docherty, 1988).

Many investigators have reported that 5-HT elicits tachycardia in anaesthetized dogs (Maxwell et al., 1959; Noble and Nanson, 1959); this effect is accompanied by an increase in noradrenaline concentration in the coronary sinus and vena caval blood and is absent after autonomic blockade (Fillion et al., 1971). Subsequently, it was shown that 5-HT is capable of eliciting tachycardiac responses (antagonized by cyproheptadine or bufetolol) in vagotomized and cardiac decentralized dogs (Kimura and Satoh, 1983), and the 5-HT-induced tachycardia in ganglion-blocked dogs is accompanied by a release of noradrenaline and adrenaline into the blood and suppressed not only by cyproheptadine and methysergide, but also by syrosingopine (a catecholamine depleting agent) and bilateral adrenalectomy (43% reduction) (Feniuk et al., 1981; Kimura and Sato, 1983). It therefore appears that 5-HT elicits tachycardia by a tyramine-like action as well as by a 5-HT_2 receptor-mediated release of catecholamines from the adrenomedullary chromaffin cells.

4.3.4 5-HT_3 receptor stimulation

The tachycardiac effect of 5-HT in the rabbit isolated atria is mediated by noradrenaline release (Trendelenburg, 1960) and detailed analyses in the perfused heart have revealed that reserpine, propranolol, cocaine, MDL 72222, ICS 205-930, but not desipramine, methiothepin or methysergide, inhibit the increase in heart rate by 5-HT (Fozard and Mwaluko, 1976; Göthert and Dührsen, 1979; Fozard, 1984b; Richardson et al., 1985). Therefore, the cardiostimulatory effect of 5-HT in the rabbit is due to a 5-HT_3 receptor-mediated release of noradrenaline from the postganglionic cardiac sympathetic nerve fibres. The action of 5-HT is mimicked by 2-methyl-5-HT and

5,7-dihydroxytryptamine (Göthert and Dührsen, 1979; Richardson et al., 1985). Recently, Wilson et al. (1990) have described that 5-HT_3 receptors mediate tachycardia in the conscious dog.

4.3.5 Stimulation of a novel 5-HT receptor

It has been previously reported that the tachycardia induced by 5-HT in the anaesthetized pig is not antagonized by drugs affecting autonomic receptors, histamine H_1 and H_2 receptors, dopamine receptors, calcium channels, 5-HT_1-like, 5-HT_2 and/or 5-HT_3 receptors (Duncker et al., 1985; Bom et al., 1988a). Moreover, selective agonists at 5-HT_1-like or 5-HT_3 receptors do not elicit tachycardia (see Table 5). Therefore, the tachycardiac action of 5-HT in the pig does not involve endogenous catecholamines or histamine, Ca^{2+} transport into the sinoatrial node cells or the 5-HT_1-like, 5-HT_2 or 5-HT_3 receptors, but seems to be mediated by a new type of 5-HT receptor (Duncker et al., 1985; Bom et al., 1988a).

In this context, Dumuis and her colleagues (1988, 1989), working with cultured neurones from mouse embryo colliculi, described a novel receptor site (designated as 5-HT_4), where 5-methoxytryptamine and certain benzamide derivatives (metoclopramide, renzapride, cisapride), but not 5-CT, α-methyl-5-HT or 2-methyl-5-HT, have high affinity and increase adenylate cyclase activity; ICS 205-930 (in high concentrations; pA_2: 6.3-6.5), but not MDL 72222 or ondansetron, behaves as an antagonist (see also Clarke et al., 1989). In the cardiovascular system this novel 5-HT receptor seems to be involved in the positive inotropic action of 5-HT on human atria; the response to 5-HT is not modified by ketanserin, methysergide, lysergide, methiothepin, yohimbine, (±)propranolol, (-)pindolol or MDL 72222, but was blocked by a high concentration (2 μM) of ICS 205-930 (Kaumann et al., 1990a,b).

Table 5: Capacity of drugs to cause tachycardia or to antagonize 5-HT-induced tachycardia in the anaesthetized pig.

Drug	Dose (μg/kg)	Drug profile	Activity as agonist or antagonist
Agonists			
5-CH_3O-tryptamine	3-30	5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, 5-HT_{1D}, 5-HT_2, 5-HT_4	Yes
Renzapride	3-100	5-HT_4	Yes
5-CT	1-100	5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}	No
8-OH-DPAT	1000	5-HT_{1A}	No
RU 24969	1000	5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}	No
BEA 1654	1000	5-HT_{1A}	No
2-CH_3-5-HT	30-100	5-HT_3	No
α-CH_3-5-HT	3-100	5-HT_{1C}, 5-HT_2, 5-HT_4	Yes
Antagonists			
Phentolamine	1000	α-Adrenoceptor	No
Propranolol	500	ß-Adrenoceptors	No
Atropine	500	Muscarinic cholinergic	No
Hexamethonium	10000	Nicotine$_1$ cholinergic	No
Mepyramine	1000	Histamine H_1	No
Cimetidine	1000	Histamine H_2	No
Haloperidol	1000	Dopamine D_1, D_2	No
Verapamil	100[a]	Calcium channels	No
Phenoxybenzamine	1000	Adrenergic, cholinergic, 5-HT	No
Methiothepin	500	5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, 5-HT_{1D}, 5-HT_2	No
Methysergide	500	5-HT_{1A}, 5-HT_{1C}, 5-HT_{1D}, 5-HT_2	No
Metergoline	500	5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, 5-HT_{1D}, 5-HT_2	No
Mesulergine	500	5-HT_{1C}, 5-HT_2	No
Mianserin	500	5-HT_{1C}, 5-HT_2	No
Pizotifen	500	5-HT_{1C}, 5-HT_2	No
Cyproheptadine	500	5-HT_2	No
Ketanserin	500	5-HT_2	No
MDL 72222	300	5-HT_3	No
ICS 205-930	300	5-HT_3	No

[a], dose followed by 0.01 mg/kg.min. Based on data from Bom et al. (1988a) and Saxena and Villalón (1990a).

4.3.6 Unidentified mechanisms

The hearts of certain lamellibranch and gastropod species (*Mercenaria mercinaria, Tapes watlingi, Patella vulgata, Helix, Aplysia*) are extremely sensitive to 5-HT (threshold concentration 0.1 nM; for references, see Walker, 1985) and in *Aplysia* heart 5-HT increases cAMP accumulation (Sawada et al., 1984). In the *Mercenaria* heart tryptamine derivatives increase the rate and contraction with the following order of activity (equipotent molar ratio; 5-HT = 1): N,N-dimethyl-5-HT (0.028), N-methyltryptamine (3.7), α-methyl-5-HT (6.0), N,N-diethyltryptamine (7.9), α-methyl-tryptamine (8.6), N-ethyltryptamine (9.1), tryptamine (9.9), N,N-dimethyltryptamine (10.7), 2-methyl-5-HT (31.4). The effect of 5-HT is antagonized by methysergide and 2-bromolysergide, but lysergide mimics 5-HT with incredible potency (threshold concentration 1 fM). There is evidence that 5-HT is an excitatory transmitter in the *Mercenaria* heart; stimulation of the cardioaccelerator nerve releases 5-HT and the its effects are reduced by methysergide, 2-bromolysergide and reserpine (Walker, 1985).

In hamster isolated atria, 5-HT increases heart rate, probably by combination of a direct effect on the myocardium and an indirect effect via the liberation of noradrenaline from cardiac sympathetic nerves, since the responses are not much affected by phentolamine + propranolol or phenoxybenzamine (González and García, 1977, 1978).

In humans, slow intravenous infusions of 5-HT elicit tachycardia before any changes in arterial blood pressure occur (Hollander et al., 1957, LeMessurier et al., 1959; Harris et al., 1960).

In conclusion, from the aforementioned studies it is clear that 5-HT can induce heart rate changes by a whole spectrum of mechanisms and receptors, whereas the mechanisms involved in 5-HT-induced changes in cardiac contractile force are still ill-understood.

CHAPTER 5

EFFECTS OF 5-HT AND RELATED DRUGS IN THE CAROTID CIRCULATION

CHAPTER 5

EFFECTS OF 5-HT AND RELATED DRUGS IN THE CAROTID CIRCULATION

5.1 INTRODUCTION

As in other vascular beds, 5-HT can elicit either vasodilatation or vasoconstriction in the carotid vascular bed. The eventual response depends -amongst other factors- upon the species, dose, vascular segment under study and, most importantly, the nature of 5-HT receptors involved.

5.2 EFFECT OF 5-HT IN THE CAROTID BLOOD FLOW OF SEVERAL SPECIES

Intracarotid administration of 5-HT in the intact dog increases external carotid blood flow, but decreases internal carotid blood flow (Vidrio and Hong, 1976), the former response being dependent on the vascular tone as can be reverted to vasoconstriction when vascular tone is abolished by vagotomy or stellectomy (Saxena, 1972; Mena and Vidrio, 1979), whereas the latter is independent of vascular tone (Vidrio and Hong, 1976; Mena and Vidrio, 1979). Similar differential effects of 5-HT have been reported in primates (Grimson et al., 1969). In contrast, 5-HT elicits a decrease of the carotid blood flow in humans (Lance et al., 1967) and cats (Saxena and Verdouw, 1983). In the latter, such effect is related to a decrease in arteriovenous anastomotic blood flow which is resistant to blockade by cyproheptadine (Saxena and Verdouw, 1983). More recently, Hong and Villalón (1988) have reported that 5-HT_1-like receptors are involved in the vasodilatation and vasoconstriction induced by 5-HT in the canine external carotid artery.

In the anaesthetized bilaterally vagotomized pig, intracarotid infusion of 5-HT decreases common carotid blood flow by reducing arteriovenous anastomotic (non-nutrient) flow, whereas arteriolar flow to the head tissues, mainly to the skin and ears, is increased (Saxena and Verdouw, 1982, 1985b).

5.3 EFFECTS OF 5-HT RECEPTOR AGONISTS AND ANTAGONISTS IN THE PORCINE CAROTID BLOOD FLOW AND ITS DISTRIBUTION

The effects of 5-HT in the porcine carotid circulation, as previously described, are mimicked by the 5-HT_1-like receptor agonist 5-carboxamidotryptamine (Saxena and Verdouw, 1985a) and antagonized by methiothepin (Saxena et al., 1986a), which blocks both 5-HT_1-like and 5-HT_2 receptors (see Hoyer, 1988a; Saxena and Villalón, 1990a), but not appreciably by the 5-HT_2 receptor antagonist ketanserin (Verdouw et al., 1984) or the 5-HT_3 receptor antagonist 1αH,3α,5αH-tropan-3yl-3,-5-dichlorobenzoate (MDL 72222) (Saxena et al., 1986a). Therefore, according to the classification proposed by Bradley et al. (1986), the constriction of arteriovenous anastomoses (AVAs) as well as the dilatation of arterioles are mediated by 5-HT_1-like receptors (Saxena et al., 1986a). However, the 5-HT_1-like receptors in the carotid circulation of the pig seem to belong to two different categories since, unlike 5-HT (Saxena and Verdouw, 1982) or 5-carboxamidotryptamine (Saxena and Verdouw, 1985a), the reductions of the arteriovenous anastomotic blood flow induced by methysergide (Saxena and Verdouw 1984), the putative 5-HT_{1A} receptor agonists 8-OH-DPAT (Bom et al., 1989a) and N-(3-acetylaminophenyl) piperazine hydrochloride (BEA 1654) (Verdouw et al., 1985), and the putative 5-HT_{1A} and 5-HT_{1B} receptor agonist RU 24969 (Bom et al., 1989b), are not or only slightly accompanied by increases in the arteriolar blood flow. Furthermore, despite the effectiveness of 8-OH-DPAT, BEA 1654 and RU 24969, the 5-HT_1-like receptor mediating arteriovenous anastomotic constriction does not appear to be related to either the 5-HT_{1A} or 5-HT_{1B} binding sites because, as we reported recently (Bom et al., 1989b), the effects of RU 24969 are not antagonized by the putative 5-HT_{1A} and 5-HT_{1B} receptor antagonist, (±)-pindolol (Hoyer et al., 1985; Hoyer, 1988a). Within this framework, it is of particular importance to characterize drugs with high selectivity to decrease cranial AVA blood flow, as this is one of the few experimental models that could make it possible to develop drugs with potential therapeutic usefulness in the treatment of migraine (see Saxena and Ferrari, 1989; Saxena, 1990). Indeed, some drugs with established antimigraine efficacy decrease porcine cranial AVA blood flow (see below; Saxena and Ferrari, 1989; Saxena and Den Boer, 1991).

5.4 MIGRAINE

Migraine is a debilitating disease which affects up to 10% of the Western World, regarded as an episodic syndrome characterized by usually unilateral headache, nausea,

vomiting and photophobia, sometimes preceded by certain premonitory aura symptoms (Wolff, 1963; Lance, 1982; Lance et al., 1989). Migraine, like hypertension, is a syndrome where the underlying causative factor(s) can vary widely. In this connection, theories to explain migraine pathophysiology have evolved from the realms of the supernatural into the scientific arena (for references, see Edmeads, 1991), where the controversy whether migraine is primarily a vascular or a neurological disfunction has been argued for over a hundred years, and the scientific community still appears to remain divided. Despite intensification of research in recent years, however, migraine aetiology remains elusive (see Lance, 1982; Lance et al., 1967, 1989; Saxena, 1987; 1990; Edmeads, 1991; Moskowitz and Buzzi, 1991).

With respect to the vascular origin of migraine, this syndrome has long been considered to result from cephalic vasodilatation, if only because of the throbbing nature of the pain (Edmeads, 1991). This concept became established as a result of the pioneering work of Graham and Wolff (1938), whose findings implicated the cranial, as opposed to the cerebral, component of the cephalic circulation. During a significant number of migraine attacks, pulsations in the superficial temporary artery were demonstrated using an external tambour, and pain could be relieved either by physical compression or by the vasoconstrictor agents ergotamine or dihydroergotamine (Graham and Wolff, 1938; Wolff, 1963).

This "vascular hypothesis" has, in recent times, fallen into disfavour largely because many patients exhibit facial pallor during attacks, not flushing (a reflection of cranial vasodilatation), and because other researchers have not been able to confirm Wolff's findings (Heyck, 1969; see below). However, the involvement of extracranial vessels has been confirmed in some patients; for example, Drummond and Lance (1983) demonstrated that temporal artery compression reduced pain intensity in about one-third of patients, while a further one-third experienced relief following common carotid artery compression. These findings are in complete agreement with those reported by Graham and Wolff (1938).

Wolff (1963) was also able to show that extracranial vascular distension was associated with perivascular oedema, resulting from sterile inflammation. In this connection, the trigeminal nerve has been implicated in the genesis of migraine (for references, see Moskowitz and Buzzi, 1991). In support of this "neural hypothesis", it has been shown that the trigeminal nerve can release a number of neuropeptides including substance P and calcitonin gene-related peptide, and that antidromic activation of the trigeminal nerve leads to plasma protein extravasation (and presumably vasodilatation) in the dura mater of rats and guinea pigs; interestingly, antimigraine drugs such as

ergotamine, dihydroergotamine and sumatriptan inhibit substance P-induced plasma protein extravasation in rats (Moskowitz and Buzzi, 1991). However, it is possible that this inhibitory effect is related to the selective vasoconstriction exerted by these drugs in the extracerebral vascular bed (see below).

5.4.1 5-HT and the pathophysiology of migraine

Amongst a host of biogenic substances implicated in the pathophysiology of migraine, none seems to have a better claim than 5-HT (for references, see Wolff, 1963; Lance, 1982; Saxena, 1987, 1990, 1991; Lance et al., 1989; Feniuk et al., 1991). Of particular importance were the clinical observations that: i) urinary excretion of 5-HIAA (5-hydroxyindoleacetic acid), the main metabolite of 5-HT, increases during migraine attacks; ii) both platelet 5-HT and whole blood 5-HT decrease during migraine attacks; iii) intramuscular injections of reserpine precipitate migraine-like headaches in migraineurs; iv) slow intravenous infusion of 5-HT alleviates both reserpine-induced and spontaneous migraine headaches (Lance et al., 1967; 1989; Sicuteri, 1977; Lance, 1982; Saxena, 1987, 1990, 1991; Feniuk et al., 1991). The above findings coupled to the fact that the 5-HT_1-like receptor agonist sumatriptan aborts migraine headaches and that 5-HT receptor antagonists, e.g. ketanserin, cyproheptadine and ICS 205-930 are poorly effective in migraine (see below), do suggest the involvement of 5-HT in migraine aetiology.

Within the bounds of the 5-HT theory, and keeping in mind the foregoing controversy about migraine pathogenesis, we would like to draw some attention to the following facts: i) distension and sensitivity of extracranial arteries can be demonstrated in about one third of migrainous patients (see above; Drummond and Lance, 1983; Lance et al., 1989), so that vascular factors cannot be ignored, whether they be primary or secondary to neural discharge; ii) the neural hypothesis of migraine is theoretical and based on animal experimentation, which argues against a purely neural hypothesis (for references, see Lance et al., 1989; Moskowitz and Buzzi, 1991; Saxena and Den boer, 1991).

5.4.2 Reduction in cranial arteriovenous anastomoses (AVAs) blood flow as a pharmacological approach for antimigraine action

The paradox between the suggested cerebral and extracerebral vasodilatation and some clinical features of migraine (facial pallor, venous engorgement and cold skin) prompted Heyck (1969) to measure oxygen difference in arterial and venous blood in migraineurs. He found that: i) the arteriovenous oxygen saturation difference between arterial and jugular venous blood decreased during migraine attacks, suggesting a higher arteriovenous shunting in the territory drained by the jugular vein; and ii) pain relief (either spontaneously or after dihydroergotamine treatment) was accompanied by a normalization of the arteriovenous oxygen saturation difference. On the basis of these results, Heyck (1969) suggested that during migraine attacks cranial AVAs opened and lower the diastolic blood pressure, causing greater pulsations and an increased AVA fraction of carotid blood flow.

From the above evidence, cranial arteriovenous anastomoses constriction has been proposed as a possible mechanism for antimigraine action (see Saxena, 1987; 1990; Saxena and Ferrari, 1989; Saxena and Den Boer, 1991). Significantly, a number of new and established antimigraine drugs interact with 5-HT receptors (see Table 6). Selective agonists at the 5-HT_1-like receptor subtype mediating contractions of cephalic vessels (AH25086, sumatriptan), which do not penetrate into the central nervous system, are effective in the treatment of acute migraine attacks (see Perrin et al., 1989; Saxena and Ferrari, 1989; Humphrey et al., 1990; Tfelt-Hanssen and Nielsen, 1990). Some antimigraine drugs behave as antagonists at 5-HT_2 (methysergide, pizotifen, ergotamine, dihydroergotamine) and/or 5-HT_1-like (methysergide, propranolol) receptors, but many 5-HT_2 and/or 5-HT_1-like receptor antagonists (ketanserin, ritanserin, cyproheptadine, mianserin, methiothepin, metergoline) have not found much use in migraine therapy. It therefore seems likely that additional properties of such antimigraine drugs, for example, the vasoconstriction in the extracerebral cephalic circulation with methysergide, ergotamine and dihydroergotamine and the antidepressant action with pizotifen, may be involved in their therapeutic action (Saxena, 1990). Though MDL 72222, a 5-HT_3 receptor antagonist, has been reported to be effective against migraine (Loisy et al., 1985), ICS 205-930 (Lataste et al., 1989) and, probably, other such drugs are not. Therefore, within the bounds of serotonergic mechanisms, the antimigraine action seems to depend mainly upon agonist action at the 5-HT_1-like receptor subtype (5-HT_{1x}; Table 1) that mediates craniovascular contraction (Saxena, 1990; Saxena and Ferrari, 1989).

Table 6: Profile of potential and proven antimigraine drugs in relation to 5-HT receptors.

Antimigraine drug	5-HT_1-like Receptor	5-HT_2 Receptor	5-HT_3 Receptor	Penetration into the CNS
AH25086	Agonist[a]	Inactive	Inactive	Poor or none
Sumatriptan	Agonist[a]	Inactive	Inactive	Poor or none
Methysergide	Partial agonist[a,b]	Antagonist[c]	Inactive	Possibly yes
Ergotamine	?	Antagonist	Inactive	Poor or none
Dihydroergotamine	?	Antagonist	Inactive	?
Pizotifen	Inactive	Antagonist	Inactive	Possibly yes
Propranolol	Weak antagonist[d]	Inactive	Inactive	Rapid
MDL 72222	Inactive	Inactive	Antagonist[e]	?

[a], Action is selective on 5-HT_1-like receptors mediating the contraction of cephalic arteries and arteriovenous anastomoses; [b], Antagonists of 5-HT_1-like receptors (methiothepin, metergoline) have no antimigraine activity; [c], Many other 5-HT_2 antagonists (ketanserin, retanserin, cyproheptadine) have not proved much effective in migraine therapy; [d], Other antimigraine ß-adrenoceptor antagonists (atenolol, timolol) have no affinity for 5-HT_1-like receptors; [e], ICS 205-930 and probably some other antagonists at 5-HT_3 receptors are not much effective in migraine. CNS, Central nervous system. (For references see Saxena, 1990; Saxena and Den Boer, 1991).

CHAPTER 6

AIMS OF THE THESIS

CHAPTER 6

AIMS OF THE THESIS

There exists now an extensive literature dealing with the nature of the 5-HT receptors involved in 5-HT-induced cardiovascular effects. For example, 5-HT-induced tachycardia is notoriously species-dependent and is mediated, directly or indirectly, either by 5-HT_1-like (cat), 5-HT_2 (rat, dog) or 5-HT_3 (rabbit, dog) receptors, or by tyramine-like (guinea-pig) or unidentified mechanisms (pig, Helix aspersa) (see Saxena, 1986; Saxena and Villalón, 1990a; 1991). In marked contrast, little is known about the nature of the myocardial receptors involved in 5-HT-induced positive inotropic effects (Kaumann et al., 1990a,b). Therefore, Chapters 7 and 8 of the present thesis deal with the mechanism(s) and characterization of the 5-HT receptor type involved in the positive chronotropic effects induced by 5-HT and some indole- and benzamide derivatives in the pentobarbitone anaesthetized pig, whereas in Chapter 9 an attempt was made to delineate the receptor involved in the positive inotropic effect by 5-HT in the same species.

On the other hand, it is has been demonstrated that 5-HT_1-like receptors mediate both the constriction of porcine cephalic arteriovenous anastomoses and the dilatation of arterioles induced by 5-HT and some 5-HT_1-like receptor agonists, including the antimigraine drug sumatriptan (Saxena et al., 1989; Saxena and Villalón, 1990a; Den Boer et al., 1991); however, it has not yet been fully identified which specific 5-HT_1 receptor subtype (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} or 5-HT_{1D}) is involved in such effects (Bom et al., 1989a,b; Saxena and Villalón, 1990a). For this reason in Chapter 10 of this dissertation we have further attempted to study the possible involvement of 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} and/or 5-HT_{1D} receptors in the distribution of common carotid artery blood flow into arteriolar (nutrient) and arteriovenous anastomotic (non-nutrient) parts in the pig by using indorenate, a tryptamine derivative with antihypertensive properties (Hong et al., 1978; Hong, 1981) as well as a high affinity for the 5-HT_{1A} binding site (Dompert et al., 1985; Hoyer et al., 1985). Ketanserin, methiothepin and metergoline were used as potential antagonists.

Finally, in view of the involvement of cranial arteriovenous anastomoses constriction as a possible mechanism for antimigraine action (see Saxena and Ferrari, 1989), the last part of the present dissertation is devoted to the analysis of the effects of dihydroergotamine (an established antimigraine drug), and S9977 (a potential

antimigraine drug) on the distribution of the porcine total common carotid artery blood flow (Chapter 11), and accordingly, highlights the possible mechanisms that might explain the action of the antimigraine drugs.

PART II

CHAPTER 7

MEDIATION OF 5-HYDROXYTRYPTAMINE-INDUCED TACHYCARDIA IN THE PIG BY THE PUTATIVE 5-HT_4 RECEPTOR

Villalón, C.M., den Boer, M.O., Heiligers, J.P.C. and Saxena, P.R.
Br. J. Pharmacol. (Special Report), 100, 665-667, 1990.

Br. J. Pharmacol. (1990), 100, 665–667 SPECIAL REPORT

Mediation of 5-hydroxytryptamine-induced tachycardia in the pig by the putative 5-HT_4 receptor

Carlos M. Villalón, Marien O. den Boer, Jan P.C. Heiligers & [1]Pramod R. Saxena

Department of Pharmacology, Faculty of Medicine and Health Sciences, Erasmus University Rotterdam, Post Box 1738, 3000 DR Rotterdam, The Netherlands

Intravenous bolus injections of 5-hydroxytryptamine (5-HT; 3, 10 and 30 $\mu g\,kg^{-1}$), 5-methoxytryptamine (5-MeO-T; 3, 10 and 30 $\mu g\,kg^{-1}$), renzapride (BRL 24924; 3, 10, 30 and 100 $\mu g\,kg^{-1}$) and isoprenaline (0.03, 0.1 and 0.3 $\mu g\,kg^{-1}$) to anaesthetized pigs increased heart rate by, respectively, 22 ± 3, 44 ± 3 and 65 ± 4 beats min^{-1} (5-HT; $n = 17$); 12 ± 1, 26 ± 2 and 44 ± 4 beats min^{-1} (5-MeO-T; $n = 15$), 5 ± 2, 11 ± 2, 18 ± 4 and 37 ± 5 beats min^{-1} (renzapride; $n = 8$) and 17 ± 2, 46 ± 3 and 75 ± 3 beats min^{-1} (isoprenaline; $n = 13$). The responses to 5-HT, 5-MeO-T and renzapride were antagonized by ICS 205-930 (1 and 3 $mg\,kg^{-1}$, i.v.), which did not modify the increases in heart rate by isoprenaline. Renzapride showed tachyphylaxis and attenuated the responses to 5-HT. These findings indicate that 5-HT elicits tachycardia in the pig by acting on a novel receptor, either similar or identical to the 5-HT_4 receptor identified in mouse brain colliculi.

Introduction The mechanism of tachycardia elicited by 5-hydroxytryptamine (5-HT) is notoriously species-dependent and is mediated, directly or indirectly, either by 5-HT_1-like (cat), 5-HT_2 (rat, dog) or 5-HT_3 (rabbit, dog) receptors, or by tyramine-like (guinea-pig) or unidentified mechanisms (see Saxena, 1986; Saxena & Villalón, 1990). In the pig, we have found that the 5-HT-induced tachycardia is mediated by a novel receptor type different from either the 5-HT_1-like, 5-HT_2 or 5-HT_3 receptor (Duncker *et al.*, 1985; Bom *et al.*, 1988). Recently, a novel 5-HT receptor site, coupled positively to adenylate cyclase and designated as 5-HT_4, has been located in cultured neurones from mouse embryo colliculi (Dumuis *et al.*, 1988; 1989; Clarke *et al.*, 1989). The purpose of this investigation was to determine whether the porcine heart 5-HT receptor is similar to the putative 5-HT_4 receptor in the mouse brain.

Methods As described in detail previously (Bom *et al.*, 1988), 21 Yorkshire pigs (15–20 kg) were, after sedation with azaperone (120 mg, i.m.) and metomidate (120–150 mg, i.v.), intubated for positive pressure ventilation with a mixture of room air (70%) and O_2 (30%). The anaesthesia was maintained with a continuous infusion of pentobarbitone sodium (15–20 $mg\,kg^{-1}\,h^{-1}$, i.v.). Both vagosympathetic trunks were cut and aortic blood pressure and heart rate were recorded. The right jugular vein was cannulated for drug injections. The body temperature of the animals was maintained around 37°C and arterial blood gases and pH were kept within the normal limits. After the animals had been in a stable haemodynamic condition for at least 30 min, they received bolus injections of 5-HT, 5-methoxytryptamine (5-MeO-T), renzapride and/or isoprenaline. Subsequently, three doses of ICS 205–930 (cumulatively: 0.3, 1.0 and 3.0 $mg\,kg^{-1}$) or equivalent volumes of physiological saline were administered and the responses to the four agonists were elicited again (for doses and number of observations in each case, see Results).

The drugs used in this study were 5-HT creatinine sulphate (Sigma Chemical Company, St. Louis, MO, U.S.A.), 5-MeO-T hydrochloride (Janssen Pharmaceutica, Beerse, Belgium), renzapride hydrochloride (BRL 24924; gift: Dr G.J. Sanger, Beecham Pharmaceuticals, Harlow, Essex, England), isoprenaline sulphate (Pharmacy Department, Erasmus University, Rotterdam, The Netherlands) and (3α-tropanyl)-1H-indole-3-carboxyl acid ester (ICS 205-930). The doses of 5-HT and 5-MeO-T are given as free base. The percentage changes from the initial agonist response in the saline- and ICS 205-930-treated animals were compared by Student's *t* test. Statistical significance was accepted at $P < 0.05$. All data are reported as mean ± s.e.mean.

Results *Initial blood pressure and heart rate changes by agonist drugs* Baseline values of mean arterial blood pressure and heart rate in the 21 pigs were 84 ± 4 mmHg and 102 ± 5 beats min^{-1}, respectively. The changes in the mean arterial blood pressure were: 5-HT (-17 ± 1, -16 ± 2 and -10 ± 3 followed by $+5 \pm 1$ mmHg after 3, 10 and 30 $\mu g\,kg^{-1}$, respectively; $n = 17$), 5-MeO-T (-18 ± 3, -17 ± 2 and -16 ± 2 followed by $+6 \pm 2$ mmHg after 3, 10 and 30 $\mu g\,kg^{-1}$, respectively; $n = 15$) and renzapride ($+1 \pm 1$, $+4 \pm 1$, $+8 \pm 1$ and $+9 \pm 1$ mmHg after 3, 10, 30 and 100 $\mu g\,kg^{-1}$, respectively; $n = 8$). These effects were not evaluated further.

As shown in Figure 1, 5-HT, 5-MeO-T, renzapride and isoprenaline caused dose-dependent increases in heart rate. Isoprenaline was about 2 log units more potent than 5-HT, which was itself more potent than 5-MeO-T or renzapride. At the doses used the duration of action of renzapride (10.8 ± 2.9, 25.7 ± 3.6, 36.8 ± 3.9 and 45.7 ± 2.8 min) was much longer than that of 5-MeO-T (4.2 ± 0.7, 8.3 ± 1.0 and 12.5 ± 1.3 min) or 5-HT (3.4 ± 0.5, 6.0 ± 0.7 and 8.4 ± 0.9 min).

Modification of heart rate responses by physiological saline or ICS 205-930 The tachycardia produced in response to 5-HT, 5-MeO-T and isoprenaline remained essentially unchanged in control animals receiving saline. In the animals where ICS 205-930 was administered, the responses to both 5-HT and 5-MeO-T remained unchanged after the lowest dose (0.3 $mg\,kg^{-1}$), but were clearly antagonized by the two higher doses (1 and 3 $mg\,kg^{-1}$). The responses to repeated doses of renzapride in the control animals were progressively reduced; nevertheless, tachycardia due to renzapride was more inhibited after ICS 205-930 than after saline. The isoprenaline-induced tachycardia was unaffected by ICS 205-930 when compared to the saline-treated animals (Figure 2).

In the saline-treated animals where 5-HT was administered before and after the last set of injections of 5-MeO-T or renzapride ($n = 5$ each), the responses to 5-HT (3, 10 and 30 $\mu g\,kg^{-1}$) remained unaltered after 5-MeO-T (14 ± 4, 39 ± 4 and 63 ± 5 beats min^{-1} before and 15 ± 4, 41 ± 6 and 59 ± 8 beats min^{-1} after 5-MeO-T, respectively), but were reduced by renzapride (27 ± 5, 50 ± 5 and 69 ± 5 beats min^{-1} before and 11 ± 3, 22 ± 3 and 31 ± 2 beats min^{-1} and after renzapride, respectively).

[1] Author for correspondence.

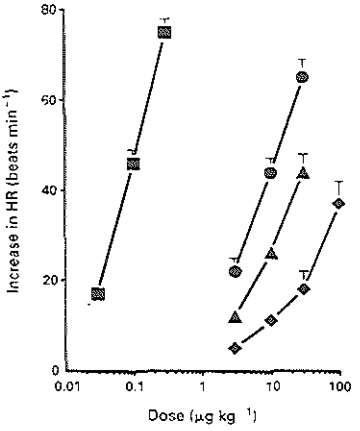

Figure 1 The initial tachycardiac responses to isoprenaline (▩), 5-hydroxytryptamine (●), 5-methoxytryptamine (▲) and renzapride (◆) in the anaesthetized pig. The number of observations with the 4 drugs were 13, 17, 15 and 8, respectively. HR, heart rate.

Discussion In previous publications (Duncker *et al.*, 1985; Bom *et al.*, 1988) we showed that the 5-HT-induced tachycardia in the pig is not antagonized by drugs that act as antagonists at various receptors: 5-HT_1 and/or 5-HT_2 (methiothepin, methysergide, ketanserin); 5-HT_3 (MDL 72222, ICS 205-930); adrenoceptors (phenoxybenzamine, propranolol); dopamine (haloperidol); histamine (mepyramine, cimetidine). Since, in addition, 5-carboxyamidotryptamine, 8-OH-DPAT, RU 24969, 2-methyl-5-HT do not mimic 5-HT and the responses to 5-HT are potentiated by the 5-HT-uptake blockers indalpine and fluvoxetine, it was concluded that 5-HT elicits tachycardia in the pig via a novel 5-HT receptor (Bom *et al.*, 1988). The present investigation showed that 5-MeO-T and, to a lesser extent, renzapride mimic 5-HT and high doses (1 and 3 mg kg^{-1}) of ICS 205-930, but not 0.3 mg kg^{-1} as also reported earlier (Bom *et al.*, 1988), antagonize the tachycardiac responses. Taken together, the above findings strongly suggest the involvement of a receptor similar or identical with the putative 5-HT_4 receptor located in the mouse brain. At this receptor, 5-MeO-T and certain benzamide derivatives (renzapride, cisapride), but not 5-carboxamidotryptamine, α-methyl-5-HT or 2-methyl-5-HT, are agonists and ICS 205-930 (in high concentrations; pA_2: 6.3–6.5), but not MDL 72222 or ondansetron, act as antagonists (Dumuis *et al.*, 1988; 1989; Clarke *et al.*, 1989). The 5-HT_4 receptor probably also medi-

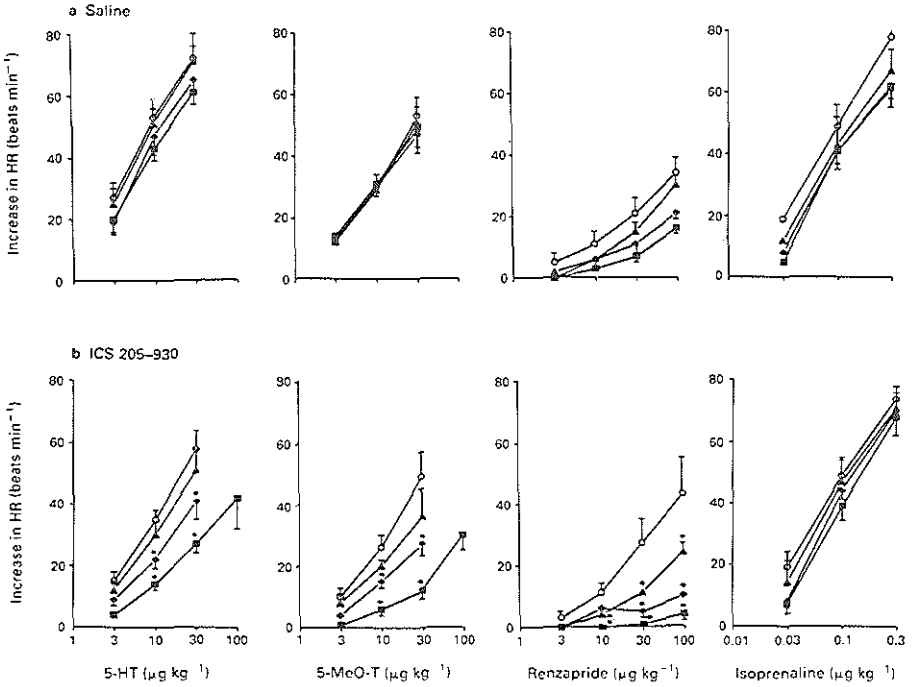

Figure 2 The effects of ICS 205-930 (b) (○, control; ▲, 0.3 mg kg^{-1}; ◆, 1.0 mg kg^{-1} and ▩, 3.0 mg kg^{-1}) or the corresponding volumes of saline (a) on the tachycardiac responses to 5-hydroxytryptamine (5-HT, $n = 5$, saline; $n = 7$, ICS 205-930), 5-methoxytryptamine (5-MeO-T, $n = 5$, saline; $n = 5$, ICS 205-930), renzapride ($n = 5$, saline; $n = 3$, ICS 205-930) and isoprenaline ($n = 3$, saline; $n = 5$, ICS 205-930). HR, heart rate. * Significant change ($P < 0.05$) in the control response to the agonist drug by ICS 205-930 as compared to the corresponding injection of saline.

ates the 5-HT-induced enhancement of cholinergic activity in the guinea-pig isolated ileum (Sanger, 1987; Craig & Clarke, 1989) and the inotropic action in the human isolated atria (Kaumann *et al.*, 1989). However, our findings are the first demonstration of a functional response to the putative 5-HT_4 receptor in an *in vivo* preparation, which is easy to set up and is not complicated by the presence of other 5-HT receptors.

C.M.V. was supported by a Science and Technology grant from the EEC.

References

BOM, A.H., DUNKER, D.J., SAXENA, P.R. & VERDOUW, P.D. (1988). 5-Hydroxytryptamine-induced tachycardia in the pig: possible involvement of a new type of 5-hydroxytryptamine receptor. *Br. J. Pharmacol.*, **93**, 663–671.

CLARKE, D.E., CRAIG, D.A. & FOZARD, J.R. (1989). The 5-HT_4 receptor: naughty but nice. *Trends Pharmacol. Sci.*, **10**, 385–386.

CRAIG, D.A. & CLARKE, D.E. (1989). 5-Hydroxytryptamine and cholinergic mechanisms in guinea-pig ileum. *Br. J. Pharmacol.*, **96**, 247P.

DUMUIS, A., BOUHELAL, R., SEBBEN, M. & BOCKAERT, J. (1988). A 5-HT receptor in the central nervous system, positively coupled with adenyl cyclase, is antagonized by ICS 205-930. *Eur. J. Pharmacol.*, **146**, 187–188.

DUMUIS, A., SEBBEN, M. & BOCKAERT, J. (1989). The gastrointestinal prokinetic benzamide derivatives are agonists at the non-classical 5-HT receptor (5-HT_4) positively coupled to adenylate cyclase in neurons. *Naunyn-Schmied. Arch. Pharmacol.*, **340**, 403–410.

DUNCKER, D.J., SAXENA, P.R. & VERDOUW, P.D. (1985). 5-Hydroxytryptamine causes tachycardia in pigs by acting on receptors unrelated to 5-HT_1, 5-HT_2 or M-type. *Br. J. Pharmacol.*, **86**, 596P.

KAUMANN, A.J., MURRAY, K.J., BROWN, A.M., SANDERS, L. & BROWN, M.J. (1989). A receptor for 5-HT in human atrium. *Br. J. Pharmacol.*, **98**, 664P.

SANGER, G.J. (1987). Increased gut cholinergic activity and antagonism of 5-hydroxytryptamine M-receptors by BRL 24924: potential clinical importance of BRL 24924. *Br. J. Pharmacol.*, **91**, 77–87.

SAXENA, P.R. (1986). Nature of the 5-hydroxytryptamine receptors in mammalian heart. *Prog. Pharmacol.*, **6**, 173–185.

SAXENA, P.R. & VILLALÓN, C.M. (1990). Cardiovascular effects of serotonin agonists and antagonists. *J. Cardiovasc. Pharmacol.* (in press).

(Received March 29, 1990
Accepted April 25, 1990)

CHAPTER 8

FURTHER CHARACTERIZATION, BY USE OF TRYPTAMINE AND BENZAMIDE DERIVATIVES, OF THE PUTATIVE 5-HT_4 RECEPTOR MEDIATING TACHYCARDIA IN THE PIG

Villalón, C.M., den Boer, M.O., Heiligers, J.P.C. and Saxena, P.R.
Br. J. Pharmacol., 102, 107-112, 1991.

Br. J. Pharmacol. (1991), 102, 107–112

Further characterization, by use of tryptamine and benzamide derivatives, of the putative 5-HT_4 receptor mediating tachycardia in the pig

Carlos M. Villalón, Marien O. den Boer, Jan P.C. Heiligers & [1]Pramod R. Saxena

Department of Pharmacology, Faculty of Medicine and Health Sciences, Erasmus University Rotterdam, Post Box 1738, 3000 DR Rotterdam, The Netherlands

1 It has recently been shown that the tachycardic response to 5-hydroxytryptamine (5-HT) in the anaesthetized pig, being mimicked by 5-methoxytryptamine and renzapride and blocked by high doses of ICS 205-930, is mediated by the putative 5-HT_4 receptor. In the present investigation we have further characterized this receptor.

2 Intravenous bolus injections of the tryptamine derivatives, 5-HT (3, 10 and 30 $\mu g\,kg^{-1}$), 5-methoxytryptamine (3, 10 and 30 $\mu g\,kg^{-1}$) and α-methyl-5-hydroxytryptamine (α-methyl-5-HT; 3, 10, 30 and 100 $\mu g\,kg^{-1}$), resulted in dose-dependent increases in heart rate of, respectively, 25 ± 2, 48 ± 3 and 68 ± 3 beats min^{-1} (5-HT: $n = 35$); 15 ± 1, 32 ± 2 and 57 ± 3 beats min^{-1} (5-methoxytryptamine: $n = 30$); 6 ± 4, 18 ± 6, 34 ± 6 and 64 ± 11 beats min^{-1} (α-methyl-5-HT; $n = 3$).

3 The increases in heart rate following i.v. administration of certain substituted benzamide derivatives were genereally less marked and not dose-dependent: 1 ± 5, 11 ± 3 and 10 ± 5 beats min^{-1} after 300, 1000 and 3000 $\mu g\,kg^{-1}$ of metoclopramide, respectively, ($n = 8$); 21 ± 4, 19 ± 2 and 2 ± 2 beats min^{-1} after 100, 300 and 1000 $\mu g\,kg^{-1}$ of cisapride, respectively, ($n = 5$); 6 ± 2, 14 ± 2, 37 ± 6, 43 ± 8 and 34 ± 10 beats min^{-1} after 10, 30, 100, 300 and 1000 $\mu g\,kg^{-1}$ of zacopride, respectively, ($n = 6$); and 1 ± 1, 2 ± 1 and 5 ± 2 beats min^{-1} after 300, 1000 and 3000 $\mu g\,kg^{-1}$ of dazopride, respectively, ($n = 4$). These drugs behaved as partial agonists, antagonizing the responses to 5-HT and 5-methoxytryptamine dose-dependently.

4 The 5-HT_3 receptor agonist 1-phenyl-biguanide (100, 300 and 1000 $\mu g\,kg^{-1}$) induced only slight increases in heart rate of 1 ± 1, 6 ± 2 and 11 ± 1 beats min^{-1}, respectively, ($n = 3$). These effects were not antagonized by the selective 5-HT_3 receptor antagonist granisetron (3 $mg\,kg^{-1}$). In addition, 1-phenyl-biguanide (1000 $\mu g\,kg^{-1}$) did not modify the tachycardia induced by either 5-HT- or 5-methoxytryptamine.

5 High doses (3 $mg\,kg^{-1}$) of ICS 205-930, a 5-HT_3 receptor antagonist with an indole group and devoid of effects on porcine heart rate *per se*, antagonized the stimulatory effects of 5-HT, 5-methoxytryptamine, α-Me-5-HT, metoclopramide, cisapride, zacopride, dazopride and 1-phenyl-biguanide. However, the 5-HT_2 receptor antagonist ketanserin (0.5 $mg\,kg^{-1}$), the 5-HT_3 receptor antagonists granisetron (3 $mg\,kg^{-1}$) and MDL 72222 (3 $mg\,kg^{-1}$) and the dopamine D_2 receptor antagonist domperidone (3 $mg\,kg^{-1}$) had no antagonist activity.

6 The above results support our contention that 5-HT, 5-methoxytryptamine, α-Me-5-HT and the substituted benzamide derivatives increase porcine heart rate by a direct action on the cardiac pacemaker, via the activation of a putative 5-HT_4 receptor. The pharmacological profile of this novel 5-HT receptor is similar (neurones from mouse brain colliculi and human heart) or, perhaps, even identical (guinea-pig cholinergic neurones) to other putative 5-HT_4 receptors.

Introduction

5-Hydroxytryptamine (5-HT) can exert multiple cardiac effects including both increases and decreases in heart rate. In most species, bradycardia induced by 5-HT is mediated by 5-HT_3 receptors, via the activation of the von Bezold Jarisch reflex. In marked contrast, the mechanism of 5-HT-induced tachycardia is notoriously species-dependent and is mediated, directly or indirectly, either by 5-HT_1-like (cat), 5-HT_2 (rat, dog) or 5-HT_3 (rabbit, dog) receptors, or by tyramine-like (guinea-pig) or unidentified mechanisms (see Saxena, 1986; Saxena & Villalón, 1990). In the pig, we have reported that the 5-HT-induced tachycardia is mediated by a novel receptor type which differs from 5-HT_1-like, 5-HT_2 and 5-HT_3 receptors (Duncker *et al.*, 1985; Bom *et al.*, 1988), but resembles the putative 5-HT_4 receptor (Villalón *et al.*, 1990) mediating stimulation of adenylate cyclase in both mouse embryo colliculi neurones and guinea-pig hippocampal membranes (Dumuis *et al.*, 1988; 1989; Clarke *et al.*, 1989). In the present study, we have further characterized the porcine heart 5-HT receptor using several agonist and antagonist drugs, including some substituted benzamide derivatives.

Methods

General

After an overnight fast, 40 young Yorkshire pigs (15–20 kg) were sedated with azaperone (120 mg, i.m.) and metomidate (120–150 mg, i.v.). After intubation, the animals were connected to a respirator for intermittent positive pressure ventilation with a mixture of room air (70%) and O_2 (30%). The anaesthesia was maintained with a continuous infusion of pentobarbitone sodium (15–20 $mg\,kg^{-1}\,h^{-1}$, i.v.). Aortic blood pressure and heart rate were recorded with, respectively, a

[1] Author for correspondence.

Statham pressure transducer and a tachograph. All drugs were injected into the right jugular vein. The body temperature of the animals was maintained around 37°C by using an electric blanket and arterial blood gases and pH were kept within the normal limits ($Po_2 > 90$ mmHg; Pco_2 30–40 mmHg; pH 7.35–7.45) by adjusting respiratory rate and tidal volume or by infusing 4.2% sodium bicarbonate solution.

Experimental protocol

After the animals had been in a stable haemodynamic condition for at least 45 min, they received intravenous bolus injections of 5-HT (3, 10 and 30 $\mu g\,kg^{-1}$) and 5-methoxytryptamine (3, 10 and 30 $\mu g\,kg^{-1}$). Subsequently, several doses of α-methyl-5-HT (3, 10, 30 and 100 $\mu g\,kg^{-1}$), 1-phenyl-biguanide (30, 100, 300 and 1000 $\mu g\,kg^{-1}$), metoclopramide (300, 1000 and 3000 $\mu g\,kg^{-1}$), cisapride (30, 100, 300 and 1000 $\mu g\,kg^{-1}$), zacopride (10, 30, 100, 300 and 1000 $\mu g\,kg^{-1}$) or dazopride (300, 1000 and 3000 $\mu g\,kg^{-1}$) were given, and after each dose or the highest dose (1-phenyl-biguanide), the responses to 5-HT and 5-methoxytryptamine were elicited again (for number of experiments and other specifications, see Results). In addition, tachycardic responses to 5-HT- and 5-methoxytryptamine were induced before and after pretreatment with 3 mg kg^{-1} of either MDL 72222, granisetron or domperidone. In another set of experiments, the tachycardic responses to 5-HT, 5-methoxytryptamine and α-methyl-5-HT were analyzed before and after pretreatment with either ketanserin (0.5 mg kg^{-1}) or ICS 205-930 (3 mg kg^{-1}). Lastly, the dose of each benzamide derivative eliciting the maximum increase in heart rate was chosen and given to a new group of animals (without previous administration of any of the benzamide derivatives) after pretreatment with 3 mg kg^{-1} of ICS 205-930.

The interval between the different doses of the compounds used as agonists and/or antagonists depended on the duration of tachycardia produced by the preceding dose, as in each case we waited until heart rate had returned completely or nearly to baseline values. The dose-intervals for the different drugs were as follows: tryptamine derivatives, between 5 and 15 min; benzamide derivatives and 1-phenyl-biguanide, usually between 15 and 30 min, but sometimes even longer than 60 min (cisapride and zacopride); and other antagonists (ICS 205-930, MDL 72222, granisetron, ketanserin and domperidone), between 10 and 15 min. The dosing with ICS 205-930 was cumulative (given as 0.3, 0.7 and 2.0 mg kg^{-1}), whereas that with all other drugs was sequential.

Drugs

The drugs used in this study were: cisapride (gift: Dr J.A.J. Schuurkes, Janssen Pharmaceutica, Beerse, Belgium), (±)-dazopride (A.H. Robbins Co., Richmond, VA, U.S.A.), domperidone (gift: Dr J.A.J. Schuurkes, Janssen Pharmaceutica, Beerse, Belgium), granisetron (gift: Dr G.J. Sanger, Smith Kline Beecham, Harlow, U.K.), 5-hydroxytryptamine creatinine sulphate (Sigma Chemical Company, St. Louis, MO, U.S.A.), ketanserin tartrate (gift: Dr J.M. Van Nueten, Janssen Pharmaceutica, Beerse, Belgium), 5-methoxytryptamine hydrochloride (Janssen Chimica, Beerse, Belgium), (±)-5-α-methyl-5-HT (gift: Dr P.P.A. Humphrey, Glaxo Group Research, Ware, U.K.), metoclopramide hydrochloride (Pharmacy Department, Erasmus University, Rotterdam, The Netherlands), 1-phenyl-biguanide (Research Biochemicals Inc., Nathick, MA, U.S.A.), 1αH,3α,5αH-tropan-3yl-3,5-dichlorobenzoate (MDL 72222; gift: Merrel-Dow Research Institute, Strasbourg, France); (3α-tropanyl)-1H-indole-3-carboxylic acid ester (ICS 205-930), and (±)-zacopride (A.H. Robbins Co., Richmond, VA, U.S.A.). The doses of cisapride, dazopride, 5-HT, 5-methoxytryptamine, α-methy-5-HT, 1-phenyl-biguanide and zacopride are given as free base.

Data presentation and analysis

All data in the text, figures and tables are presented as mean ± s.e.mean. The peak changes in heart rate induced by the different doses of both tryptamine- and benzamide derivatives were determined. The increases in heart rate just before and after a particular antagonist drug were compared by Duncan's new multiple range test, once an analysis of variance (randomized block design) revealed that the samples represented different populations (Saxena, 1985). The effects of agonist drugs in the different groups of animals were compared by use of the unpaired Student's t test. A P value of 0.05 or less (two-tailed) was considered statistically significant.

Results

Initial blood pressure and heart rate changes by 5-HT agonist drugs

Baseline values of mean arterial blood pressure and heart rate in the 40 pigs were 84 ± 4 mmHg and 101 ± 4 beats min^{-1}, respectively. The changes induced in mean arterial blood pressure by each 5-HT agonist drug were: 5-HT (−18 ± 1, −18 ± 1 and −16 ± 1 followed by +2 ± 1 mmHg after 3, 10 and 30 $\mu g\,kg^{-1}$, respectively; $n = 35$), 5-methoxytryptamine (−20 ± 1, −18 ± 2 and −15 ± 2 followed by +2 ± 1 mmHg after 3, 10 and 30 $\mu g\,kg^{-1}$, respectively; $n = 30$), α-methyl-5-HT (−20 ± 1, −14 ± 2, +10 ± 3 and +41 ± 8 mmHg after 3, 10, 30 and 100 $\mu g\,kg^{-1}$, respectively; $n = 3$), metoclopramide (+5 ± 2, +8 ± 1 and +3 ± 3 mmHg after 300, 1000 and 3000 $\mu g\,kg^{-1}$, respectively; $n = 8$), cisapride (+4 ± 4, +11 ± 2, +5 ± 3 and −8 ± 5 mmHg after 30, 100, 300 and 1000 $\mu g\,kg^{-1}$, respectively; $n = 5$), zacopride (+5 ± 2, +10 ± 2, +14 ± 3 and +12 ± 2 mmHg after 10, 30, 100 and 300 $\mu g\,kg^{-1}$, respectively; $n = 6$), dazopride (+1 ± 1, +2 ± 1 and +4 ± 2 mmHg after 300, 1000 and 3000 $\mu g\,kg^{-1}$, respectively; $n = 4$), and 1-phenyl-biguanide (0 ± 0, 0 ± 0, +4 ± 1 and +9 ± 2 after 30, 100, 300 and 1000 $\mu g\,kg^{-1}$, respectively; $n = 3$). These effects were not evaluated further.

As shown in Figure 1, intravenous bolus injections of the above-mentioned 5-HT agonist drugs caused increases in heart rate of diverse magnitude; the order of potency was 5-HT ≥ 5-methoxytryptamine > α-methyl-5-HT > zacopride > cisapride > metoclopramide = 1-phenyl-biguanide >

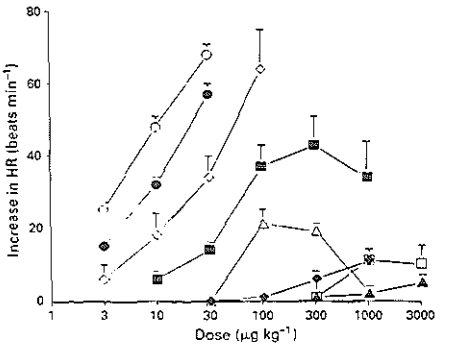

Figure 1 The tachycardic responses to 5-HT (○, $n = 35$), 5-methoxytryptamine (●, $n = 30$), α-methyl-5-HT (◇, $n = 3$), zacopride (■, $n = 6$), cisapride (△, $n = 5$), metoclopramide, (□, $n = 8$), 1-phenyl-biguanide (◆, $n = 3$) and dazopride (▲, $n = 4$) in the anaesthetized pig.

dazopride. At the doses used, the duration of action of cisapride (>60 min at 100 μg kg^{-1}) was longer than that of zacopride (17 ± 1, 23 ± 1, 43 ± 17 and 50 ± 8 min), metoclopramide (11 ± 1, 15 ± 2 and 21 ± 5 min), dazopride (1 ± 1, 5 ± 2 and >30 min), 1-phenyl-biguanide (0 ± 0, 0 ± 0, 5 ± 3 and 30 ± 3 min), 5-methoxytryptamine (5.9 ± 0.3, 9.6 ± 0.5 and 13.7 ± 0.6 min), 5-HT (2.2 ± 0.2, 4.5 ± 0.3 and 7.6 ± 0.7 min) or α-methyl-5-HT (0.4 ± 0.1, 0.9 ± 0.1, 4.3 ± 1.3 and 7.3 ± 1.3 min).

Modification of tachycardia in response to 5-HT and 5-methoxytryptamine induced by benzamide derivatives

In a previous publication, we have reported that the tachycardia induced by repeated administrations of 5-HT and 5-methoxytryptamine remained essentially unchanged in control animals receiving physiological saline (Villalón *et al.*, 1990). In marked contrast, the administration of several doses of either zacopride, cisapride, metoclopramide or dazopride antagonized the tachycardia induced by 5-HT (Figure 2) or 5-methoxytryptamine (Figure 3) in a dose-dependent manner; the order of potency for blockade of both 5-HT- and 5-methoxytryptamine-induced tachycardia was similar to that of their tachycardic response (see above): zacopride = cisapride ⩾ metoclopramide > dazopride.

Tachycardia induced by benzamide derivatives after ICS 205-930

Because of the fact that the responses to the higher doses of the benzamide derivatives were usually less than the maximum response achieved (see Figure 1), the dose of each benzamide derivative eliciting the maximum increase in heart rate was administered to animals after treatment with 3 mg kg^{-1} of ICS 205-930. This dose of ICS 205-930 antagonizes the tachycardic responses to 5-HT, 5-methoxytryptamine and renzapride, but not that to isoprenaline (Villalón *et al.*, 1990). As shown in Figure 4, the increase in heart rate induced by either zacopride, cisapride, dazopride or metoclopramide was markedly antagonized by ICS 205-930.

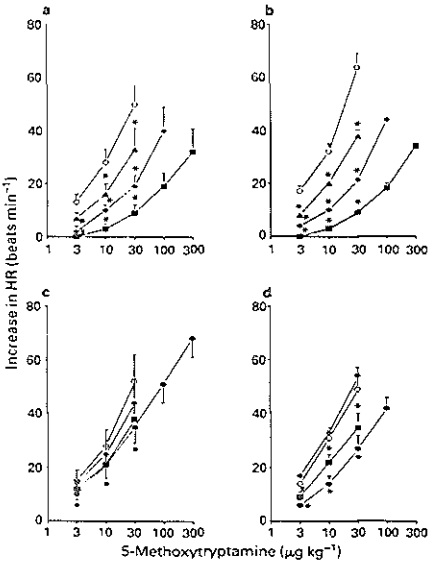

Figure 3 The effects of (a) zacopride (n = 4), (b) cisapride (n = 4), (c) dazopride (n = 4) and (d) metoclopramide (n = 5) on the tachycardic responses to 5-methoxytryptamine. The doses of the antagonists were: (○), 0 mg kg^{-1} (control); (▲) 0.1 mg kg^{-1}; (◆) 0.3 mg kg^{-1}; (■) 1.0 mg kg^{-1} and (●) 3.0 mg kg^{-1}. *Significantly different from the corresponding control response to 5-methoxytryptamine (P < 0.05).

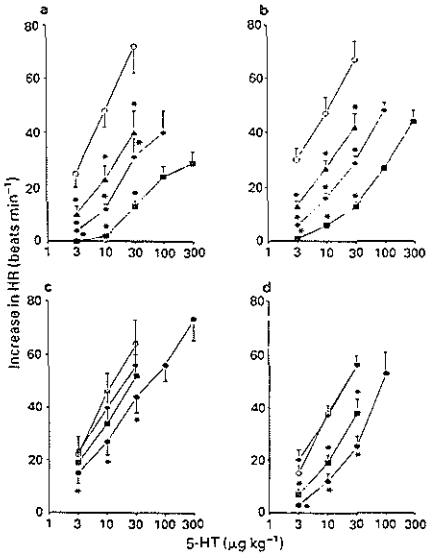

Figure 2 The effects of (a) zacopride (n = 5), (b) cisapride (n = 5), (c) dazopride (n = 4) and (d) metoclopramide (n = 8) on the tachycardic responses to 5-HT. The doses of the antagonists were: (○) 0 mg kg^{-1} (control); (▲) 0.1 mg kg^{-1}; (◆) 0.3 mg kg^{-1}, (■) 1.0 mg kg^{-1} and (●) 3.0 mg kg^{-1}. *Significantly different from the corresponding control response to 5-HT (P < 0.05).

Tachycardia induced by 5-HT or 5-methoxytryptamine after administration of some agonist and antagonist drugs

Inasmuch as all putative antagonists at this novel cardiac receptor also display high affinity for the 5-HT$_3$ receptors, we decided to investigate the effect of high doses of other selective 5-HT$_3$ receptor agonists and antagonists on the tachycardic responses induced by 5-HT or 5-methoxytryptamine; the

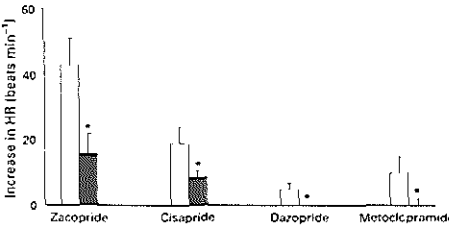

Figure 4 The tachycardic responses to zacopride (0.3 mg kg^{-1}), cisapride (0.3 mg kg^{-1}), metoclopramide (3 mg kg^{-1}) and dazopride (3 mg kg^{-1}) in untreated control pigs (open columns, n = 6, 5, 8 and 4, respectively) and pigs treated with ICS 205-930 (solid columns, n = 6, 6, 5 and 6, respectively). *Significantly different from the corresponding response in the untreated animals (P < 0.05).

Table 1 Effect of MDL 72222, granisetron, 1-phenyl-biguanide or domperidone on 5-HT- and 5-methoxytryptamine-induced increases in heart rate in the pig

Antagonist	*Dose* ($mg\,kg^{-1}$)	n	*Increase in heart rate* (beats min^{-1}) $3\,\mu g\,kg^{-1}$ Before	After	$10\,\mu g\,kg^{-1}$ Before	After	$30\,\mu g\,kg^{-1}$ Before	After
5-HT								
MDL 72222	3.0	6	26 ± 5	23 ± 6	49 ± 9	40 ± 8	69 ± 12	63 ± 11
Granisetron	3.0	3	32 ± 10	31 ± 10	58 ± 14	51 ± 13	77 ± 17	69 ± 16
1-Phenyl-biguanide	1.0	3	32 ± 10	33 ± 10	58 ± 14	56 ± 15	77 ± 17	74 ± 18
Domperidone	3.0	6	26 ± 5	29 ± 6	49 ± 9	50 ± 7	69 ± 12	69 ± 6
5-Methoxytryptamine								
MDL 72222	3.0	6	18 ± 4	18 ± 4	35 ± 6	33 ± 5	61 ± 10	57 ± 10
Granisetron	3.0	3	15 ± 2	14 ± 2	32 ± 7	28 ± 5	62 ± 8	54 ± 8
1-Phenyl-biguanide	1.0	3	15 ± 2	14 ± 3	32 ± 7	30 ± 7	62 ± 8	60 ± 8
Domperidone	3.0	6	18 ± 4	18 ± 5	35 ± 6	38 ± 5	61 ± 10	58 ± 7

All data are mean ± s.e.mean. None of the responses after antagonist drugs differed significantly from those before antagonist ($P > 0.05$).

selective dopamine$_2$ (D_2) receptor antagonist domperidone, as some benzamide derivatives (metoclopramide) also show affinity for D_2 receptors. As shown in Table 1, the responses to both 5-HT and 5-methoxytryptamine remained unchanged after administration of MDL 72222 ($3\,mg\,kg^{-1}$), granisetron ($3\,mg\,kg^{-1}$), 1-phenyl-biguanide ($1\,mg\,kg^{-1}$) or domperidone ($3\,mg\,kg^{-1}$).

Since α-methyl-5-HT (a 5-HT$_2$ and, to some extent, 5-HT$_1$-like receptor agonist) did induce quite consistent increases in heart rate (see Figure 1), we explored by pharmacological means the possible mechanisms involved in such an effect. In control animals where 5-HT and 5-methoxytryptamine were administered before and after the last set of injections of α-methyl-5-HT (3, 10, 30 and $100\,\mu g\,kg^{-1}$), the tachycardic responses induced by both 5-HT (3, 10 and $30\,\mu g\,kg^{-1}$) and 5-methoxytryptamine (3, 10 and $30\,\mu g\,kg^{-1}$) remained unchanged after α-methyl-5-HT [for 5-HT: 40 ± 9, 66 ± 11, and 88 ± 13 beats min^{-1} before and 36 ± 5, 62 ± 10 and 82 ± 12 beats min^{-1} after α-methyl-5-HT ($n = 3$), respectively; for 5-methoxytryptamine: 21 ± 7, 37 ± 8 and 67 ± 11 beats min^{-1} before and 17 ± 5, 32 ± 7 and 59 ± 11 beats min^{-1} after α-methyl-5-HT ($n = 3$), respectively]. Likewise, the increases in heart rate induced by 5-HT, 5-methoxytryptamine and α-methyl-5-HT were unaffected by ketanserin ($0.5\,mg\,kg^{-1}$), but were markedly antagonized by ICS 205-930 ($3\,mg\,kg^{-1}$) (Figure 5).

Lastly, it may be noted that the 5-HT$_3$ receptor agonist 1-phenyl-biguanide (30, 100, 300 and $1000\,\mu g\,kg^{-1}$) induced a small increase in porcine heart rate ($n = 3$); this effect was not blocked after administration of $3\,mg\,kg^{-1}$ of the selective 5-HT$_3$ receptor antagonist granisetron (0 ± 0, 1 ± 1, 6 ± 2 and 11 ± 1 beats min^{-1} before and 0 ± 0, 5 ± 3, 11 ± 3 and 16 ± 1 beats min^{-1} after granisetron, respectively). Notwithstanding, this effect appeared to be antagonized by $3\,mg\,kg^{-1}$ of ICS 205-930 (0 ± 0, 5 ± 3, 11 ± 3 and 16 ± 1 beats min^{-1} before and 0 ± 0, 3 ± 1, 4 ± 1 and 7 ± 1 beats min^{-1} after ICS 205-930, respectively).

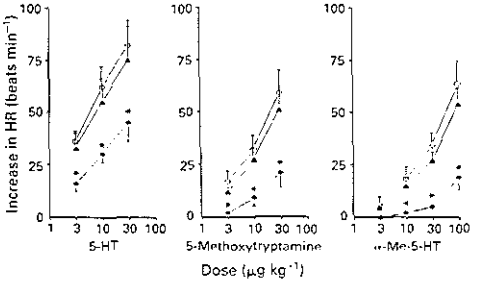

Figure 5 The tachycardic responses to 5-HT ($n = 3$), 5-methoxytryptamine ($n = 3$) and α-methyl-5-HT (α-Me-5-HT) ($n = 3$) before (control, ○) and after injections of ketanserin ($0.5\,mg\,kg^{-1}$, ▲), and ICS 205-930 ($3\,mg\,kg^{-1}$, ◆). *Significantly different from response in untreated animals ($P < 0.05$).

Discussion

We have shown that the 5-HT-induced tachycardia in the pig is neither mimicked by agonists at 5-HT$_1$-like (5-carboxamidotryptamine, 8-hydroxy-2-(di-n-propylamino)tetralin, RU 24969) and 5-HT$_3$ (2-methyl-5-HT) receptors, nor antagonized by drugs that act at various receptors: 5-HT$_1$ and/or 5-HT$_2$ (methiothepin, methysergide, ketanserin); 5-HT$_3$ (MDL 72222, ICS 205-930); adrenoceptors (phenoxybenzamine, propranolol); dopamine (haloperidol); histamine (mepyramine, cimetidine) (see Duncker *et al.*, 1985; Bom *et al.*, 1988). More recently, we found that the tachycardic effects of 5-HT in the pig, being mimicked by 5-methoxytryptamine and renzapride (Villalón *et al.*, 1990), but not by indorenate or sumatriptan (Villalón *et al.*, 1991), and blocked by high doses ($> 1\,mg\,kg^{-1}$) of ICS 205-930 (Villalón *et al.*, 1990), are mediated by a putative 5-HT$_4$ receptor which resembles the one mediating increases in adenosine 3':5'-cyclic monophosphate (cyclic AMP) in mouse embryo colliculi neurones and guinea-pig hippocampal membranes (Dumuis *et al.*, 1988; 1989; Clarke *et al.*, 1989). The present investigation extends these findings and clearly demonstrates that the porcine heart 5-HT receptor (i) can be stimulated by α-methyl-5-HT and some benzamide derivatives; (ii) does not resemble either 5-HT$_2$, 5-HT$_3$ or dopamine receptors; and (iii) resembles that present on the guinea-pig enteric neurones (Craig & Clarke, 1990) and human heart (Kaumann *et al.*, 1990).

Agonist action of α-methyl-5-HT and some benzamide derivatives on the porcine heart 5-HT receptor

Like 5-HT and 5-methoxytryptamine, it was observed that α-methyl-5-HT behaved as a potent agonist and elicited a dose-dependent tachycardia in the pig. The drug was also short-lasting in action and was devoid of any antagonist action against 5-HT or 5-methoxytryptamine. In contrast, the tachycardic action of the benzamide derivatives zacopride, cisapride, metoclopramide and dazopride, was less marked, but longer-lasting, and not strictly dose-dependent. In addition, each of these drugs antagonized the effects of 5-HT and 5-methoxytryptamine in a dose-dependent manner. It has to be emphasized that the tachycardic effects of 5-HT and 5-methoxytryptamine were not 'masked' by the increase in heart rate induced by the benzamide derivatives as the responses to 5-HT and 5-methoxytryptamine were elicited at the time when

the tachycardic effect of the benzamides had worn off (data not shown). Therefore, as previously found with renzapride (Villalón *et al.*, 1990), the benzamide derivatives employed here also behaved as partial agonists at the 5-HT$_4$ receptors in the porcine heart.

In our previous investigation, tachyphylaxis was observed with the tachycardic effect of renzapride (Villalón *et al.*, 1990). Though the present study was not designed for this purpose, we did observe tachyphylaxis in some preliminary experiments with the benzamide derivatives used here. Indeed, for this reason the antagonist effect of ICS 205-930 against the tachycardia induced by zacopride, cisapride, dazopride and metoclopramide was analyzed separately in control animals and in animals pretreated with ICS 205-930 (see Figure 4).

Lack of resemblance of the porcine heart 5-HT receptor with either 5-HT$_2$, 5-HT$_3$ or dopamine D$_2$ receptors

Both 5-methoxytryptamine and α-methyl-5-HT can interact with 5-HT$_2$ (and 5-HT$_1$-like) receptors (Richardson & Engel, 1986; Martin *et al.*, 1987; Hoyer, 1988). However, the tachycardic action of 5-methoxytryptamine and α-methyl-5-HT, as well as that of 5-HT (see also Bom *et al.*, 1988), was not modified after a dose of ketanserin (0.5 mg kg^{-1}) that is sufficient to block 5-HT$_2$ receptors (Van Nueten *et al.*, 1981; Saxena & Lawang, 1985). Therefore, the possibility that 5-HT$_2$ receptors might be involved in these effects is practically ruled out. Unlike ketanserin, 3 mg kg^{-1} of ICS 205-930 markedly antagonized the tachycardic responses to 5-HT, 5-methoxytryptamine and α-methyl-5-HT (see Figure 5), which again suggests the involvement of the 5-HT$_4$ receptor.

Admittedly, ICS 205-930 and the benzamide derivatives used here have the ability to block potently 5-HT$_3$ receptors (for references see Fozard, 1990). However, as previously discussed (Bom *et al.*, 1988; Villalón *et al.*, 1990), several results of this study clearly indicate that this novel 5-HT receptor in the pig heart does not belong to the 5-HT$_3$ receptor family. Firstly, besides the potency of 5-HT as a distinguishing factor, both α-methyl-5-HT and 5-methoxytryptamine are totally inactive at 5-HT$_3$ receptors (Richardson *et al.*, 1985; Richardson & Engel, 1986; Fozard, 1990). Secondly, the selective 5-HT$_3$ receptor agonists 2-methyl-5-HT and 1-phenyl-biguanide (Fozard, 1990) were practically inactive in the stimulation of 5-HT$_3$ receptors (Bom *et al.*, 1988; present results). Thirdly, high doses of other selective 5-HT$_3$ receptor antagonists (except ICS 205-930) such as granisetron (an indazole derivative; Sanger & Nelson, 1989; Fozard, 1990) or MDL 72222 (a dichlorobenzoate derivative; Fozard, 1984; 1990) were completely inactive (see Table 1). Lastly, it must be taken into consideration that the affinity of ICS 205-930 for the 5-HT$_4$ receptor involved in the stimulation of cyclic AMP production in mouse embryo colliculi neurones is much lower (pK_i = 6–6.3; Dumuis *et al.*, 1989) than its affinity for 5-HT$_3$ receptors (pA_2 = 8–10; Richardson *et al.*, 1985; Richardson & Engel, 1986).

Most of the benzamide derivatives analyzed in the present study are currently used as prokinetic drugs (Schuurkes *et al.*, 1985; Alphin *et al.*, 1986; Cooper *et al.*, 1986; van Daele *et al.*, 1986; Sanger, 1987). Apart from metoclopramide, which also displays high affinity for central dopamine receptors (Cooper *et al.*, 1986), the other benzamide derivatives are devoid of important dopamine blocking activity. However, ICS 205-930, zacopride and other 5-HT$_3$ receptor antagonists are able to inhibit the release of dopamine by 5-HT and 2-methyl-5-HT in the central nervous system (Blandina *et al.*, 1988; Tricklebank, 1989). It is for these reasons, although haloperidol had been found ineffective (Bom *et al.*, 1988), that we decided to determine whether domperidone, a potent D$_2$ receptor antagonist (Kohli *et al.*, 1983) with gastrokinetic action, antagonizes the 5-HT-induced tachycardia or itself causes tachycardia in the pig. As shown in Table 1, domperidone (3 mg kg^{-1}) did not modify the tachycardic responses to either 5-HT or 5-methoxytryptamine. Therefore, the positive chronotropic effect induced by the tryptamine- and benzamide derivatives in the pig heart is unrelated to a possible action via dopaminergic pathways and/or receptors. Moreover, since domperidone failed to affect basal heart rate in the pig, the drug does not interact with the pig heart 5-HT$_4$ receptor.

Resemblance of the porcine heart 5-HT receptor to other putative 5-HT$_4$ receptors

At the 5-HT$_4$ receptor identified in the neurones from mouse embryo colliculi on the basis of increase in cyclic AMP, 5-methoxytryptamine, 5-carboxamidotryptamine (low affinity) and certain benzamide derivatives (renzapride, metoclopramide, cisapride), but not α-methyl-5-HT or 2-methyl-5-HT, are agonists; and ICS 205-930 (in high concentrations), but not MDL 72222, granisetron or ondansetron, acts as an antagonist (Dumuis *et al.*, 1988; 1989; Clarke *et al.*, 1989). The pharmacological characteristics of this receptor, though exhibiting several similarities, differ in some important respects. For example, 5-carboxamidotryptamine, apparently because of its low affinity, does not show activity in the pig heart (Duncker *et al.*, 1985; Bom *et al.*, 1988) in doses which are highly active in the cat heart (Saxena *et al.*, 1985; Connor *et al.*, 1986). Secondly, α-methyl-5-HT, which has little activity on the neurones from mouse embryo colliculi (Dumuis *et al.*, 1988; 1989), is highly active in our experiments. Thirdly, the agonist potency order reported by Dumuis *et al.* (1989) using mouse embryo colliculi (cisapride > renzapride > zacopride > 5-HT > metoclopramide), differs from that found in the pig heart (5-HT > 5-methoxytryptamine > α-methyl-5-HT > zacopride ≥ renzapride > cisapride > metoclopramide > dazopride; indorenate and sumatriptan, inactive at 1 and 3 mg kg^{-1}) (Villalón *et al.*, 1990; 1991; present results). Lastly, the benzamide derivatives cisapride and renzapride, which are full agonists at the mouse brain receptor, behaved as partial agonists at the pig heart receptor. Several possible explanations for these differences in agonist potencies may include: use of 'second messenger' (cyclic AMP) and functional (tachycardia) responses; tissue-dependent factors such as the number of receptors and coupling efficiency; and/or drug-dependent factors such as the affinity of 5-HT and related agonists for each of these novel receptors.

The 5-HT$_4$ receptor may also mediate the 5-HT-induced enhancement of cholinergic activity in the guinea-pig isolated ileum (Sanger, 1987; Craig & Clarke, 1990) and ascending colon (Elswood *et al.*, 1990), as well as relaxation of the rat oesophagus (Baxter & Clarke, 1990). As in the present experiments, the tryptamine derivatives 5-methoxytryptamine, α-methyl-5-HT and some benzamides mimic, and ICS 205-930 antagonizes 5-HT at the 5-HT$_4$ receptor in the guinea-pig gastrointestinal tract (Craig & Clarke, 1990; Elswood *et al.*, 1990) and the rat oesophagus (Baxter & Clarke, 1990). Moreover, the order of potency at the cholinergic neurones in the guinea-pig ileum (5-HT > 5-methoxytryptamine > renzapride > α-methyl-5-HT > zacopride = cisapride; Craig & Clarke, 1990) is practically identical to that found by us in the porcine heart.

The 5-HT$_4$ receptor is also apparently involved in the inotropic action of 5-HT, mediated via cyclic AMP increase in the human atria. The positive inotropic response to 5-HT is not modified by ketanserin, methysergide, lysergide, methiothepin, yohimbine (±)-propranol, (−)-pindolol or MDL 72222, but is blocked by a high concentration (2 μM) of ICS 205-930 (Kaumann *et al.*, 1990). The precise role of these receptors in cardiac function and cardiovascular pathologies remains to be determined.

In summary, the present investigation demonstrates that the tachycardic response to i.v. administered 5-HT in the anaesthetized pig can be mimicked by the tryptamine derivatives 5-methoxytryptamine and α-methyl-5-HT, and to a lesser extent by the partial agonist benzamide derivatives (in order of potency) zacopride, cisapride, metoclopramide and dazop-

ride. High doses of ICS 205-930, but not ketanserin, granisetron or MDL 72222, acted as an antagonist. These results further confirm the involvement of a putative 5-HT_4 receptor in the positive chronotropic action of 5-HT in the anaesthetized pig.

C.M.V. was supported by a Science and Technology grant from the EEC.

References

ALPHIN, R.S., SMITH, W.L., JACKSON, C.B., DROPPLEMAN, D.A. & SANCILIO, L.F. (1986). Zacopride (AHR-11190 B): a unique and potent gastrointestinal prokinetic and antiemetic agent in laboratory animals. *Dig. Dis. Sci.*, **31**, 482S.

BAXTER, G.S. & CLARKE, D.E. (1990). Putative 5-HT_4 receptors mediate relaxation of rat oesophagus. *Proceedings of the 2nd IUPHAR Satellite Meeting on Serotonin*, Basel, July 11–13, 1990. Abstract No. P85.

BLANDINA, P., GOLDFARB, J. & GREEN, P.J. (1988). Activation of a 5-HT_3 receptor releases dopamine from rat striatal slice. *Eur. J. Pharmacol.*, **155**, 349–350.

BOM, A.H., DUNKER, D.J., SAXENA, P.R. & VERDOUW, P.D. (1988). 5-Hydroxytryptamine-induced tachycardia in the pig: possible involvement of a new type of 5-hydroxytryptamine receptor. *Br. J. Pharmacol.*, **93**, 663–671.

CLARKE, D.E., CRAIG, D.A. & FOZARD, J.R. (1989). The 5-HT_4 receptor: naughty but nice. *Trends Pharmacol. Sci.*, **10**, 385–386.

CONNOR, H.E., FENIUK, W., HUMPHREY, P.P.A. & PERREN, M.J. (1986). 5-Carboxamidotryptamine is a selective agonist at 5-hydroxytryptamine receptors mediating vasodilatation and tachycardia in anaesthetized cats. *Br. J. Pharmacol.*, **87**, 417–426.

COOPER, S.M., MCCLELLAND, M., MCRITCHIE, B. & TURNER, D.H. (1986). BRL 24924: a new and potent gastric motility stimulant. *Br. J. Pharmacol.*, **88**, 383P.

CRAIG, D.A. & CLARKE, D.E. (1990). Pharmacological characterization of a neuronal receptor for 5-hydroxytryptamine in guinea pig ileum with properties similar to the 5-hydroxytryptamine$_4$ receptor. *J. Pharmacol. Exp. Ther.*, **225**, 1378–1386.

DUMUIS, A., BOUHELAL, R., SEBBEN, M. & BOCKAERT, J. (1988). A 5-HT receptor in the central nervous system, positively coupled with adenyl cyclase, is antagonized by ICS 205-930. *Eur. J. Pharmacol.*, **146**, 187–188.

DUMUIS, A., SEBBEN, M. & BOCKAERT, J. (1989). The gastrointestinal prokinetic benzamide derivatives are agonists at the non-classical 5-HT receptor (5-HT_4) positively coupled to adenylate cyclase in neurons. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **340**, 403–410.

DUNCKER, D.J., SAXENA, P.R. & VERDOUW, P.D. (1985). 5-Hydroxytryptamine causes tachycardia in pigs by acting on receptors unrelated to 5-HT_1, 5-HT_2 or M-type. *Br. J. Pharmacol.*, **86**, 596P.

ELSWOOD, C.J., BUNCE, K.T. & HUMPHREY, P.P.A. (1990). Identification of 5-HT_4 receptors in guinea-pig ascending colon. *Proceedings of the 2nd IUPHAR Satellite Meeting on Serotonin*, Basel, July 11–13, 1990. Abstract No. P86.

FOZARD, J.R. (1984). MDL 72222: a potent and highly selective antagonist at neuronal 5-hydroxytryptamine receptors. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **326**, 36–44.

FOZARD, J.R. (1990). Agonists and antagonists at 5-HT_3 receptors. In *The Cardiovascular Pharmacology of 5-Hydroxytryptamine: Prospective Therapeutic Applications*. ed. Saxena, P.R., Wallis, D.I., Wouters, W. & Bevan, P. pp. 101–115. Dordrecht: Kluwer Academic Publishers.

HOYER, D. (1988). Functional correlates of serotonin 5-HT_1 recognition sites. *J. Receptor Res.*, **8**, 59–81.

KAUMANN, A.J., SANDERS, L., BROWN, A.M., MURRAY, K.J. & BROWN, M.J. (1990). A 5-hydroxytryptamine receptor in human atrium. *Br. J. Pharmacol.*, **100**, 879–885.

KOHLI, J.D., GLOCK, D. & GOLDBERG, L.I. (1983). Selective DA_2 versus DA_1 antagonist activity of domperidone in the periphery. *Eur. J. Pharmacol.*, **89**, 137–141.

MARTIN, G.R., LEFF, P., CAMBRIDGE, D. & BARRETT, V.J. (1987). Comparative analysis of two types of 5-hydroxytryptamine receptor mediating vasorelaxation: differential classification using tryptamines. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **336**, 365–373.

RICHARDSON, B.P. & ENGEL, G. (1986). The pharmacology and function of 5-HT_3 receptors. *Trends Neurosci.*, **9**, 424–428.

RICHARDSON, B.P., ENGEL, G., DONATSCH, P. & STADLER, P.A. (1985). Identification of serotonin M-receptor subtypes and their blockade by a new class of drugs. *Nature*, **316**, 126–131.

SANGER, G.J. (1987). Increased gut cholinergic activity and antagonism of 5-hydroxytryptamine M-receptors by BRL 24924: potential clinical importance of BRL 24924. *Br. J. Pharmacol.*, **91**, 77–87.

SANGER, G.J. & NELSON, D.R. (1989). Selective and functional 5-hydroxytryptamine$_3$ receptor antagonism by BRL 43694 (granisetron). *Eur. J. Pharmacol.*, **159**, 113–124.

SAXENA, P.R. (1985). An interactive computer program for data management and parametric and non-parametric statististical analysis. *Br. J. Pharmacol.*, **86**, 818P.

SAXENA, P.R. (1986). Nature of the 5-hydroxytryptamine receptors in mammalian heart. *Prog. Pharmacol.*, **6**, 173–185.

SAXENA, P.R. & LAWANG, A. (1985). A comparison of cardiovascular and smooth muscle effects of 5-hydroxytryptamine and 5-carboxamidotryptamine, a selective agonist at 5-HT_1 receptors. *Arch. Int. Pharmacodyn.*, **277**, 235–252.

SAXENA, P.R. & VILLALÓN, C.M. (1990). Cardiovascular effects of serotonin agonists and antagonists. *J. Cardiovasc. Pharmacol.*, **15** (Suppl. 7), S17–S34.

SAXENA, P.R., MYLECHARANE, E.J. & HEILIGERS, J. (1985). Analysis of the heart rate effects of 5-hydroxytryptamine in the cat; mediation of tachycardia by 5-HT_1-like receptors. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **330**, 121–129.

SCHUURKES, J.A.J., VAN NUETEN, J.M., VAN DAELE, P.G.H., REYNTJES, A.J. & JANSSEN, P.A.J. (1985). Motor-stimulating properties of cisapride on isolated gastrointestinal preparations of the guinea pig. *J. Pharmacol. Exp. Ther.*, **234**, 775–783.

TRICKLEBANK, M.D. (1989). Interactions between dopamine and 5-HT_3 receptors suggest new treatments for psychosis and drug addiction. *Trends Pharmacol. Sci.*, **10**, 127–129.

VAN DAELE, G.H.P., DE BRUYN, M.F.L., SOMMEN, F.M., JANSSEN, M., VAN NUETEN, J.M., SCHUURKES, J.A.J., NIEMEGEERS, C.J.E. & LEYSEN, J.E. (1986). Synthesis of cisapride, a gastrointestinal stimulant derived from cis-4-amino-3-methoxypiperidine. *Drug. Dev. Res.*, **8**, 225–232.

VAN NUETEN, J.M., JANSSEN, P.A.J., VAN BEEK, J., XHONNEUX, R., VERBEUREN, T.J. & VANHOUTTE, P.M. (1981). Vascular effects of ketanserin (R 41 468), a novel antagonist of 5-HT_2 serotonergic receptors. *J. Pharmacol. Exp. Ther.*, **218**, 217–230.

VILLALÓN, C.M., DEN BOER, M.O., HEILIGERS, J.P.C. & SAXENA, P.R. (1990). Mediation of 5-hydroxytryptamine-induced tachycardia in the pig by the putative 5-HT_4 receptor. *Br. J. Pharmacol.*, **100**, 665–667.

VILLALÓN, C.M., DEN BOER, M.O., HEILIGERS, J.P.C. & SAXENA, P.R. (1991). The 5-HT_4 receptor mediating tachycardia in the pig. In *Serotonin: Molecular Biology, Receptors and Function* ed. Fozard, J.R. & Saxena, P.R. Basel: Birkhauser (in press).

(Received June 8, 1990
Revised September 11, 1990
Accepted September 14, 1990)

CHAPTER 9

ROLE OF THE PUTATIVE 5-HT$_4$ RECEPTOR IN THE POSITIVE INOTROPIC EFFECT OF 5-HT IN THE PIG

Villalón, C.M., Heiligers, J.P.C., Verdouw, P.D., Duncker, D.J.G.M. and Saxena, P.R.

To be submitted.

ROLE OF THE PUTATIVE 5-HT$_4$ RECEPTOR IN THE POSITIVE INOTROPIC EFFECT OF 5-HT IN THE PIG

Summary

1 It has recently been shown that the tachycardiac response to 5-HT in the anaesthetized pig, being mimicked by tryptamine- (5-methoxytryptamine and α-methyl-5-HT) and benzamide- (renzapride, cisapride, zacopride, metoclopramide and dazopride) derivatives, and blocked by high doses of ICS 205-930, is mediated by the putative 5-HT$_4$ receptor. This study analyzes the inotropic effects of 5-HT, 5-methoxytryptamine and renzapride using the maximum rate of rise in left ventricular pressure (LVdP/dt$_{max}$) in anaesthetized pigs during or in the absence of atrial pacing (150 beats min^{-1}).

2 In the absence of atrial pacing, intravenous bolus injections of 5-HT and 5-methoxytryptamine (both 3, 10 and 30 μg kg^{-1}) caused dose-dependent increases in heart rate (24±3, 46±3 and 65±3 and 9±1, 27±3 and 40±1 beats min^{-1}, respectively), LVdP/dt$_{max}$ (484±117, 712±123 and 843±120 and 181±42, 367±58 and 560±95 mmHg sec^{-1}, respectively) and cardiac output (210±60, 360±70 and 310±100 and 130±70, 150±80 and 260±110 ml min^{-1}). The effects of renzapride (3, 10, 30, 100 and 300 g kg^{-1}) were generally longer-lasting, but less marked (heart rate: 1±1, 2±1, 8±2, 14±2 and 20±2 beats min^{-1}; LVdP/dt$_{max}$: 30±20, 80±51, 180±77, 307±93 and 530±152 mmHg sec^{-1}; and cardiac output: 20±20, 40±20, 80±40, 130±100 and 200±80 ml min^{-1}). Renzapride behaved as a partial agonist, antagonizing the responses to both 5-HT and 5-methoxytryptamine, but not those to isoprenaline.

3 When the heart rate was kept constant by atrial pacing (150 beats min^{-1}), both 5-HT and 5-methoxytryptamine still increased LVdP/dt$_{max}$ and cardiac output, but the effects were slightly less than in unpaced animals. Renzapride was devoid of any effect when dosed for a second time during atrial pacing, probably because of tachyphylaxis. However, when given for the first time during atrial pacing, renzapride did increase LVdP/dt$_{max}$ and cardiac output and behaved again as a partial agonist, attenuating the responses to both 5-HT and 5-methoxytryptamine.

4 The present results are consistent with the contention that 5-HT, 5-methoxytryptamine and renzapride increase $LVdP/dt_{max}$ and cardiac output by a positive inotropic effect via an interaction with the putative 5-HT_4 receptor.

Introduction

5-Hydroxytryptamine (5-HT) exerts profound effects on heart rate and cardiac contractile force in a variety of animal species including humans. Although the mechanisms and receptors involved in 5-HT-induced positive chronotropic effects in several species have been extensively reviewed (see Saxena & Villalón, 1990; 1991), the knowledge about the mechanisms involved in 5-HT-induced changes in cardiac contractile force is rather limited. For example, in molluscs (Venus mercenaria, Aplysia kurodai, Helix aspersa, etc.) 5-HT increases myocardial contractions with high potency (Greenberg, 1960) through receptors that seem to be positively coupled to the adenylate cyclase system (Sawada *et al.*, 1984), but which apparently do not resemble any of the previously described four categories for vertebrate 5-HT receptors (Cadogan & Humphrey, 1991). With respect to mammals, 5-HT elicits positive inotropic effects in the rabbit atria indirectly by releasing noradrenaline via stimulation of neuronal 5-HT_3 receptors (Trendelenburg, 1960; Fozard, 1984). 5-HT has also been reported to induce positive inotropic effects in human right atria via stimulation of a cyclic AMP-dependent protein kinase (Kaumann *et al.*, 1990a,b) by the putative 5-HT_4 receptor.

Interestingly, in the cat heart, the receptors involved in 5-HT-induced positive inotropic effects on left atrium (sensitive to blockade by phenoxybenzamine and methysergide) and papillary muscle (right ventricle; resistant to blockade by phenoxybenzamine, methysergide, ketanserin or MDL 72222) do not display the same pharmacological profile (Kaumann, 1985; Kaumann *et al.*, 1990b). This information coupled to the fact that the putative 5-HT_4 receptor mediates both positive inotropic effects in the piglet left atria (Kaumann *et al.*, 1991) and positive chronotropic effects in the anaesthetized pig (Duncker *et al.*, 1985; Bom *et al.*, 1988; Villalón *et al.*, 1990; 1991a) raise questions as to the nature of the mechanisms and receptors involved in 5-HT-induced positive inotropic effects in the porcine myocardium. However, to our knowledge, the possible effects of 5-HT and related drugs on myocardial contractility *in vivo* have not yet been investigated and, as in the cat heart, the nature of the contractile 5-HT myocardial receptor in the pig remains elusive. Therefore, in the present study, we set out to investigate the haemodynamic actions of 5-HT in anaesthetized, open-chest pigs, and compare these to those of 5-methoxytryptamine,

renzapride and isoprenaline, paying particular attention to the maximum rate of rise in left ventricular pressure ($LVdP/dt_{max}$), which can be considered as an *index* of myocardial contractility. A preliminary account of these results has been presented to the British Pharmacological Society (Villalón *et al.*, 1991b).

Methods

General

After an overnight fast, young Yorkshire pigs (16-18 kg) were sedated with ketamine (20 mg/kg, i.m.). Subsequently, they received 150 mg metomidate via a dorsal ear vein. After intubation, the animals were connected to a respirator for intermittent positive pressure ventilation with a mixture of O_2 (33%) and N_2O (67%). The anaesthesia was maintained with a continuous infusion of pentobarbitone sodium (15-20 mg kg^{-1} h^{-1}, i.v.). All drugs were injected into the left jugular vein. The body temperature of the animals was maintained around 37°C by using an electric blanket and arterial blood gases and pH were kept within the normal limits (PO_2>90 mmHg; PCO_2 30-40 mmHg; pH 7.35-7.45) by adjusting respiratory rate and tidal volume. A catheter was placed in the aortic arch via a femoral artery, connected to a Statham pressure transducer (P23 Dc, Hato Rey, Puerto Rico) for the measurement of arterial blood pressure. Left ventricular pressure was measured with a microtipped catheter placed into the left ventricle via the left carotid artery (Honeywell-Phyllips, Best, the Netherlands). The catheters were flushed with heparin to prevent clotting. The ECG leads were monitored throughout the experiment in order to determine heart rate.

After the heart had been exposed via the left third intercostal space, an electromagnetic flow probe (Skalar, Delft, the Netherlands) was placed around the ascending aorta. Finally, a pacing wire was fixed on the left atrium.

Experimental protocol

Once the surgical procedures were completed and the animals (n=8) had been in a stable haemodynamic condition for at least 45 min, baseline data were obtained at the animal's own sinus rhythm. These data consisted of: electrocardiogram, arterial blood pressure, left ventricular pressure (and its first derivative; $LVdP/dt_{max}$) and cardiac output (ascending aortic blood flow).

After collection of these data, in 6 animals chosen at random, the effect of atrial pacing (120, 150 and 180 beats min^{-1} for 3 min each) on $LVdP/dt_{max}$ and cardiac output

was determined before administration of any drug. These values of atrial pacing were selected on the basis of our previous findings concerning the dose-dependent tachycardiac responses induced by isoprenaline (Villalón et al., 1990).

Once the atrial pacing was interrupted, a stabilization period of 15 minutes was allowed. The animals received intravenous bolus injections of 5-HT (3, 10 and 30 μg kg^{-1}), 5-methoxytryptamine (3, 10 and 30 μg kg^{-1}) and isoprenaline (0.01, 0.03 and 0.10 μg kg^{-1}). Subsequently, a 3 ml bolus of physiological saline was given, and the responses to the different doses of 5-HT, 5-methoxytryptamine and isoprenaline were elicited again. After recovery of the haemodynamic baseline values, the heart rate was raised to 150 beats min^{-1} by atrial pacing, and the responses to 5-HT, 5-methoxytryptamine and isoprenaline were elicited after physiological saline following the same procedure, as described above.

As of here, the animals were divided into 2 groups. In the first group (n=5), the atrial pacing was interrupted, and a 10 min interval was allowed for recovery at the animal's own sinus rhythm. Subsequently, intravenous bolus injections of renzapride (3, 10, 30, 100 and 300 μg kg^{-1}, given cumulatively) were administered. Once the haemodynamic parameters returned to baseline values, the responses to the different doses of 5-HT, 5-methoxytryptamine and isoprenaline were analyzed (for other specifications, see Results). In the second group (n=3), the above-mentioned doses of renzapride were given **during atrial pacing**, and after recovery of the haemodynamic parameters to baseline level, the responses to 5-HT, 5-methoxytryptamine and isoprenaline were elicited again.

The interval between the different doses of the compounds used as agonists and/or antagonists depended on the duration of tachycardia and/or the increase in $LVdP/dt_{max}$ produced by the preceding dose, as in each case we waited until such parameters had returned completely or nearly to the baseline values. The dose-intervals for the different drugs were as follows: tryptamine derivatives, between 5 and 15 min; renzapride usually between 15 and 30 min, but as its actions were longer-lasting, we decided to dose cumulatively between doses, and therefore, we will refer to the recovery period after the last dose, which was longer than 90 min). The doses mentioned in the text are cumulative for renzapride (given as 3, 7 and 20, 70 and 200 μg kg^{-1}); and sequential for 5-HT, 5-methoxytryptamine and isoprenaline.

Drugs

The drugs used in this study were: 5-hydroxytryptamine creatinine sulphate (Sigma Chemical Company, St. Louis, MO, U.S.A.), 5-methoxytryptamine hydrochloride (Janssen Chimica, Beerse, Belgium), renzapride hydrochloride (BRL 24924; gift:

Dr. G.J. Sanger, Beecham Pharmaceuticals, Harlow, Essex, England), isoprenaline sulphate (Pharmacy Department, Erasmus University, Rotterdam, The Netherlands) and heparin sodium (Thromboliquine, Organon Teknika B.V., Boxtel, The Netherlands). All drugs were dissolved in physiological saline, which had no effect on the haemodynamic parameters. The doses of 5-HT and 5-methoxytryptamine are mentioned in terms of the free base.

Data presentation and analysis

All data in the text, figures and tables are presented as mean±s.e.mean. The peak change in heart rate (in the absence of atrial pacing) and/or $LVdP/dt_{max}$ and cardiac output induced by the different doses of 5-HT, 5-methoxytryptamine, renzapride and isoprenaline were determined. The increases in heart rate, $LVdP/dt_{max}$ and cardiac output just before and after a particular (partial) antagonist drug were compared by Duncan's new multiple range test, once an analysis of variance (randomized block design) revealed that the samples represented different populations (Saxena, 1985). The effects of agonist drugs in the different groups of animals were compared by using unpaired Student's t-test. A P value of 0.05 or less (two-tailed) was considered statistically significant.

Results

Initial blood pressure and heart rate changes by 5-HT agonist drugs

Baseline values of mean arterial blood pressure, heart rate, $LVdP/dt_{max}$ and cardiac output in the 8 pigs were 81±4 mmHg, 103±3 beats min^{-1}, 1692±83 mmHg sec^{-1} and 2.0±0.3 l min^{-1}, respectively. The changes induced in mean arterial blood pressure by each 5-HT agonist drug were: 5-HT (-17±1, -16±1 and -14±1 followed by +2±1 mmHg after 3, 10 and 30 $\mu g\ kg^{-1}$, respectively; n=8); 5-methoxytryptamine (-19±1, -17±2 and -14±2 followed by +2±1 mmHg after 3, 10 and 30 $\mu g\ kg^{-1}$, respectively; n=8), and renzapride (+1±1, +3±2, +7±3, +10±3 and +13±5 mmHg after 3, 10, 30, 100 and 300 $\mu g\ kg^{-1}$, respectively; n=5). These effects were not evaluated further.

In the *absence* of atrial pacing, intravenous bolus injections of either 5-HT, 5-methoxytryptamine, renzapride or isoprenaline caused dose-dependent increases in cardiac output (see Table 1), $LVdP/dt_{max}$ and heart rate (Figure 1) of diverse magnitude. Isoprenaline was much more potent than 5-HT, which was itself more potent than 5-methoxytryptamine or renzapride. At the doses used, the duration of action of renzapride on $LVdP/dt_{max}$ (> 60 min at 300 $\mu g\ kg^{-1}$) was much longer than that of the

three doses of 5-methoxytryptamine (6.2±0.4, 10.3±0.6 and 14.8±0.7 min) or 5-HT (2.4±0.2, 4.5±0.3 and 8.3±0.7 min). The duration of action of these drugs on cardiac output and heart rate was practically similar (data not shown).

Table 1 Effect of renzapride (0.3 mg kg^{-1}) on the increases in cardiac out (ml min^{-1}) by 5-HT, 5-methoxytryptamine (5-MeO-T) and isoprenaline in pigs without or during atrial pacing (150 beats min^{-1}).

Agonist	Dose ($\mu g\ kg^{-1}$)	Unpaced (n=5)		Paced (n=3)	
		Before	After renzapride	Before	After renzapride
5-HT	3	210±60	90±40*	183±44	183±44
	10	360±70	110±80*	283±44	200±58
	30	310±100	80±80*	483±101	150±76*
5-MeO-T	3	130±70	50±50*	167±44	67±33*
	10	150±80	50±50*	267±44	217±44
	30	260±110	100±60*	433±109	153±29*
Isoprenaline	0.01	360±120	310±70	357±54	300±50
	0.03	700±150	610±100	550±153	500±104
	0.1	860±110	1000±110	952±198	933±233

*, $P < 0.05$, after vs before.

Modification of 5-HT- and 5-methoxytryptamine-induced increases in $LVdP/dt_{max}$, cardiac output and heart rate by physiological saline or renzapride in the absence of atrial pacing

At the animal's own heart rate, intravenous administration of either 5-HT, 5-methoxytryptamine or isoprenaline elicited dose-dependent increases in heart rate (see Figure 1 and Table 2). These responses remained essentially unchanged in control animals after receiving physiological saline, as previously reported (Villalón *et al.*, 1990).

Similarly, 5-HT-, 5-methoxytryptamine- and isoprenaline-induced increases in both $LVdP/dt_{max}$ and cardiac output remained unaltered after the administration of physiological saline (Figure 2; see Table 1 for cardiac output). In marked contrast, the increases in heart rate, $LVdP/dt_{max}$ and cardiac output induced by 3, 10, 30, 100 and 300 $\mu g\ kg^{-1}$ of renzapride under baseline conditions (1±1, 2±1, 8±2, 14±2 and 20±2 beats min^{-1}; 30±20, 80±51, 180±77, 307±93 and 530±152 mmHg sec^{-1}; and 20±20, 40±20, 80±40, 130±100 and 200±80 ml min^{-1}, respectively; n=5) disappeared when given again after administration of physiological saline (0±0, 0±0, 0±0, 0±0 and 0±0 for heart rate, $LVdP/dt_{max}$ and cardiac output, respectively; n=5).

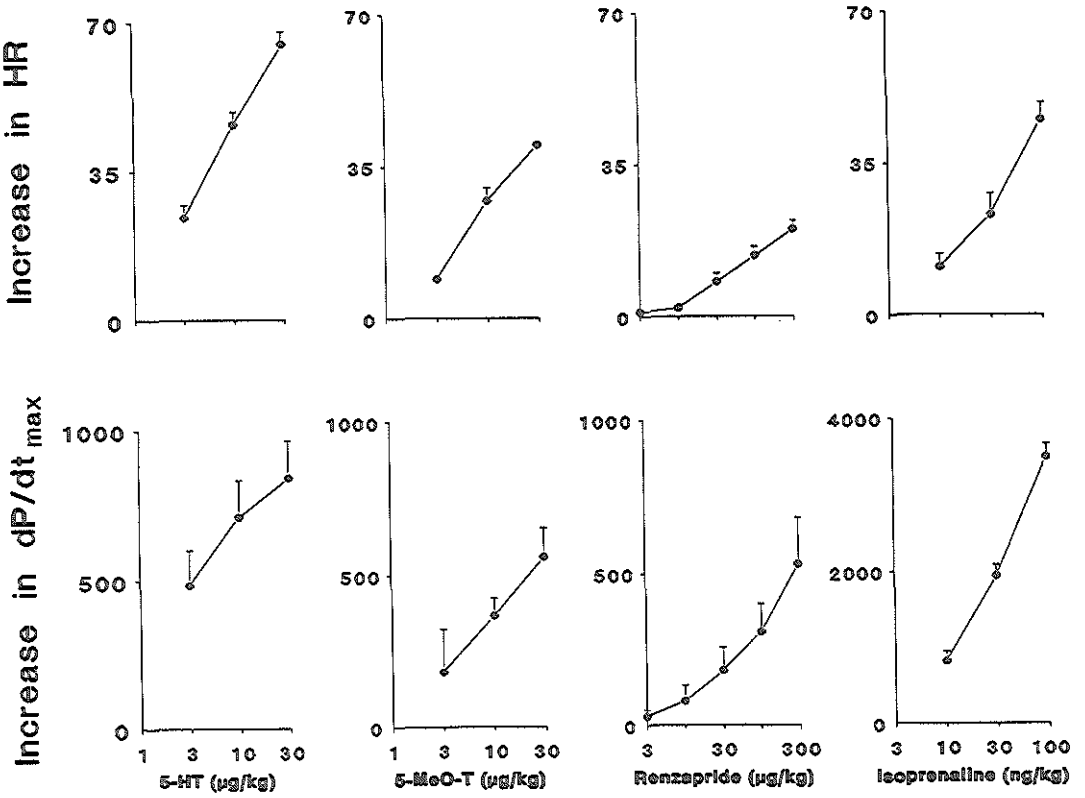

Figure 1. The effects of 5-HT, 5-methoxytryptamine (5-MeO-T), renzapride and isoprenaline in the anaesthetized pig (n=5) on heart rate (HR; beats min^{-1}) and $LVdP/dt_{max}$ (mmHg sec^{-1}) in the absence of atrial pacing.

Notwithstanding, renzapride (300 μg kg^{-1}) did potently antagonize both 5-HT- and 5-methoxytryptamine-induced increases in heart rate (Table 2), $LVdP/dt_{max}$ (Figure 2) and cardiac output (Table 1), but not isoprenaline-induced increases in heart rate (Table 2), $LVdP/dt_{max}$ (Figure 2) or cardiac output (Table 1).

It may be noted that unlike renzapride, in the saline-treated animals where 5-HT was administered before and after the last set of injections of 5-methoxytryptamine (n=5; see description of experimental protocol), the increases in $LVdP/dt_{max}$ induced by 3, 10 and 30 μg kg^{-1} of 5-HT (484±117, 712±123 and 843±120 mmHg sec^{-1}, respectively; n=5) remained unaltered after 5-methoxytryptamine (460±142, 715±161 and 897±154 mmHg sec^{-1}, respectively; n=5). Similarly, the increases in both cardiac output and heart rate induced by 5-HT were not modified after 5-methoxytryptamine (not shown, but see baseline data in Tables 1 and 2, respectively).

Table 2 Effect of renzapride (0.3 mg kg^{-1}) on the increases in heart rate (beats min^{-1}) by 5-HT, 5-methoxytryptamine (5-MeO-T) and isoprenaline (ISO) in pigs without atrial pacing.

Agonist	3 μg kg^{-1}		10 μg kg^{-1}		30 μg kg^{-1}	
	Before	After renzapride	Before	After renzapride	Before	After renzapride
5-HT	24±3	5±2*	46±3	16±3*	65±3	29±3*
5-MeO-T	9±1	5±2*	27±3	12±2*	40±1	23±1*
ISO[1]	11±6	5±4	23±9	17±7	45±9	34±11

[1], The doses of isoprenaline were 0.01, 0.03 and 0.1 μg kg^{-1}. *, $P < 0.05$, after renzapride vs before renzapride.

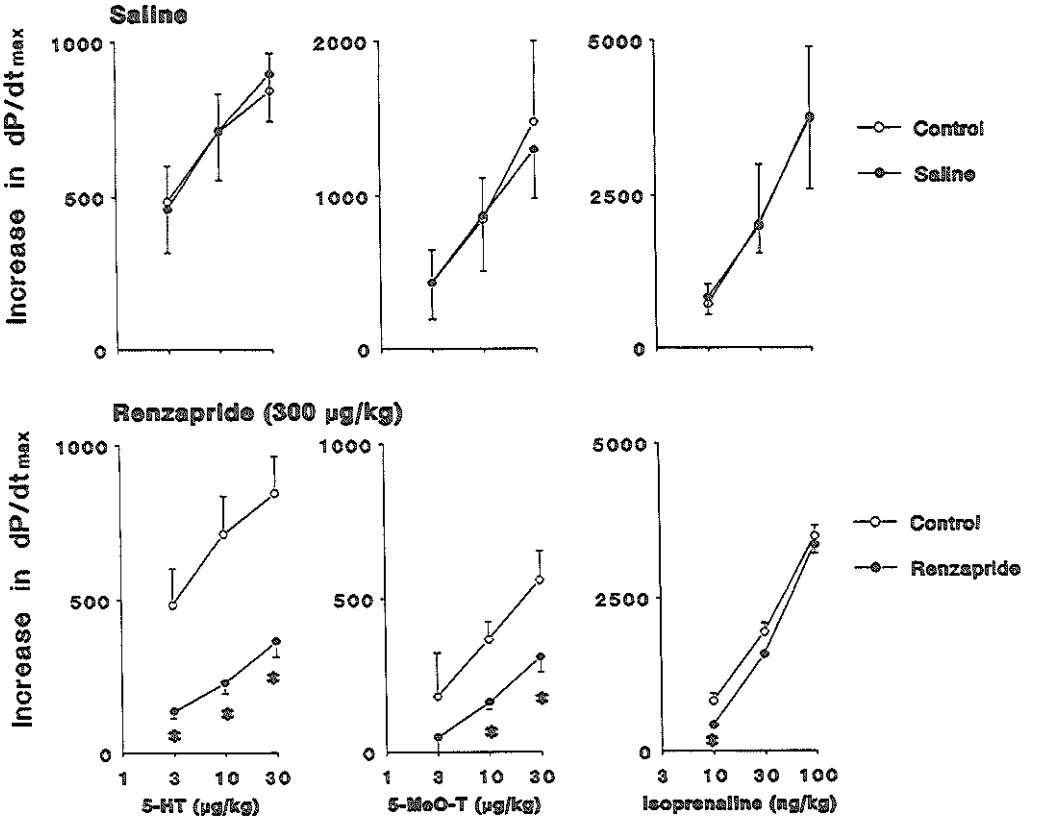

Figure 2. The effects of saline (n=5) or renzapride (300 μg kg^{-1}; n=5) on the increases in LVdP/dt$_{max}$ (mm sec^{-1}) induced by 5-HT, 5-methoxytryptamine (5-MeO-T) and isoprenaline in the absence of atrial pacing. *, Significantly different from the corresponding control response to the agonist drug ($P<0.05$).

Effect of the increase in heart rate by electrical stimulation of the atrium (atrial pacing) at different rates on myocardial contractility (LVdP/dt$_{max}$) and cardiac output

Since the increase in heart rate by the above drugs can have a direct influence on the increase in LVdP/dt$_{max}$, we decided to investigate the effect of increasing heart rate by electrical stimulation of the atrium at different rates (120, 150 and 180 beats min^{-1}) on LVdP/dt$_{max}$ and cardiac output. As shown in Figure 3, the selected rates of atrial pacing exerted only a weak effect on LVdP/dt$_{max}$ and cardiac output, which was not dependent on the rate of stimulation. Furthermore, it is important to highlight that atrial pacing

at 180 beats min^{-1} was not tolerated by the animals when maintained for more than 5 min, since intense tremor was observed. This finding coupled to the fact that the absolute values of increase in heart rate induced by the different agonist drugs were not higher than 150 beats min^{-1} (see Table 2), led us to select the atrial pacing rate of 150 beats min^{-1} in order to investigate the effects of agonist drugs on $LVdP/dt_{max}$ and cardiac output.

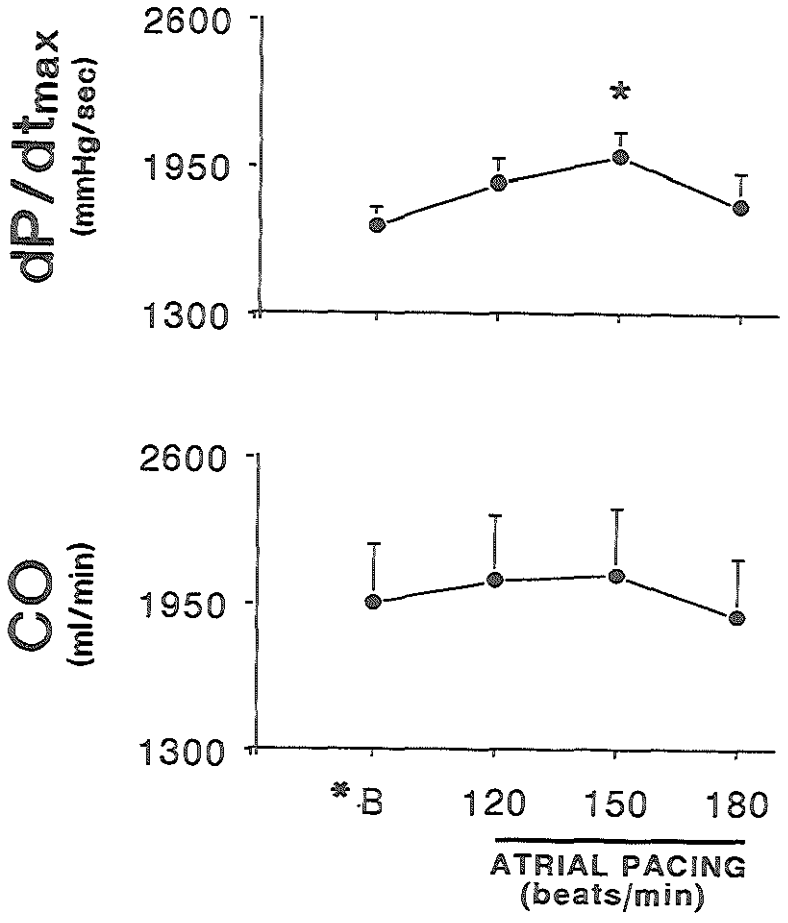

Figure 3. Effect of atrial pacing on $LVdP/dt_{max}$ and cardiac output (CO). B. Baseline heart rate (100±3 beats.min^{-1}); *, $P < 0.05$ vs baseline value.

Modification of 5-HT- and 5-methoxytryptamine-induced increases in LVdP/dt$_{max}$ by physiological saline or renzapride during atrial pacing

During atrial pacing to a frequency of 150 beats min^{-1}, intravenous bolus injections of 5-HT, 5-methoxytryptamine, renzapride and isoprenaline were also able to induce dose-dependent increases in cardiac output (Table 1) and LVdP/dt$_{max}$ (Figure 4). As shown in Figure 4, there was an apparent attenuation of these responses when compared with those previously induced in the absence of atrial pacing (Villalón *et al.*, 1991b).

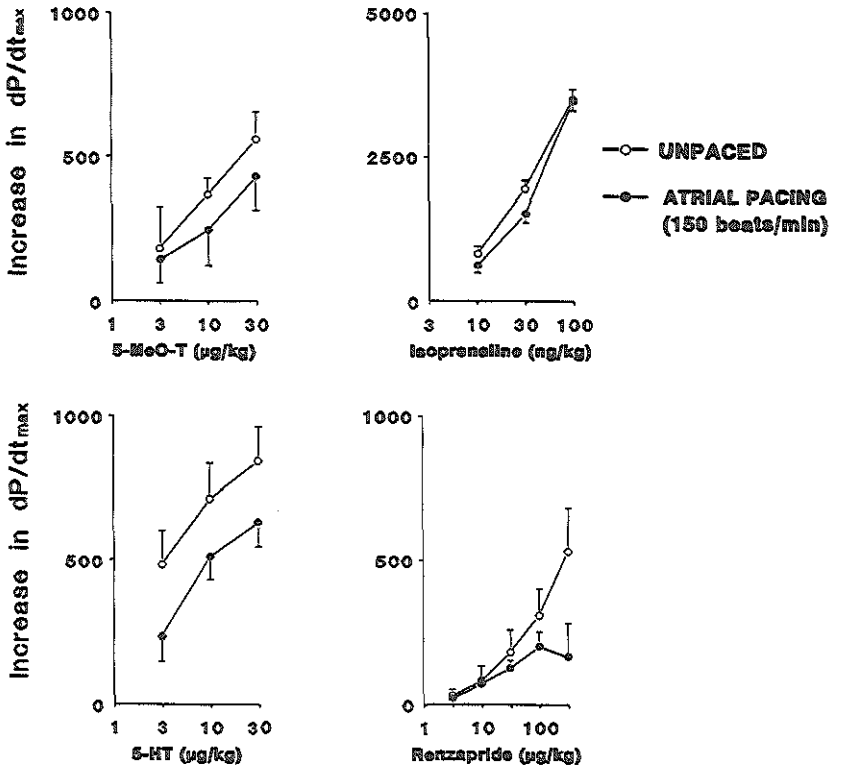

Figure 4. The effects of 5-HT, 5-methoxytryptamine (5-MeO-T), renzapride, and isoprenaline on LVdP/dt$_{max}$ (mm Hg sec^{-1}) in the absence or during atrial pacing at 150 beats min^{-1} .

Furthermore, the increases in $LVdP/dt_{max}$ induced by 5-HT, 5-methoxytryptamine and isoprenaline during atrial pacing remained essentially unchanged after administration of physiological saline (Figure 5; n=3). In marked contrast, renzapride-induced increases in $LVdP/dt_{max}$ (20±20, 70±20, 125±25, 200±50 and 165±115 mmHg min^{-1}) and cardiac output (0±0, 20±20, 40±40, 100±0, 100±50 ml min^{-1}) disappeared when given after administration of physiological saline (0±0, 0±0, 0±0, 0±0 and 0±0 for both $LVdP/dt_{max}$ and cardiac output; n=2). Interestingly, renzapride (300 $\mu g\ kg^{-1}$) slightly blunted the increases in $LVdP/dt_{max}$ induced by either 5-HT or 5-methoxytryptamine, but not those by isoprenaline (Figure 5).

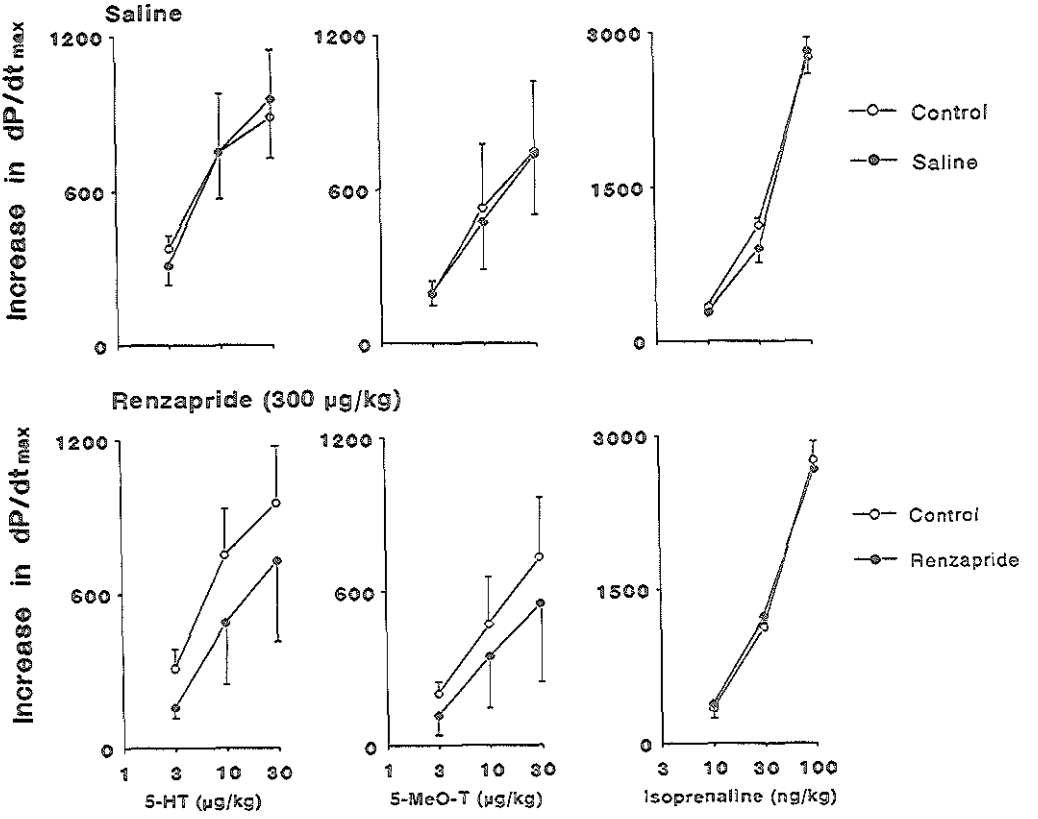

Figure 5. The effects of saline (n=5) or renzapride (300 $\mu g\ kg^{-1}$; n=3)) on the increases in $LVdP/dt_{max}$ (mm Hg sec^{-1}) induced by 5-HT, 5-methoxytryptamine (5-MeO-T) and isoprenaline during atrial pacing (150 beats min^{-1}).

It is prudent to note that, in contrast to the ability of renzapride to elicit increases in heart rate, $LVdP/dt_{max}$ and cardiac output at the animal's own heart rate (see Figure 1 and the above data), the responses to the benzamide virtually disappeared when given again during atrial pacing (0±0, 0±0, 0±0, 0±0 and 0±0 for heart rate, cardiac output and $LVdP/dt_{max}$ after 3, 10, 30, 100 and 300 $\mu g\ kg^{-1}$ of renzapride, respectively; n=5). For this reason, the effects of renzapride during atrial pacing were analyzed separately in control animals at their own sinus rhythm and in animals during atrial pacing. Under the latter experimental circumstances, renzapride did induce dose-dependent increases in $LVdP/dt_{max}$ (see Figure 4). The rank order of potency was 5-HT > 5-methoxytryptamine > renzapride, as previously described in the absence of atrial pacing.

Lastly, the possible attenuation of the responses to 5-HT by 5-methoxytryptamine was investigated. In the saline-treated animals where 5-HT was administered before and after the last set of injections of 5-methoxytryptamine (see description of experimental protocol), the increases in $LVdP/dt_{max}$ induced by 3, 10 and 30 $\mu g\ kg^{-1}$ of 5-HT (233±86, 511±80 and 631±84 $mmHg\ sec^{-1}$, respectively; n=3) remained unaltered after 5-methoxytryptamine (325±80, 492±126 and 603±136 $mmHg\ sec^{-1}$, respectively; n=3), indicating that 5-methoxytryptamine does not behave as a partial agonist.

Discussion

The putative $5\text{-}HT_4$ receptor is involved in 5-HT-induced positive chronotropic effects in the anaesthetized pig (Duncker *et al.*, 1985; Bom *et al.*, 1988; Villalón *et al.*, 1990, 1991a) and in 5-HT-induced positive inotropic effects in human right atria, via stimulation of the adenylate cyclase system (Kaumann *et al.*, 1990a,b,d). The putative $5\text{-}HT_4$ receptor also mediates positive inotropic effects in the piglet left atrium (Kaumann *et al.*, 1991), but it is still unknown whether 5-HT is able to increase myocardial contractility *in vivo* in the pig. The present investigation in anaesthetized pigs clearly demonstrates that 5-HT, 5-methoxytryptamine and renzapride increased $LVdP/dt_{max}$ and that renzapride also antagonized the responses to 5-HT and 5-methoxytryptamine. Thus, the ventricular 5-HT receptors in the pig seem to have a pharmacological profile resembling that of the putative $5\text{-}HT_4$ receptor present on the porcine cardiac pacemaker (Duncker *et al.*, 1985; Bom *et al.*, 1988; Villalón *et al.*, 1990; 1991a), human right atrium (Kaumann *et al.*, 1990a,b,d), piglet left atrium (Kaumann *et al.*, 1991) and guinea-pig enteric neurons (Craig & Clarke, 1990).

Interpretation of the increases induced in LVdP/dt$_{max}$ and cardiac output by 5-HT, 5-methoxytryptamine and renzapride

In pentobarbital anaesthetized, open-chest pigs, intravenous bolus injections of 5-HT, 5-methoxytryptamine and renzapride elicited dose-dependent increases in heart rate (as previously reported; Villalón *et al.*, 1990, 1991a), together with dose-dependent increases in LVdP/dt$_{max}$ and cardiac output in the absence of atrial pacing (Figures 1 and 2; Table 1); the increases in cardiac output induced by the above agonist drugs were due to both positive chronotropic and positive inotropic effects. Since these drugs also induced dose-dependent increases in LVdP/dt$_{max}$ and cardiac output even when the heart rate was fixed at 150 beats min^{-1} by atrial pacing (see Figures 4 and 5 and Table 1), it could be suggested that the effects of these drugs on LVdP/dt$_{max}$ were due to their positive inotropic effects. Indeed, this contention is further supported by the fact that electrical pacing of the heart rate (atrial pacing) at 120, 150 and 180 beats min^{-1} did not elicit similar increases in LVdP/dt$_{max}$ and cardiac output (see Figure 3). The rank order of potency for the increase in myocardial contractility was: 5-HT > 5-methoxytryptamine > renzapride (either in the absence or during atrial pacing).

Partial agonist action of renzapride

In contrast to 5-HT and 5-methoxytryptamine, the increases in LVdP/dt$_{max}$ and cardiac output induced by renzapride (both in the absence and during atrial pacing) were less marked, but longer-lasting, and not strictly dose-dependent. In addition, renzapride antagonized the effects of 5-HT and 5-methoxytryptamine (either in the absence or during atrial pacing). This antagonism was apparently not due to a 'masking' of the effect as a result of the renzapride-induced increase in LVdP/dt$_{max}$ as 5-HT and 5-methoxytryptamine had been administered at the time when the inotropic effect of renzapride had worn off. Therefore, as previously found with several benzamide derivatives, including renzapride, with regard to the tachycardiac action of 5-HT and 5-methoxytryptamine in the pig (Villalón *et al.*, 1990; 1991a), renzapride also behaved as a partial agonist at the porcine 5-HT receptor mediating the positive inotropic action. It should be noted that in the absence of atrial pacing isoprenaline-induced increases in heart rate and contractility were little affected by 300 μg kg^{-1} of renzapride.

Resemblance of the porcine myocardial 5-HT receptor to other putative 5-HT$_4$ receptors

At the 5-HT$_4$ receptor identified in the neurones from mouse embryo colliculi on the basis of the increase in cAMP, the tryptamine derivatives 5-methoxytryptamine,

5-carboxamidotryptamine (low affinity) and certain benzamide derivatives (renzapride, metoclopramide, cisapride), but not α-methyl-5-HT or 2-methyl-5-HT, are agonists; and ICS 205-930 (in high concentrations), but not MDL 72222, granisetron or ondansetron, acts as an antagonist (Dumuis *et al.*, 1988; 1989; Clarke *et al.*, 1989). These pharmacological characteristics exhibit several similarities with those of other 5-HT_4 receptors. However, the rank order of potency for tryptamine- and benzamide derivatives reported by Dumuis *et al.* (1989) using mouse embryo colliculi neurons (cisapride > renzapride > zacopride > 5-HT > metoclopramide), differs from that found at the porcine cardiac pacemaker either *in vivo* (5-HT > 5-methoxytryptamine > α-methyl-5-HT > renzapride ≥ zacopride > cisapride > metoclopramide > dazopride; indorenate and sumatriptan, both inactive at 1 and 3 mg kg^{-1} (Villalón *et al.*, 1990; 1991a) or *in vitro* (5-HT > renzapride > cisapride > 5-CT; Kaumann, 1990c); piglet left atrium (5-HT > renzapride; Kaumann *et al.*, 1991); human right atrium (5-HT > renzapride; Kaumann *et al.*, 1990d); and cholinergic neurons of guinea-pig ileum (5-HT > 5-methoxytryptamine > renzapride > α-methyl-5-HT > zacopride = cisapride; Craig & Clark, 1990). Several possible explanations for these differences in agonist potencies may include: use of "second messenger" (cAMP) and functional (positive chronotropic and/or inotropic) responses; tissue-dependent factors such as the number of receptors and coupling efficiency; and/or drug-dependent factors such as the affinity of 5-HT and related agonists for each of these novel receptors. The 5-HT_4 receptor may also mediate the 5-HT-induced relaxation of the rat oesophagus (Baxter & Clarke, 1990) and enhancement of cholinergic activity in guinea-pig ascending colon (Elswood *et al.*, 1991); in these tissues, the tryptamine derivatives 5-methoxytryptamine, α-methyl-5-HT and some benzamides mimic, and ICS 205-930 acts as an antagonist.

In the present experiments the positive inotropic effects of 5-HT were mimicked by 5-methoxytryptamine and renzapride (potency order: 5-HT > 5-methoxytryptamine > renzapride), and renzapride selectively antagonized the responses to 5-HT and 5-methoxytryptamine without affecting the responses to isoprenaline. Furthermore, tachyphylaxis was observed with the inotropic effects of renzapride both in the absence and during atrial pacing. In this connection, a similar fading pattern with renzapride has been reported earlier with renzapride-induced increases in cyclic AMP in mouse embryo colliculi neurons (Dumuis *et al.*, 1989) and heart rate in the pig (Villalón *et al.*, 1990, 1991a). These findings suggest that the porcine 5-HT receptor mediating cardiotonic responses also belongs to the putative 5-HT_4 receptor category.

References

BAXTER, G.S. & CLARKE, D.E. (1990) Putative 5-HT_4 receptors mediate relaxation of rat oesophagus. *Proceedings of the 2nd IUPHAR Satellite Meeting on Serotonin, Basel, July 11-13, 1990.* Abstract No. P85.

BOM, A.H., DUNCKER, D.J., SAXENA, P.R., & VERDOUW, P.D. (1988). 5-HT-induced tachycardia in the pig: possible involvement of a new type of 5-HT receptor. *Br. J. Pharmacol.*, **93**, 663-671.

CADOGAN, A., & HUMPHREY, P.P.A. (1991). Classification of the 5-HT receptor mediating cardio-excitation in the snail, Helix aspersa. *Br. J. Pharmacol.*, **102**, 97P.

CLARKE, D.E., CRAIG, D.A. & FOZARD, J.R. (1989). The 5-HT_4 receptor: naughty but nice. *Trends Pharmac. Sci.*, **10**, 385-386.

CRAIG, D.A. & CLARKE, D.E. (1990). Pharmacological characterization of a neuronal receptor for 5-hydroxytryptamine in guinea pig ileum with properties similar to the 5-hydroxytryptamine$_4$ receptor. *J. Pharmac. Exp. Ther.*, **252**, 1378-1386.

DUMUIS, A., BOUHELAL, R., SEBBEN, M. & BOCKAERT, J. (1988). A 5-HT receptor in the central nervous system, positively coupled with adenyl cyclase, is antagonized by ICS 205-930. *Eur. J. Pharmac.*, **146**, 187-188.

DUMUIS, A., SEBBEN, M. & BOCKAERT, J. (1989). The gastrointestinal prokinetic benzamide derivatives are agonists at the non-classical 5-HT receptor (5-HT_4) positively coupled to adenylate cyclase in neurons. *Naunyn-Schmied. Arch. Pharmac.*, **340**, 403-410.

DUNCKER, D.J., SAXENA, P.R. & VERDOUW, P.D. (1985). 5-hydroxytryptamine causes tachycardia in pigs by acting on receptors unrelated to 5-HT_1, 5-HT_2 or M-type. *Br. J. Pharmacol.*, **86**, 596P.

ELSWOOD, C.J., BUNCE, K.T. & HUMPHREY, P.P.A. (1991). Identification of putative 5-HT_4 receptors in guinea-pig ascending colon. *Eur. J. Pharmac.*, **196**, 149-155.

FOZARD, J.R. (1984). MDL 72222: a potent and highly selective antagonist at neuronal 5-hydroxytryptamine receptors. *Naunyn-Schmied. Arch. Pharmac.*, **326**, 36-44.

GREENBERG, M.J. (1988). Structure-activity relationship of tryptamine analogues on the heart of Venus mercenaria. *Br. J. Pharmacol.*, **15**, 375-388.

KAUMANN, A.J. (1985). Two classes of myocardial 5-hydroxytryptamine receptors that are neither 5-HT_1 nor 5-HT_2. *J. Cardiovasc. Pharmac.*, **7(Suppl.7)**, S76-S78.

KAUMANN, A.J., SANDERS, L., BROWN, A.M., MURRAY, K.J. & BROWN, M.J. (1990a). A 5-hydroxytryptamine receptor in human atrium. *Br. J. Pharmac.*, **100**, 879-885.

KAUMANN, A.J., MURRAY, K.J., BROWN, A.M., FRAMPTON, J.E., SANDERS, L. & BROWN, M.J. (1990b). Heart 5-HT receptors. A novel 5-HT receptor in human atrium. In *Serotonin: From Cell Biolology to Pharmacology and Therapeutics*. eds. Paoletti *et al.*, pp. 347-354, Dordrecht: Kluwer Academic Publishers.

KAUMANN, A.J. (1990c). Piglet sinoatrial 5-HT receptors resemble human atrial 5-HT_4-like receptors. Naunyn-Schmied. Arch. Pharmac., **342**, 619-622.

KAUMANN, A.J., SANDERS, L., BROWN, A.M., MURRAY, K.J. & BROWN, M.J. (1990d). Human atrial 5-HT receptors: similarity to rodent neuronal 5-HT_4 receptors. *Br. J. Pharmac.*, **100**, 319.

KAUMANN, A.J., BROWN, A.M. & RAVAL, P. (1991). Putative 5-HT_4-like receptors in piglet left atrium. *Br. J. Pharmac.*, **102**, 98P.

SAWADA, M., ICHINOSE, M., ITO, I., MAENO, T. & McDOO, D.J. (1984). Effects of 5-hydroxytryptamine on membrane potential, contractility, accumulation of cyclic AMP and Ca^{2+} movements in anterior aorta and ventricle of aplysia. *J. Neurophysiol.*, **51**, 361-374.

SAXENA, P.R. (1985). An interactive computer program for data management and parametric and non-parametric statistical analysis. *Br. J. Pharmac.*, **86**, 818P.

SAXENA, P.R. & VILLALóN, C.M. (1990). Cardiovascular effects of serotonin agonists and antagonists. *J. Cardiovasc. Pharmac.*, **15(Suppl.7)**, S17-S34.

SAXENA, P.R. & VILLALóN, C.M. (1991). 5-Hydroxytryptamine, a chamaleon in the heart. *Trends Pharmac. Sci.*, 12, 223-227.

TRENDELENBURG, U. (1960). The action of hystamine and 5-hydroxytryptamine on isolated mamalian atria. *J. Pharmacol. Exp Ther.*, 130, 450-460.

VILLALóN, C.M., DEN BOER, M.O., HEILIGERS, J.P.C. & SAXENA, P.R. (1990). Mediation of 5-hydroxytryptamine-induced tachycardia in the pig by the putative 5-HT_4 receptor. *Br. J. Pharmac.*, **100**, 665-667.

VILLALóN, C.M., DEN BOER, M.O., HEILIGERS, J.P.C. & SAXENA, P.R. (1991a). Further characterization, by use of tryptamine and benzamide derivatives, of the putative 5-HT_4 receptor mediating tachycardia in the pig. *Br. J. Pharmac.*, **102**, 107-112.

VILLALóN, C.M., DEN BOER, M.O., HEILIGERS, J.P.C., VERDOUW, P.D., DUNCKER, D.J.G.M. & SAXENA, P.R. (1991b). Role of the putative 5-HT_4 receptor in positive inotropic effect of 5-HT in the pig. *Fund. Clin. Pharmac.* (in press).

CHAPTER 10

CONSTRICTION OF PORCINE ARTERIOVENOUS ANASTOMOSES BY INDORENATE IS UNRELATED TO 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} OR 5-HT_{1D} RECEPTOR SUBTYPES

Villalón, C.M., Bom, A.H., Heiligers, M.O., den Boer, M.O. and Saxena, P.R.

Eur. J. Pharmacol., 190, 167-176, 1990.

European Journal of Pharmacology, 190 (1990) 167–176
Elsevier

EJP 51573

Constriction of porcine arteriovenous anastomoses by indorenate is unrelated to 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} or 5-HT_{1D} receptor subtypes [1]

Carlos M. Villalón [2], Anton H. Bom, Jan P.C. Heiligers, Marien O. Den Boer and Pramod R. Saxena

Department of Pharmacology, Faculty of Medicine and Health Sciences, Erasmus University Rotterdam, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands

Received 2 April 1990, revised MS received 31 July 1990, accepted 14 August 1990

The study concerns the effects of indorenate, a tryptamine derivative with antihypertensive properties as well as high affinity for the 5-HT_{1A} binding site, on carotid haemodynamics in anaesthetized pigs. Intracarotid infusions of indorenate (0.3, 1.0, 3.0 and 10.0 $\mu g \cdot kg^{-1} \cdot min^{-1}$ for 10 min each) caused dose-related decreases in total common carotid artery blood flow due almost exclusively to a reduction in arteriovenous anastomotic flow. These effects of indorenate were not appreciably modified after treatment with the 5-HT_2 receptor antagonist ketanserin (0.5 $mg \cdot kg^{-1}$ i.a.), but were markedly reduced after treatment with methiothepin (1.0 $mg \cdot kg^{-1}$ i.a.), which antagonizes not only 5-HT_2 receptors, but also the putative 5-HT_{1A}, 5-HT_{1B} 5-HT_{1C} and 5-HT_{1D} subtypes of 5-HT_1-like receptors. Nonetheless, metergoline (1 $mg \cdot kg^{-1}$ i.a.), a drug with higher affinity than methiothepin for the above 5-HT_1 receptor subtypes, failed to significantly modify the responses to indorenate. It is therefore concluded that, like 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) and 5-methoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1H-indole (RU 24969), indorenate reduces both total common carotid and cephalic arteriovenous anastomotic blood flow in the pig by stimulating 5-HT_1-like receptors; these receptors, however, do not seem to correspond to either 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} or 5-HT_{1D} binding sites.

Arteriovenous anastomoses; Blood flow (carotid); 5-HT (5-hydroxytryptamine, serotonin); 5-HT_1-like receptors; Indorenate; Migraine

1. Introduction

It has been demonstrated that intracarotid infusion of 5-hydroxytryptamine (5-HT) into anaesthetized pigs decreases common carotid blood flow by reducing arteriovenous anastomotic (non-nutrient flow, whereas arteriolar flow to the head tissues, mainly to the skin and ears, is increased (Saxena and Verdouw, 1982). These effects of 5-HT are mimicked by the 5-HT_1-like receptor agonist 5-carboxamidotryptamine (Saxena and Verdouw, 1985) and antagonized by methiothepin (Saxena et al., 1986), which blocks both 5-HT_1-like and 5-HT_2 receptors, but are not appreciably antagonized by the 5-HT_2 receptor antagonist ketanserin (Verdouw et al., 1984) or the 5-HT_3 receptor antagonist 1αH,3α,5αH-tropan-3yl-3,5-dichlorobenzoate (MDL 72222) (Saxena et al., 1986). Therefore, according to the classification

[1] Partially supported by a grant from Glaxo Group Research, Ware, England.
[2] Supported by a Science and Technology fellowship from the EEC.
Correspondence to: P.R. Saxena, Department of Pharmacology, Faculty of Medicine and Health Sciences, Erasmus University Rotterdam, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands.

proposed by Bradley et al. (1986), the constriction of arteriovenous anastomoses as well as the dilatation of arterioles are mediated by 5-HT_1-like receptors (Saxena et al., 1986). However, the 5-HT_1-like receptors in the carotid circulation of the pig seem to belong to two different categories since, unlike 5-HT (Saxena and Verdouw, 1982) or 5-carboxamidotryptamine (Saxena and Verdouw, 1985), the reduction in arteriovenous anastomotic blood flow induced by methysergide (Saxena and Verdouw 1984), the putative 5-HT_{1A} receptor agonists 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) (Bom et al., 1989a) and N-(3-acetylaminophenyl) piperazine hydrochloride (BEA 1654) (Verdouw et al., 1985), and the putative 5-HT_{1A} and 5-HT_{1B} receptor agonist 5-methoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1H-indole (RU 24969) (Bom et al., 1989b) is not or only slightly accompanied by increases in the arteriolar blood flow. Furthermore, despite the effectiveness of 8-OH-DPAT, BEA 1654 and RU 24969, the 5-HT_1-like receptor mediating arteriovenous anastomotic constriction does not appear to be related to either 5-HT_{1A} or 5-HT_{1B} binding sites because, as we reported recently (Bom et al., 1989b), the effects of RU 24969 are not antagonized by the putative 5-HT_{1A} and 5-HT_{1B} receptor antagonist, (±)-pindolol (Hoyer et al., 1985; Hoyer, 1988). In this investigation, we have studied the possible involvement of 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} and/or 5-HT_{1D} receptors in the distribution of common carotid artery blood flow in the pig by using indorenate, a tryptamine derivative with antihypertensive properties (Hong et al., 1978; Hong, 1981) and high affinity for the 5-HT_{1A} binding site (Dompert et al., 1985; Hoyer et al., 1985). Ketanserin, methiothepin and metergoline were used as potential antagonists. Preliminary results of this investigation have been communicated to the British Pharmacological Society (Villalón et al., 1990).

2. Materials and methods

2.1. General

After an overnight fast, young Yorkshire pigs (body weight: 18-22 kg; n = 24) were sedated with 120 mg (i.m.) azaperone and 120-150 mg (i.v.) metomidate, intubated and connected to a respirator for intermittent positive pressure ventilation with a mixture (1 : 2) of oxygen and nitrous oxide. A continuous i.v. infusion of pentobarbitone sodium (20-26 $mg \cdot kg^{-1} \cdot h^{-1}$) completed the anaesthesia. Respiratory rate and tidal volume were adjusted to keep arterial blood gases and pH within normal limits (pH, 7.35-7.45; pO_2, 90-150 mm Hg; pCO_2, 35-45 mm Hg). Aortic blood pressure was recorded with a Statham pressure transducer (P23 1D) connected to a cannula inserted via the right femoral artery. The common carotid arteries were dissected free and the cervical vagosympathetic trunks were cut to reduce reflex influences on the carotid circulation. Blood flow in one of the common carotid arteries was measured with a calibrated flow probe (internal diameter: 2.5 or 3 mm) connected to a sine-wave electromagnetic blood flow meter (Transflow 601-system, Skalar, Delft, The Netherlands). After its hub had been removed, a 0.5 mm (external diameter) needle, connected to a suitable polyethylene tubing, was inserted into this common carotid artery for the administration of microspheres and drugs. The body temperature of the animals was kept around 37 °C with an electric blanket.

2.2. Distribution of common carotid blood flow

The distribution of common carotid blood flow was determined with the radioactive microsphere method using 15 ± 1 (s.d.) μm diameter microspheres labelled with either ^{141}Ce, ^{113}Sn, ^{103}Ru, ^{95}Nb or ^{46}Sc (NEN Company, Dreieich, Federal Republic of Germany). For each measurement, a suspension of 100 000-150 000 microspheres, labelled with one of the nuclides, was mechanically agitated and injected against the direction of the carotid blood flow to facilitate uniform mixing with the blood. At the end of the experiment the animal was killed and the heart, kidneys, lungs and the various tissues of the head were dissected out, weighed and placed in vials. The radioactivity in these vials was counted for 5-10 min in a gamma-scintillation counter (Packard, Minaxi Autogamma 5000) with suitable windows to detect the different isotopes. The amount of carotid blood

flow distributed to the individual tissues ($Q_{ts[car]}$) was calculated as described earlier (Saxena et al., 1980; Saxena and Verdouw, 1982) by: $Q_{ts[car]}$ (ml $\cdot$ min^{-1}) = (I_{ts}/I_{tot}) $\times$ $Q_{[car]}$, where I_{ts} and I_{tot} are the radioactivity (c.p.m.) in a particular tissue and that detected in all tissues, respectively, and $Q_{[car]}$ is carotid blood flow (ml $\cdot$ min^{-1}). Because microspheres passing through the cephalic arteriovenous anastomoses in the capillaries of the lungs are trapped completely, the values determined for the lungs provide an *index* of the amount of the common carotid arterial blood flow that passed through the arteriovenous anastomoses (see Johnston and Saxena, 1978; Saxena et al., 1980; Saxena and Verdouw, 1982).

2.3. Experimental protocol

In all experiments baseline values of heart rate, blood pressure and carotid blood flow were obtained after the animal had been stable for at least 45 min after completion of the surgical procedure. The animals were then divided into four groups (n = 6 each): untreated control group and groups treated with either ketanserin (0.05 mg $\cdot$ kg^{-1} $\cdot$ min^{-1}), methiothepin (0.1 mg $\cdot$ kg^{-1} $\cdot$ min^{-1}) or metergoline (0.1 mg $\cdot$ kg^{-1} $\cdot$ min^{-1}, administered into the carotid artery at a rate of 1.0 ml $\cdot$ min^{-1} during a period of 10 min (total dose: 0.5, 1.0 and 1.0 mg $\cdot$ kg^{-1} i.a., respectively). Drug administration was followed by a 10 min period of intracarotid infusion of physiological saline. After the measurement of heart rate, blood pressure and carotid blood flow and its distribution, all animals received consecutive 10 min intracarotid infusions of indorenate (0.3, 1.0, 3.0 and 10.0 μg $\cdot$ kg^{-1} $\cdot$ min^{-1} at the rate of 1.0 ml $\cdot$ min^{-1}) with an interval of about 5 min. The haemodynamic measurements were repeated at the end of each infusion period.

2.4. Data presentation and statistical evaluation

Except where stated otherwise, all data in the text and illustrations are presented as means $\pm$ S.E.M. The significance of the differences between the variables at baseline and after indorenate infusions was evaluated by Duncan's new multiple-range test once an analysis of variance (randomized block design) had revealed that the samples represented different populations (Steel and Torrie, 1980). The percent changes by indorenate in the antagonist-treated groups were compared with those in the untreated control group using Student's t-test. Statistical significance was accepted at $P < 0.05$ (two-tailed).

2.5. Drugs

Apart from the anaesthetics, the drugs used in this study were: indorenate (5-methoxytryptamine-β-methylcarboxylate hydrochloride or TR3369 (a gift from Prof. E. Hong, CINVESTAV-I.P.N., Mexico City, Mexico), ketanserin tartrate (a gift from Dr. J.M. Van Nueten, Janssen Pharmaceutica, Beerse, Belgium), methiothepin maleate (a gift from Hoffman La Roche B.V., Mijdrecht, The Netherlands) and metergoline base (courtesy of Farmitalia Carlo Erba, Torino, Italy). Indorenate was dissolved in physiological saline. Ketanserin, methiothepin and metergoline were dissolved in distilled water to which 5% propylene glycol (ketanserin and methiothepin) or 1% ascorbic acid (metergoline) was added. The vehicles had no effect on heart rate, blood pressure or carotid blood flow. The doses of indorenate, ketanserin and methiothepin are mentioned in terms of the salt.

3. Results

3.1. Systemic haemodynamics

The values for heart rate, mean arterial blood pressure and carotid artery blood flow before and after the infusion of either ketanserin, methiothepin or metergoline are shown in table 1. Both mean arterial blood pressure and heart rate decreased after intracarotid infusion of ketanserin (0.5 mg $\cdot$ kg^{-1}), whereas these variables remained unchanged after infusion of methiothepin or metergoline (each 1.0 mg $\cdot$ kg^{-1}). There was some increase in the common carotid blood flow after the three antagonists, but the change in each case was small and/or short-lasting.

TABLE 1

Mean arterial blood pressure, heart rate and carotid blood flow before and after ketanserin (total dose: 0.5 mg·kg^{-1}; n = 6), methiothepin (total dose: 1.0 mg·kg^{-1}; n = 6) or metergoline (total dose: 1.0 mg·kg^{-1}; n = 6). Ketanserin, methiothepin and metergoline were infused into the carotid artery at a rate of 1 ml·min^{-1} for 10 min. Abbreviations and units: MAP, mean arterial blood pressure (mm Hg); HR, heart rate (beats·min^{-1}); CBF, carotid blood flow (ml·min^{-1}) measured by electromagnetic flow probe.

	Antagonist treatment	Baseline value	Immediately after antagonist	10 min after antagonist
MAP	None	92± 6	92± 6	96± 7
	Ketanserin	100± 6	90± 6 [a]	86± 5 [a]
	Methiothepin	90±11	83±10	83±11
	Metergoline	91±10	87± 9	89± 7
HR	None	101± 6	101± 6	102± 6
	Ketanserin	107± 6	101± 6 [a]	98± 6 [a]
	Methiothepin	85± 8	82± 8	82± 8
	Metergoline	86± 6	80± 6	83± 4
CBF	None	127±13	127±13	128±13
	Ketanserin	118±11	128± 7	134± 9 [a]
	Methiothepin	93±15	102±14 [a]	98±13
	Metergoline	159±20	176±23 [a]	162±20

[a] Significantly different ($P < 0.05$) from the baseline value.

Mean arterial blood pressure and heart rate recorded during the intracarotid infusions of indorenate are summarized in table 2. Only a small decrease in mean arterial blood pressure was observed with indorenate in metergoline-treated animals. Heart rate decreased during the intracarotid infusion of increasing doses of indorenate in all four groups.

3.2. Carotid haemodynamics

3.2.1. Distribution between arteriovenous anastomotic and arteriolar flow

The effects of intracarotid infusions of indorenate on the total common carotid blood flow and its distribution between arteriovenous anastomotic (non-nutrient) and arteriolar (nutrient) flow are shown in fig. 1. A large fraction of the total carotid blood flow was diverted through cephalic arteriovenous anastomoses in the untreated as well as in the ketanserin-, methiothepin- or metergoline-treated animals before infusion of indorenate. In the untreated animals, indorenate induced a dose-dependent decrease in total common carotid blood flow. This effect was directly related to the decrease in arteriovenous anastomotic blood flow, since arteriolar blood flow remained practically unchanged at all doses tested (no change in the skin colour was observed). The effects of indorenate on total common carotid and arteriovenous anastomotic blood flow appeared to have been slightly attenuated in the ketanserin-treated animals (total dose: 0.5 mg·kg^{-1}) and, at the highest dose of indorenate, a small increase in

TABLE 2

Effect of intracarotid infusions of indorenate on mean arterial blood pressure and heart rate in untreated control animals and in animals treated with ketanserin (0.5 mg·kg^{-1}; n = 6), methiothepin (1.0 mg·kg^{-1}; n = 6) or metergoline (1.0 mg·kg^{-1}; n = 6). Indorenate was infused in 10 min periods at a rate of 1.0 ml·min^{-1}. Abbreviations and units: MAP, mean arterial blood pressure (mm Hg); HR, heart rate (beats·min^{-1}).

	Antagonist treatment	Baseline value [a]	Indorenate (μg·kg^{-1}·min^{-1})			
			0.3	1.0	3.0	10.0
MAP	None	96± 7	92± 4	88±4	90±5	92±5
	Ketanserin	86± 5	86± 4	84±3	86±2	88±3
	Methiothepin	83±11	84±10	83±8	78±7	77±8
	Metergoline	89± 7	83± 6	79±6 [b]	77±6 [b]	75±6 [b]
HR	None	102± 6	97± 6 [b]	94±6 [b]	90±6 [b]	87±5 [b]
	Ketanserin	98± 6	96± 6	94±6	91±6 [b]	90±6 [b]
	Methiothepin	82± 8	80± 7	77±6 [b]	75±6 [b]	72±6 [b]
	Metergoline	83± 4	79± 5	79±6	77±6 [b]	74±6 [b]

[a] After antagonist treatment; [b] Significantly different ($P < 0.05$) from the baseline value.

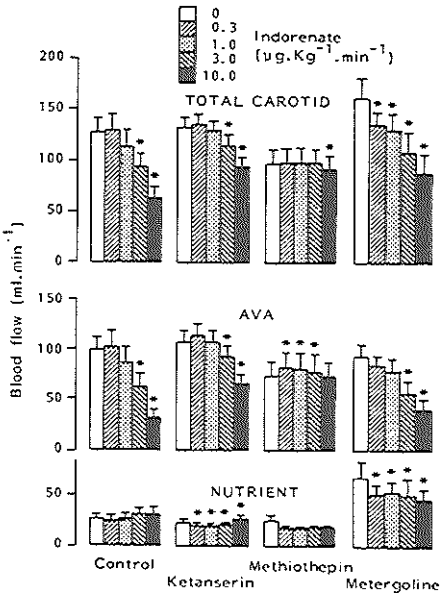

Fig. 1. Effects of intracarotid infusions of indorenate (0.3, 1.0, 3.0 and 10.0 $\mu g \cdot kg^{-1} \cdot min^{-1}$) on total carotid blood flow and its distribution into arteriovenous anastomotic (AVA) and arteriolar (nutrient) flow in untreated (control) and ketanserin (0.5 $mg \cdot kg^{-1}$ i.a.)-, methiothepin (1 $mg \cdot kg^{-1}$ i.a.)- or metergoline (1.0 $mg \cdot kg^{-1}$, i.a.)-treated pigs (n = 6 each). * Significantly different ($P < 0.05$) from the baseline value.

arteriolar blood flow was noticed. In contrast, in the animals treated with methiothepin (total dose: 1.0 $mg \cdot kg^{-1}$), indorenate caused no or a small (highest dose) decrease in total carotid blood flow, a slight increase in arteriovenous anastomotic blood flow and no change in arteriolar blood flow. In the animals treated with metergoline (total dose: 1.0 $mg \cdot kg^{-1}$), the effects of indorenate on total common carotid and arteriovenous anastomotic blood flow remained essentially unchanged. The total arteriolar blood flow was higher in the metergoline-treated animals and, in these animals, infusion of indorenate apparently decreased the arteriolar blood flow.

The effects of intracarotid infusions of indorenate on vascular conductance, approximated by the ratio of blood flow and mean arterial blood pressure, are shown in fig. 2. The effects of indorenate were essentially similar to its effects on blood flow.

Figure 3 presents the percentage changes induced by different doses of indorenate in the total carotid vascular conductance and its arteriovenous anastomotic and arteriolar components in the four groups of animals. The decrease in the total carotid conductance by indorenate was comparable in ketanserin- and metergoline-treated animals, but was markedly inhibited by methiothepin. The decrease in the arteriovenous anastomotic flow induced by the highest dose of indorenate was

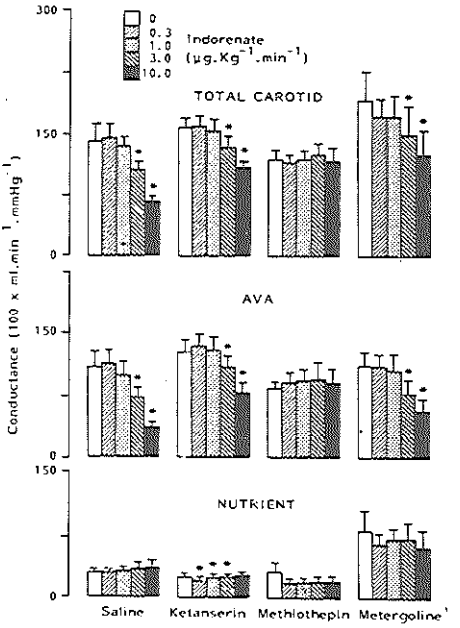

Fig. 2. Effects of intracarotid infusions of indorenate (0.3, 1.0, 3.0, 10.0 $\mu g \cdot kg^{-1} \cdot min^{-1}$) on the total carotid conductance and arteriovenous anastomotic (AVA) and arteriolar (nutrient) flow in untreated (control) and ketanserin (0.5 $mg \cdot kg^{-1}$ i.a.)-, methiothepin (1 $mg \cdot kg^{-1}$ i.a.)- or metergoline (1.0 $mg \cdot kg^{-1}$ i.a.)-treated pigs (n = 6 each). * Significantly different ($P < 0.05$) from the baseline value.

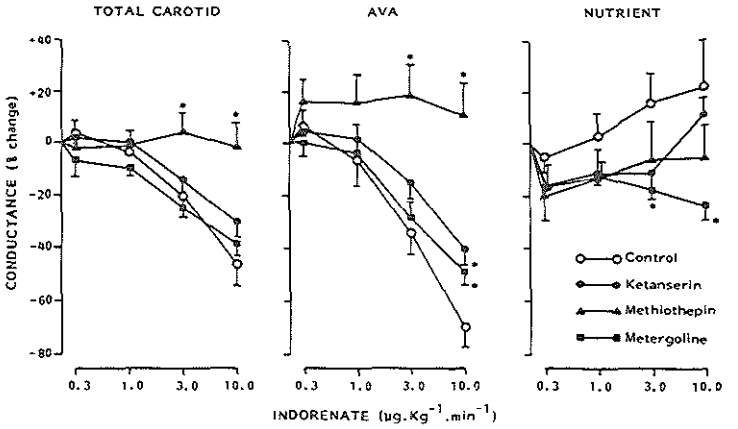

Fig. 3. Percent changes induced by intracarotid infusions of indorenate (0.3, 1.0, 3.0, 10.0 $\mu g \cdot kg^{-1} \cdot min^{-1}$) in the total carotid conductance and arteriovenous anastomotic (AVA) and arteriolar (nutrient) flow in untreated (control) and ketanserin (0.5 $mg \cdot kg^{-1}$ i.a.)-, methiothepin (1 $mg \cdot kg^{-1}$ i.a.)- or metergoline (1.0 $mg \cdot kg^{-1}$ i.a.)-treated pigs (n = 6 each). * $P < 0.05$ vs. changes in the control group.

slightly inhibited by ketanserin and metergoline. Methiothepin, on the other hand, potently antagonized the effects of indorenate on arteriovenous anastomotic blood flow. The arteriolar (nutrient) fraction of the carotid blood flow tended to increase after indorenate in the untreated control animals ($P > 0.05$), whereas it decreased in the metergoline-treated animals. This decrease, however, may be related to the fact that arteriolar flow was conspicuously higher in the metergoline-treated animals than in the other groups (see fig. 1).

3.2.2. Distribution of arteriolar blood flow between the different tissues

The effect of indorenate on regional carotid blood flow distribution into the different tissues, presented in fig. 4 (ear, skin, muscles and tongue) and fig. 5 (bones, eyes, brain, fat and salivary glands), were rather small in all four groups of animals. In the untreated control animals, indorenate increased blood flow to the ears (highest dose) and slightly decreased flow to the eyes (two highest doses). In the ketanserin-treated animals, blood flow to the skin, tongue and brain increased at the highest does of indorenate; the drug, however, slightly decreased blood flow to the ears, muscles and bones. Finally, after treatment with metergoline, the overall arteriolar blood flow was higher than in the other groups before infusion of indorenate. Indorenate apparently induced a small decrease in blood flow to the muscles, tongue, bones and eyes, but increased flow to the skin.

4. Discussion

4.1. Systemic haemodynamic changes

In contrast to its effect in anaesthetized cats (Hong et al., 1978) as well as renal hypertensive dogs (Hong, 1981) and rats (Safdy et al., 1982), indorenate did not lower blood pressure in the anaesthetized pigs, except for a small decrease in animals that had been treated with metergoline. 8-OH-DPAT also does not lower blood pressure in anaesthetized pigs (Bom et al., 1989a). It is possible that the central 5-HT_{1A} receptors mediating hypotensive responses (see Saxena and Villalón, 1990) are not present in the pig. How-

ever, it cannot be ruled out that the absence of a clear hypotensive response to 5-HT_{1A} receptor stimulation is due to the use of deep anaesthesia in our experiments. The decrease in heart rate observed by us is probably not, or only partly, attributable to indorenate, since a modest decrease in heart rate has also been noticed during the course of experiments in which only physiological saline was infused; the heart rate (beats · min^{-1}) was 109 ± 13 at baseline and 109 ± 12, 105 ± 12, 102 ± 11 and 99 ± 10 after the four consecutive infusions of physiological saline (Bom et al., 1989a).

4.2. Carotid haemodynamic changes

It has already been shown that carotid blood flow and its distribution remains unchanged when measurements are made with microspheres injected into anaesthetized pigs receiving four consecutive infusions of physiological saline for a period of 10 min each (Bom et al., 1989a). Therefore, the major findings in this study are: (i) indorenate caused dose-related decreases in the total carotid blood flow and conductance, which were selective for arteriovenous anastomotic flow as arteriolar (nutrient) flow was not significantly modified, and (ii) the responses to indorenate were only slightly attenuated by ketanserin or metergoline, but were potently antagonized by methiothepin. Apart from the implications discussed below, these findings show that indorenate acts as an agonist mainly at 5-HT_1-like receptors which mediate the constriction of arteriovenous anastomoses. However, as described previously for 5-HT (Saxena and Verdouw, 1982; Verdouw et al., 1984), we cannot completely exclude the possibil-

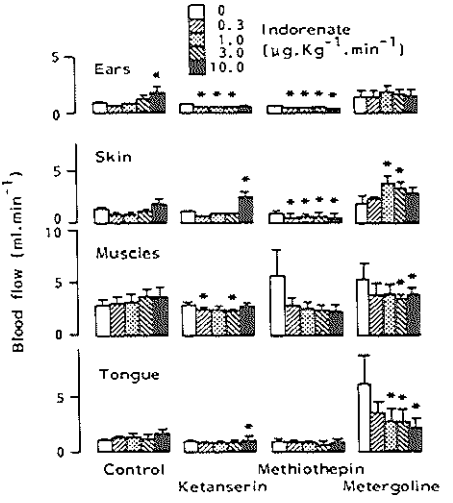

Fig. 4. Effect of intracarotid infusions of indorenate (0.3, 1.0, 3.0 and 10.0 $\mu g \cdot kg^{-1} \cdot min^{-1}$) on the fraction of carotid blood flow distributed to the ears, head skin, head muscles and tongue in untreated (control) and ketanserin (0.5 $mg \cdot kg^{-1}$ i.a.)-, methiothepin (1 $mg \cdot kg^{-1}$ i.a.)- or metergoline (1.0 $mg \cdot kg^{-1}$ i.a.)-treated pigs ($n = 6$ each). * Significantly different ($P < 0.05$) from the baseline value.

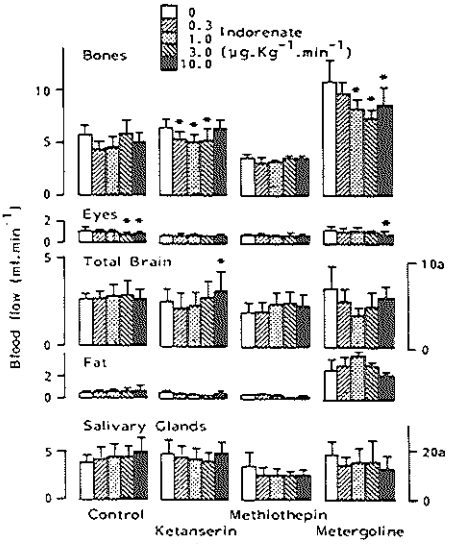

Fig. 5. Effect of intracarotid infusions of indorenate (0.3, 1.0, 3.0 and 10.0 $\mu g \cdot kg^{-1} \cdot min^{-1}$) on the fraction of carotid blood flow distributed to the bones, eyes, total brain, fat and salivary glands in untreated (control) and ketanserin (0.5 $mg \cdot kg^{-1}$ i.a.)-, methiothepin (1 $mg \cdot kg^{-1}$ i.a.)- or metergoline (1.0 $mg \cdot kg^{-1}$ i.a.)-treated pigs ($n = 6$ each). [a] Scale for the metergoline group. * Significantly different ($P < 0.05$) from the baseline value.

ity that 5-HT_2 receptors might also be involved to a lesser extent, or that ketanserin has some affinity for these 5-HT_1-like receptors. Indeed, this conclusion is also supported by the affinity profile of indorenate, which has pK_D values of 7.80, 5.39, 6.49, 6.69 and < 5.0, respectively, for 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, 5-HT_{1D} and 5-HT_2 binding sites (Hoyer et al., 1985; Hoyer, personal communication).

4.2.1. Heterogeneity of the 5-HT_1-like receptors in the carotid circulation

We have demonstrated that, in the porcine carotid vascular bed, 5-HT (Saxena and Verdouw, 1982) and 5-carboxamidotryptamine (Saxena and Verdouw, 1985) decrease arteriovenous anastomotic blood flow, but increase arteriolar blood flow, particularly to the skin, which becomes red. Both the constriction of arteriovenous anastomoses and the dilatation of arterioles are mediated by 5-HT_1-like receptors (Saxena et al., 1986). However, these 5-HT_1-like receptors seem to be heterogeneous (Saxena et al., 1989) because several other 5-HT-related drugs – methysergide (Saxena and Verdouw, 1984), BEA 1654 (Verdouw et al., 1985), sumatriptan (GR43175) (Feniuk et al., 1987; 1989; Den Boer et al., 1990), 8-OH-DPAT (Bom et al., 1989a) and RU 24969 (Bom et al., 1989b) – reduce arteriovenous anastomotic blood flow without increasing arteriolar blood flow or changing skin colour. The selective arteriovenous anastomotic response to indorenate further strengthens the concept of heterogeneous 5-HT_1-like receptors within the porcine carotid circulation.

4.2.2. Are the 5-HT_1-like receptors mediating cephalic arteriovenous anastomotic constriction related to the known 5-HT_1 binding sites subtypes?

It is now well recognized that 5-HT_1-like receptors are heterogeneous and four different subtypes of 5-HT_1 binding sites (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} and 5-HT_{1D}) have been described (for recent reviews, see Saxena, 1989; Feniuk and Humphrey, 1990). Although we acknowledge that, except for the 5-HT_{1A} binding site, no selective agents are available for the other subtypes, we still considered it important to attempt to establish the nature of the 5-HT_1-like receptors mediating constriction of the cephalic arteriovenous anastomoses. In this connection, a number of drugs with high affinity for 5-HT_{1A} binding sites (see Middlemiss and Fozard, 1983; Hoyer et al., 1985; Hoyer, 1988), namely 8-OH-DPAT (Bom et al., 1989a), BEA 1654 (Verdouw et al., 1985), methysergide (Saxena and Verdouw, 1984), RU 24969 (Bom et al., 1989b) and indorenate (present results), decrease porcine arteriovenous anastomotic blood flow, which suggests that the 5-HT_1-like receptors involved might correspond to 5-HT_{1A} binding sites. However, this seems unlikely because ipsapirone (Dompert et al., 1985; Hoyer et al., 1985) and some other 5-HT_{1A} ligands (unpublished observations) are inactive in the pig carotid circulation (Bom et al., 1988), and the reduction of carotid and arteriovenous anastomotic blood flow induced by RU 24969 is not affected by a high dose of (±)-pindolol (Bom et al., 1989b); both these compounds display high affinity for 5-HT_{1A} and 5-HT_{1B} binding sites (Hoyer et al., 1985; Hoyer, 1988). The present observation that the indorenate-induced decrease in both total common carotid and arteriovenous anastomotic blood flow was not markedly affected by metergoline (pK_D values: 8.2, 7.6, 9.3 and 9.1 for 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} and 5-HT_{1D} binding sites, respectively; Hoyer et al., 1985; Hoyer, 1988) further reveals that the 5-HT_1-like receptors involved cannot be related to either 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} or 5-HT_{1D} subtypes. It should be realized that metergoline has higher affinity for these subtypes than methiothepin (pK_D values: 7.1, 7.3, 7.6 and 6.3 for 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} and 5-HT_{1D} binding sites, respectively; Hoyer et al., 1985; Hoyer, 1988). The inactivity of metergoline is probably not related to differences in pharmacokinetics since, at the 5-HT_1-like receptor on the dog saphenous vein (Humphrey et al., 1988; 1990), the responses to 5-HT, 8-OH-DPAT and/or sumatriptan are antagonized by methiothepin but not by metergoline.

It should be noted that the *percentage* of carotid blood flow distributed to the arteriovenous anastomoses at baseline is less in the metergoline-treated group than in the other groups (data not presented, but can be deduced from fig. 1). It is

not very likely that this difference in baseline values will affect the comparison between the effects of indorenate in the saline-treated and metergoline-treated animals. The absolute arteriovenous anastomotic blood flow in the different groups was similar, and despite higher absolute values of total and arteriolar blood flow in the metergoline group, indorenate caused more prominent reductions in these variables (fig. 1).

4.3. Possible clinical implications

Lastly, we would like to consider the possible clinical relevance of the constriction of the arteriovenous anastomoses induced by indorenate. It has been suggested that dilatation of the arteriovenous anastomoses probably plays a role in the pathophysiology of migraine (Heyck, 1969; Saxena, 1987; Saxena and Ferrari, 1989). Indeed, a number of effective antimigraine drugs, including ergotamine, methysergide and isometheptene (Johnston and Saxena, 1978; Spierings and Saxena, 1980a,b; Saxena and Verdouw, 1984; Saxena, 1987), constrict arteriovenous anastomoses. In addition, sumatriptan, a newly developed agonist at 5-HT_1-like receptors (Humphrey et al., 1988; 1990), selectively reduces carotid and arteriovenous anastomotic blood flow in cats, dogs and pigs (Feniuk et al., 1987; 1989; Den Boer et al., 1990) and has shown considerable potential in the treatment of acute attacks of migraine (Doenicke et al., 1988; Perrin et al., 1989). It is therefore suggested that indorenate may also have an antimigraine action.

In conclusion, the present study demonstrates that indorenate, which has high affinity for 5-HT_{1A} binding sites, and to a lesser degree also for the 5-HT_{1C} and 5-HT_{1D} binding sites, decreases common carotid arterial blood flow in the pig by selectively reducing arteriovenous anastomotic blood flow. This effect of indorenate, which is highly susceptible to methiothepin, but much less susceptible to ketanserin or metergoline, is mediated by a subtype of 5-HT_1-like receptors that does not seem to correspond to either 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} or 5-HT_{1D} subtypes.

References

Bom, A.H., P.R. Saxena and P.D. Verdouw, 1988, Further characterization of the 5-HT_1-like receptors in the carotid circulation of the pig, Br. J. Pharmacol. 94, 327P.

Bom, A.H., P.R. Saxena and P.D. Verdouw, 1989a, Carotid haemodynamics in pigs during infusions of 8-OH-DPAT: Reduction in arteriovenous shunting is mediated by 5-HT_1-like receptors, Br. J. Pharmacol. 96, 125.

Bom, A.H., C.M. Villalón, P.D. Verdouw and P.R. Saxena, 1989b, The 5-HT_1-like receptor mediating reduction of porcine carotid arteriovenous shunting by RU 24969 is not related to either the 5-HT_{1A} or the 5-HT_{1B} subtype, European J. Pharmacol. 171, 87.

Bradley, P.B., G. Engel, W. Feniuk, J.R. Fozard, P.P.A. Humphrey, D.N. Middlemiss, E.J. Mylecharane, B.P. Richardson and P.R. Saxena, 1986, Proposals for the classification and nomenclature of functional receptors for 5-hydroxytryptamine, Neuropharmacology 25, 563.

Den Boer, M.O., C.M. Villalón, J.P.C. Heiligers, P.R. Saxena and P.P.A. Humphrey, 1990, The craniovascular effects of sumatriptan, Br. J. Pharmacol. 99, 37P.

Doenicke, A., J. Brand and V.L. Perrin, 1988, Possible benefit of GR43175, a novel 5-HT_1-like receptor agonist for the treatment of severe migraine, Lancet 1, 1309.

Dompert, W.V., T. Glaser and J. Traber, 1985, ^{3}H-TVX Q 7821: Identification of 5-HT_1 binding sites as target for a novel putative anxiolytic, Naunyn-Schmiedeb. Arch Pharmacol. 328, 467.

Feniuk, W. and P.P.A. Humphrey, 1990, Putative agonists and antagonists at 5-HT_1-like receptors, in: Cardiovascular Pharmacology of 5-Hydroxytryptamine: Prospective Therapeutic Applications, eds. P.R. Saxena, D.I. Wallis, W. Wouters and P. Bevan (Kluwer Academic Publishers, Dordrecht) p. 69.

Feniuk, W., P.P.A. Humphrey and M.J. Perren, 1987, Selective vasoconstrictor action of GR 43175 on arteriovenous anastomoses (AVAs) in the anaesthetized cat, Br. J. Pharmacol. 92, 756P.

Feniuk, W., P.P.A. Humphrey and M.J. Perren, 1989, The selective carotid arterial vasoconstrictor action of GR43175 in anaesthetized dogs, Br. J. Pharmacol. 96, 83.

Heyck, H., 1969, Pathogenesis of migraine, Res. Clin. Stud. Headache 2, 1.

Hong, E., 1981, A serotonergic antihypertensive agent, in: Molecular Basis of Drug Action, eds. T.P. Singer and R.N. Ondarza (Elsevier/North Holland, Amsterdam) p. 247.

Hong, E., P. Nava-Félix and H. Vidrio, 1978, On the central antihypertensive effect of a new tryptamine derivative, Pharmacologist 20, 188.

Hoyer, D., 1988, Functional correlates of serotonin 5-HT_1 recognition sites, J. Rec. Res. 8, 59.

Hoyer, D., G. Engel and H.O. Kalkman, 1985, Molecular pharmacology of 5-HT_1 and 5-HT_2 recognition sites in rat and pig brain membranes, Radioligand binding studies

with [^{3}H]5-HT, [^{3}H]8-OH-DPAT, (−)[^{125}I]iodocyanopindolol, [^{3}H]mesulergine and [^{3}H]ketanserin. European J. Pharmacol. 118, 13.

Humphrey, P.P.A., E. Apperley, W. Feniuk and M.J. Perren, 1990. A rational approach to identifying a fundamentally new drug for the treatment of migraine, in: Cardiovascular Pharmacology of 5-Hydroxytryptamine: Prospective Therapeutic Applications, eds. P.R. Saxena, D.I. Wallis, W. Wouters and P. Bevan (Kluwer Academic Publishers, Dordrecht) p. 417.

Humphrey, P.P.A., W. Feniuk, M.J. Perren, H. Connor, A.W. Oxford, I.H. Coates and D. Butina, 1988. GR 43175, a selective agonist for the 5-HT$_1$-like receptor in the dog isolated saphenous vein. Br. J. Pharmacol. 94, 1123.

Johnston, B.M. and P.R. Saxena, 1978. The effect of ergotamine on tissue blood flow and the arteriovenous shunting of radioactive microspheres in the head. Br. J. Pharmacol. 63, 541.

Middlemiss, D. and J.R. Fozard, 1983. 8-Hydroxy-2-(di-n-propylamino)-tetralin discriminates between subtypes of the 5-HT$_1$ recognition site. European J. Pharmacol. 90, 151.

Perrin, V.L., M. Fürkkilä, J. Goasguen, A. Doenicke, J. Brand and P. Tfelt-Hansen, 1989. Overview of initial clinical studies with intravenous and oral GR43175 in acute migraine. Cephalalgia 9 (Suppl. 9), 63.

Safdy, M.E., E. Kurchacova, R.N. Schut, H. Vidrio and E. Hong, 1982. Tryptophan analogues. 1. Synthesis and antihypertensive activity of positional isomers. J. Med. Chem. 25, 723.

Saxena, P.R., 1987. The arteriovenous anastomoses and veins in migraine research, in: Migraine Therapeutic, Conceptual and Research Aspects, ed. J.N. Blau (Elsevier, Amsterdam) p. 581.

Saxena, P.R., 1989. Cardiovascular effects from stimulation of 5-hydroxytryptamine receptors. Fund. Clin. Pharmacol. 3, 245.

Saxena, P.R., A.H. Bom and P.D. Verdouw, 1989. Characterization of 5-hydroxytryptamine receptors in the cranial vasculature. Cephalalgia 9 (Suppl. 9), 15.

Saxena, P.R., D.J. Duncker, A.H. Bom, J. Heiligers and P.D. Verdouw, 1986. Effects of MDL 72222 and methiothepin on carotid vascular responses to 5-hydroxytryptamine in the pig: Evidence for the presence of '5-hydroxytryptamine$_1$-like' receptors. Naunyn-Schmiedeb. Arch. Pharmacol. 333, 198.

Saxena, P.R. and M.D. Ferrari, 1989. 5-HT$_1$-like receptor agonists and the pathophysiology of migraine. Trends Pharmacol. Sci. 10, 200.

Saxena, P.R., H.C. Schamhardt, R.P. Forsyth and J. Loeve, 1980. Computer programs for the radioactive microsphere technique. Determination of regional blood flows and other haemodynamic variable in different experimental circumstances. Comp. Program Biomed. 12, 63.

Saxena, P.R. and P.D. Verdouw, 1982. Redistribution by 5-hydroxytryptamine of arterial carotid blood at the expense of arteriovenous blood flow. J. Physiol. 332, 501.

Saxena, P.R. and P.D. Verdouw, 1984. Effects of methysergide and 5-hydroxytryptamine on carotid blood flow distribution in pigs: Further evidence for the presence of atypical 5-HT receptors. Br. J. Pharmacol. 82, 817.

Saxena, P.R. and P.D. Verdouw, 1985. 5-Carboxamidotryptamine, a compound with high affinity for 5-hydroxytryptamine$_1$ binding sites, dilates arterioles and constricts arteriovenous anastomoses. Br. J. Pharmacol. 84, 533.

Saxena, P.R. and C.M. Villalón, 1990. 5-HT$_{1A}$ receptor agonism as a novel mechanism for antihypertensive action. Trends Pharmacol. Sci. 11, 95.

Spierings, E.L.H. and P.R. Saxena, 1980a. Effect of isometheptene on the distribution and shunting of 15 μm microspheres throughout the cephalic circulation of the cat. Headache 20, 103.

Spierings, E.L.H. and P.R. Saxena, 1980b. Antimigraine drugs and cranial arteriovenous shunting in the cat. Neurology (Minn.) 30, 696.

Steel, R.G.D. and J.H. Torrie, 1980. Principles and Procedures of Statistics, a Biomedical Approach, 2nd edn. (McGraw-Hill Kogakusha, Ltd., Tokyo).

Verdouw, P.D., H.M. Jennewein, J. Heiligers, D.J. Duncker and P.R. Saxena, 1984. Redistribution of carotid artery blood flow by 5-HT: Effects of the 5-HT$_2$ receptor antagonists ketanserin and WAL 1307. European J. Pharmacol. 102, 499.

Verdouw, P.D., H.M. Jennewein, J. Mierau and P.R. Saxena, 1985. N-(3-Acetylaminophenyl)piperazine hydrochloride (BEA 1654), a putative 5-HT$_1$ agonist, causes constriction of arteriovenous anastomoses and dilatation of arterioles. European J. Pharmacol. 107, 337.

Villalón, C.M., A.H. Bom, J. Heiligers, M.O. Den Boer and P.R. Saxena, 1990. Reduction of porcine carotid arteriovenous shunting by indorenate is unrelated to 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1C}$ or 5-HT$_{1D}$ receptor subtype. Br. J. Pharmacol. 99, 36P.

CHAPTER 11

EFFECTS OF S9977 AND DIHYDROERGOTAMINE IN AN EXPERIMENTAL MODEL FOR MIGRAINE

Villalón, C.M., Bom, A.H., den Boer, M.O., Heiligers, J.P.C. and Saxena, P.R.
Pharmacol. Res., In Press.

EFFECTS OF S9977 AND DIHYDROERGOTAMINE IN AN ANIMAL EXPERIMENTAL MODEL FOR MIGRAINE

SUMMARY

The present study concerns the effects of S9977, a trimethylxanthine derivative with potential antimigraine characteristics, on the distribution of carotid blood flow in the anaesthetized pig. Furthermore, the effects of dihydroergotamine have been analyzed for comparison. Dihydroergotamine (3, 10, 30 and 100 μg/kg, i.v.) elicited dose-dependent pressor and bradycardic responses which were probably mediated by its partial agonist action on α-adrenoceptors and dopamine$_2$ receptors. In contrast, S9977 (0.3, 1, 3 and 10 mg/kg, i.v.) caused a moderate hypotension and bradycardia. The carotid haemodynamic effects of dihydroergotamine (3, 10, 30 and 100 μg/kg, i.v.) consisted of a dose-dependent reduction of arteriovenous anastomotic blood flow and conductance and an increase in nutrient (tissue) blood flow and conductance. As a consequence, jugular venous pO_2 decreased. These findings, demonstrating an active constriction of arteriovenous anastomoses, are in agreement with earlier findings in cats. Though S9977 (0.3, 1, 3 and 10 mg/kg, i.v.) decreased carotid (two highest doses) and arteriovenous anastomotic (highest dose) blood flow, there was no concomitant decrease in the vascular conductances. Therefore, the effects of S9977 seem to be related to a decrease in arterial blood pressure and not to an active vasoconstriction of arteriovenous anastomoses. These results are discussed in terms of the potential therapeutical usefulness of S9977 in the treatment of migraine.

INTRODUCTION

Migraine is a vascular headache but its pathophysiology is complex and multifactorial. Of the many factors that have been implicated in migraine, the changes in *cerebral* as well as *non-cerebral cranial circulation* and the decrease in blood 5-hydroxytryptamine (5-HT) concentrations have been best documented (1). In some migraine patients a zone of slowly spreading reduced cerebral blood flow appears during the so-called aura phase (2). In the non-cerebral cranial circulation, vasodilatation and increased pulsations have been reported on the side affected during the headache phase of migraine (1). The idea of simple vasodilatation in the non-cerebral cranial circulation is, however, in direct contrast to the facial pallor noticed during headache in the majority of migraine patients. Heyck (3) therefore measured the O_2 content in the arterial and jugular venous blood and reported that the arterio-jugular venous oxygen difference decreased on the side of the migraine headache and this difference normalized after spontaneous or drug-induced alleviation of the headache. The vascular and biochemical changes in migraine are

supported by pharmacological experiments showing that 5-HT (4) and a number of proven and effective antimigraine drugs (ergotamine, dihydroergotamine, methysergide, isometheptene) constrict arteriovenous anastomoses to decrease arteriovenous shunting (5-6). More recently, a newly developed agonist at one of the subtypes of 5-HT_1-like receptors (GR43175; sumatriptan) contracts large cranial arteries, reduces arteriovenous shunting and seems to be highly effective in aborting acute attacks of migraine (7-10).

Taken together, the above findings suggest that migraine headaches may be, at least partly, due to a sudden opening of non-cerebral cranial arteriovenous anastomoses, which leads to an increase in arteriovenous shunting and arterial pulsations, and a decrease in diastolic arterial pressure and tissue blood flow (3,5,6). The dilatation of cranial arteries and arteriovenous anastomoses and the resulting ischaemia and oedema may also initiate a nociceptive reflex (7).

Apart from the use of 5-HT_1-like receptor agonists, a new approach for decreasing arteriovenous anastomoses shunting may be via antagonism towards dopamine and/or purinergic mechanisms. In this context, there is evidence that arteriovenous anastomoses are innervated by both dopaminergic (11,12) and purinergic (13) neurones which, upon stimulation, appear to cause a selective increase in arteriovenous anastomotic blood flow. Moreover, it is interesting to remark that hypersensitivity to dopamine-like drugs has been reported in migraine patients (14,15). Therefore, it was proposed to investigate the effects of a novel trimethylxanthine derivative S9977 (Figure 1), which antagonizes the dopaminergic hypersensitivity and increase in adenosine receptors induced by food sensitization in the rat (16,17), on the fractionation of carotid arterial blood into arteriovenous anastomotic and nutrient parts in the anaesthetized pigs. This animal experimental model has shown that a number of proven antimigraine drugs are effective in reducing arteriovenous anastomotic flow (4-7, 10) and, therefore, for comparison we have also included dihydroergotamine in our investigation. A part of this study has been presented recently at the XI International Congress of Pharmacology, Amsterdam (18).

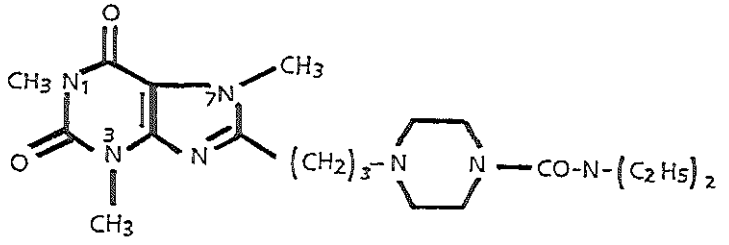

Figure 1. Chemical structure of S9977.

METHODS

Experimental set-up

After an overnight fast, young Yorkshire pigs (body weight: 18-25 Kg; n = 24) were sedated with 120 mg (i.m.) azaperone and 120-150 mg (i.v.) metomidate, intubated and connected to a respirator for intermittent positive pressure ventilation with a mixture (1:2) of oxygen and nitrous oxide. A continuous infusion of pentobarbitone sodium (20 mg/kg.h, i.v.) completed the anaesthesia. Respiratory rate and tidal volume were adjusted to keep arterial blood gases and pH within normal limits (pH, 7.35-7.45; pO_2, 90-150 mmHg; pCO_2, 35-45 mmHg). The right femoral artery and vein were cannulated for the measurement of aortic blood pressure (Statham transducer, model P23 ID) and the administration of drugs, respectively. Heart rate was derived from the electrocardiogram signals. The common carotid arteries were dissected free and a bilateral cervical vagosympathectomy was performed to reduce reflex influences on the carotid circulation. Blood flow in one of the common carotid arteries was measured with a precalibrated flow probe (2.5 or 3 mm internal diameter) connected to a sine-wave electromagnetic blood flow meter (Transflow 601 system, Skalar, Delft, The Netherlands). After removing its hub, a 0.5 mm (external diameter) needle, connected to a suitable polyethylene tubing, was inserted into this common carotid artery for the administration of radioactive microspheres (see later). The animals were wrapped in a thermo-insulating blanket to maintain body temperature between 37-38°C.

Distribution of common carotid blood flow

The distribution of common carotid blood flow was determined with the radioactive microsphere method using 15 ± 1 (s.d.) μm diameter spheres labelled with either ^{141}Ce, ^{113}Sn, ^{103}Ru, ^{95}Nb or ^{46}Sc (NEN Company, Dreieich, F.R.G.). For each measurement a suspension of microspheres, labelled with one of the nuclides, was mechanically agitated and injected against the direction of the carotid blood flow to facilitate uniform mixing with the blood. At the end of the experiment the animal was killed and the heart, kidneys, lungs and the various tissues of the head were dissected out, weighed and placed in vials. The radioactivity in these vials was counted for 5-10 min in a gamma-scintillation counter (Packard Minaxi Autogamma 5000) using suitable windows for discriminating the different isotopes. The amount of carotid blood flow distributed to the individual tissues ($Q_{tis[car]}$) was calculated as described earlier (4,19) by: $Q_{tis[car]}(ml/min) = (I_{tis}/I_{tot}) x Q_{[car]}$,

where I_{tis} and I_{tot} are, respectively, the radioactivity (c.p.m.) in a particular tissue and that detected in all tissues, and $Q_{[car]}$ is carotid blood flow (ml/min). Because there was a

complete entrapment of the microspheres passing through the cranial circulation in the capillaries of the lungs (no significant amounts of radioactivity were detected in the heart or kidneys), the values determined for the lungs provide an index of the arteriovenous anastomotic fraction of the common carotid blood flow (4,20).

Experimental protocol

Three series of experiments (n=8 each) were performed. In the first series the effects of 4 consecutive intracarotid infusions of physiological saline on carotid blood flow and its distribution were determined. In the following series, the effects of i.v. infusions of dihydroergotamine (3, 10, 30 and 100 μg/kg) or S9977 (0.3, 1.0, 3.0 and 10.0 mg/kg) in cumulative doses at intervals of 15 min were assessed; the drugs were administered at a rate of 5 ml/min during a period of 2 min, whereas the physiological saline was infused at a rate of 1 ml/min during a period of 10 min. In all experiments the baseline values were determined after the preparation had been stable for at least 45 min after completion of the surgical procedures. The measurements consisted of the recording of the electrocardiogram, arterial blood pressure and common carotid blood flow, while a batch of microspheres (100,000 - 150,000) was injected for the determination of tissue (arteriolar) and arteriovenous anastomotic blood flows. Subsequently, these measurements were repeated about 15 min after the i.v. infusion of each dose of S9977 or dihydroergotamine.

Statistical analysis

The significance of the differences between variables was evaluated by Duncan's new multiple range test, once an analysis of variance (randomized block design) had revealed that the samples represented different populations (21). Statistical significance was accepted at $p<0.05$ (two tailed). All data are expressed as means ± s.e. mean.

Drugs

Apart from the anaesthetics, the drugs used in this study were: 1,3,7-trimethyl-8-[3-(4-diethylaminocarbonylpiperazino)propyl]xanthine hydrochloride (S9977-2; Servier, Paris) and dihydroergotamine mesylate (Dihydergot^{R}, Sandoz, Basel). The doses of the drugs mentioned in the text refer to the respective salts.

RESULTS

Arterial and jugular venous blood gases

Baseline values of arterial and jugular venous blood gases are shown in Table 1. None of these variables showed any significant change during the course of the experiment in the saline-treated animals. Except the jugular venous pO_2, this was also true for the animals treated with dihydroergotamine and S9977. Jugular venous pO_2 decreased significantly with all four doses of dihydroergotamine (from 77±3 to, respectively, 69±3, 58±2, 58±3 and 54±6 mmHg) and with the two highest of the four doses of S9977 (from 66±3 to, respectively, 63±3, 62±4, 57±4 and 53±3 mmHg). These changes appear to correspond with the decrease in the arteriovenous anastomotic part of the carotid blood flow (see below).

TABLE 1. Baseline values of arterial and jugular venous blood gases in pigs subsequently treated with physiological saline, dihydroergotamine (DHE) or S9977 (n=8 each).

	Saline	DHE	S9977
Arterial pH	7.4±0.0	7.4±0.0	7.4±0.0
Arterial pCO_2 (mmHg)	38±1	38±1	35±2
Arterial pO_2 (mmHg)	171±11	188±7	178±10
Arterial Hb (g %)	8.5±0.2	7.8±0.2	7.4±0.3
Venous pH	7.4±0.0	7.4±0.0	7.4±0.0
Venous pCO_2 (mmHg)	39±1	40±3	38±2
Venous pO_2 (mmHg)	79±8	77±3	66±3
Venous Hb (g %)	7.9±0.3	7.8±0.2	7.6±0.4

Systemic haemodynamic variables

As shown in Figure 2, both mean arterial blood pressure and heart rate gradually decreased by about 10% from 92±9 to 82±9 mmHg and 106±10 to 95±8 beats/min, respectively, in the saline-treated group. In the dihydroergotamine-treated group arterial blood pressure increased and heart rate decreased (15% and 17%, respectively by the highest dose), but during treatment with S9977 both arterial blood pressure and heart

rate decreased (36% and 20%, respectively by the highest dose). The changes induced by the two drugs were more than those observed in the saline-treated group (Figure 2).

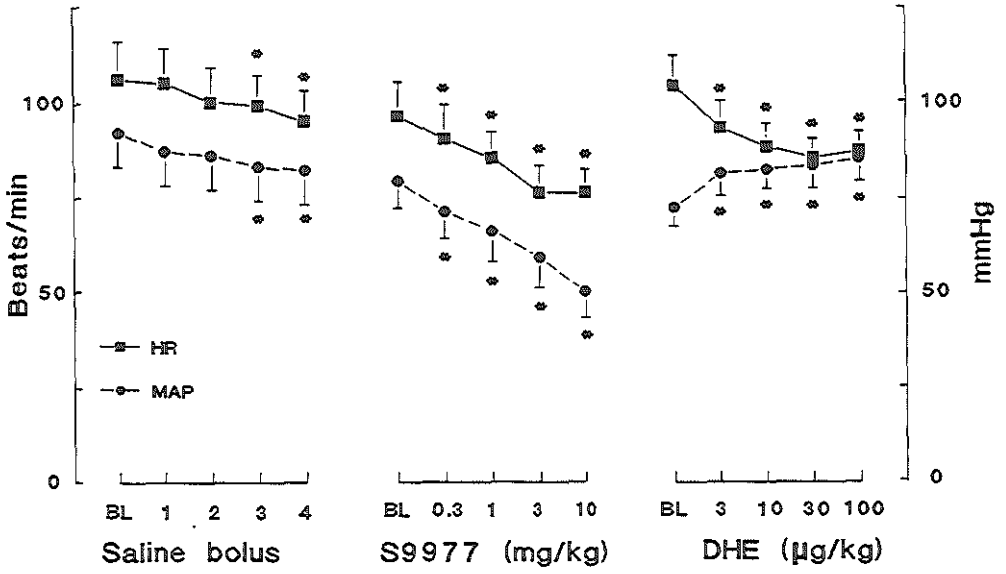

Figure 2. Effects of bolus injections of physiological saline, S9977 and dihydroergotamine on heart rate and mean arterial blood pressure in anaesthetized pigs. BL, Baseline; DHE, dihydroergotamine. *, $P<0.05$ vs. baseline.

Carotid haemodynamics

The effects of four consecutive infusions of either physiological saline, dihydroergotamine or S9977 on the total common carotid blood flow and conductance and their fractionation into arteriovenous anastomotic and nutrient parts are shown in Figures 3 and 4, respectively. In the saline-treated group the carotid haemodynamics remained fairly constant, except that there was some tendency for the total carotid and the arteriovenous anastomotic vascular conductances to increase slightly. Dihydroergotamine (3, 10, 30 and 100 μg/kg) caused a clear dose-dependent decrease in the total carotid and arteriovenous anastomotic blood flow and conductance, but the nutrient blood flow and conductance increased. Treatment with S9977 also decreased carotid (two highest doses) and arteriovenous anastomotic (highest dose) blood flows, but the respective conductances did not change. Except for an increase with the highest dose in the vascular conductance, S9977 did not affect the nutrient circulation.

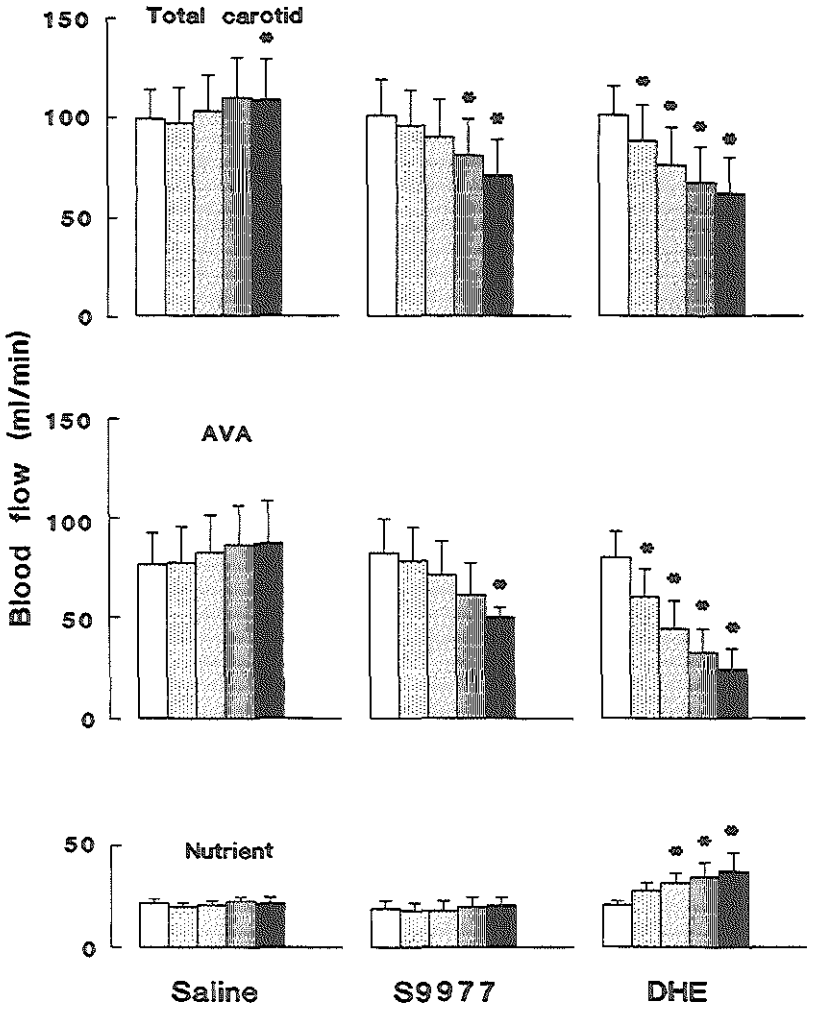

Figure 3. Effects of bolus injections of physiological saline, S9977 and dihydroergotamine on total carotid artery blood flow and its fractionation into arteriovenous anastomotic (AVA) and nutrient (tissue) parts in anaesthetized pigs. The five columns in each of the three panels represent, sequentially, baseline values, and values after the four i.v. bolus injections of saline, S9977 (0.3, 1, 3 and 10 mg/kg) or dihydroergotamine (DHE, 3, 10, 30 and 100 μg/kg). *, $P<0.05$ vs. baseline.

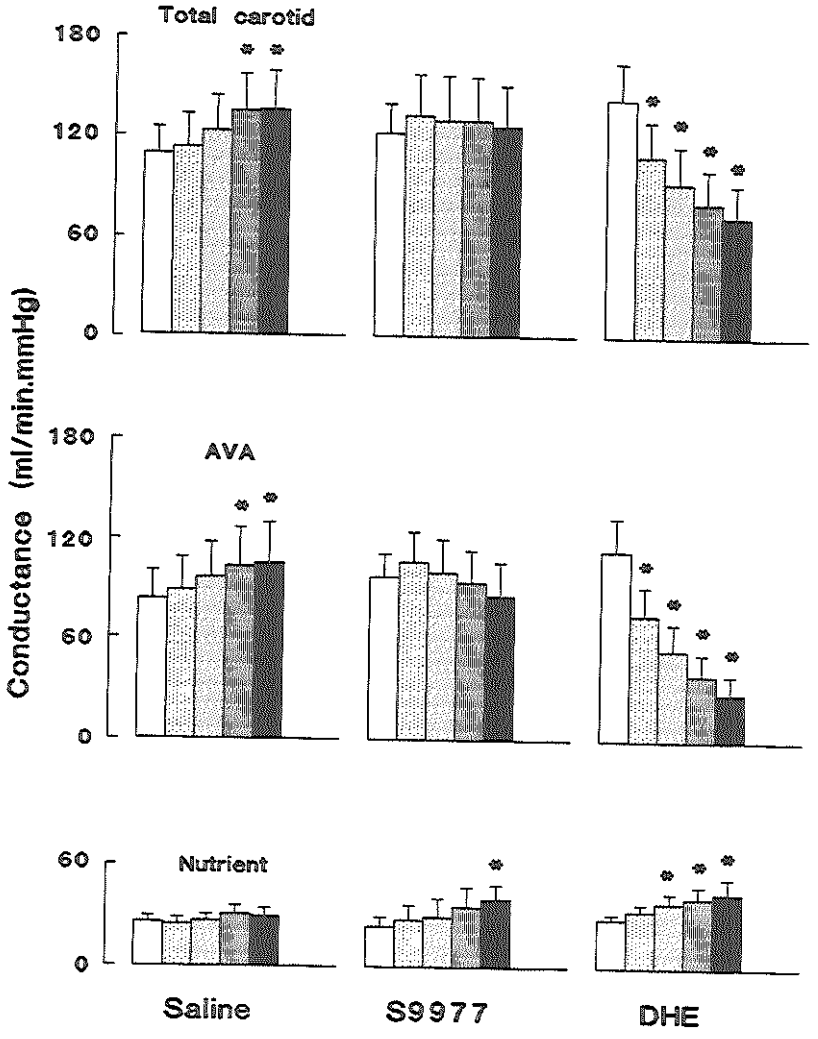

Figure 4. Effects of bolus injections of physiological saline, S9977 and dihydroergotamine on total carotid artery vascular conductance and its fractionation into arteriovenous anastomotic (AVA) and nutrient (tissue) parts in anaesthetized pigs. The five columns in each of the three panels represent, sequentially, baseline values, and values after the four i.v. bolus injections of saline, S9977 (0.3, 1, 3 and 10 mg/kg) or dihydroergotamine (DHE, 3, 10, 30 and 100 μg/kg). *, $P<0.05$ vs. baseline.

Analysis of the regional distribution of carotid blood flows (Figure 5) and conductances (Figure 6) showed that the infusions of physiological saline were devoid of any important effects. Infusions of S9977 caused only modest changes in regional blood flows; ocular blood flow was reduced in a dose-dependent manner and, at the highest dose, blood flows to the tongue and salivary glands increased (Figure 5). S9977 increased the conductance to the skin, muscles, tongue, fat and salivary glands (Figure 6). In contrast, dihydroergotamine dose-dependently increased both blood flows and conductances to the ears, skin, head muscles, tongue, salivary glands and total brain; blood flows to the eyes and fat remained essentially unchanged, but vascular conductance in the eye was slightly reduced (Figures 5 and 6).

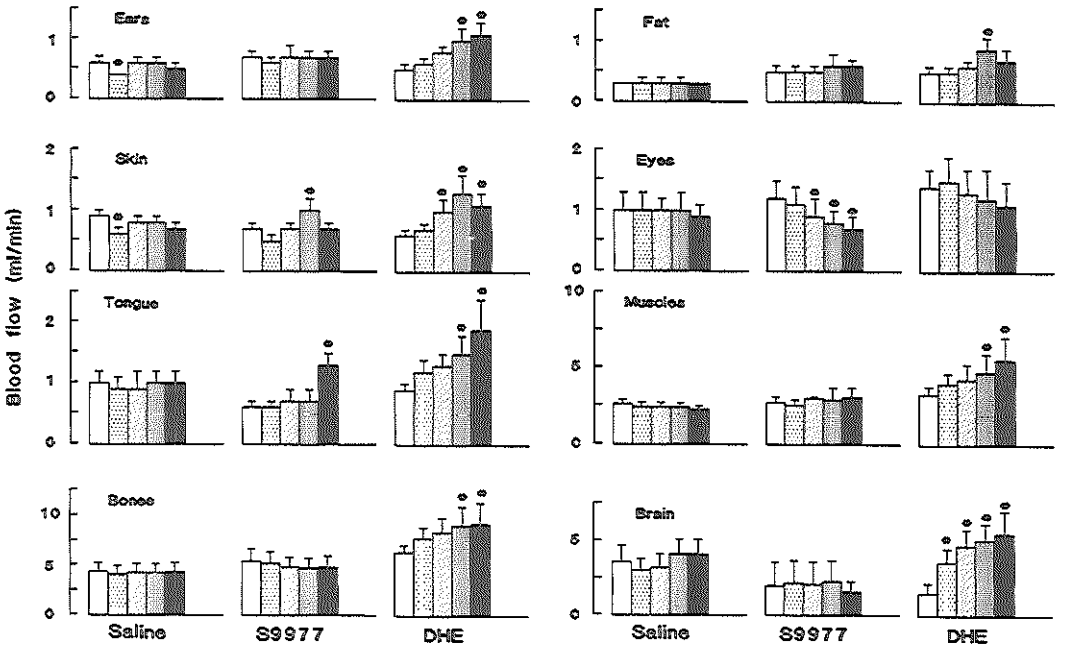

Figure 5. Effects of bolus injections of physiological saline, S9977 and dihydroergotamine on the regional (tissue) distribution of carotid artery blood flow in anaesthetized pigs. The five columns in each of the three panels represent, sequentially, baseline values, and values after the four i.v. bolus injections of saline, S9977 (0.3, 1, 3 and 10 mg/kg) or dihydroergotamine (DHE, 3, 10, 30 and 100 μg/kg). *, $P<0.05$ vs. baseline.

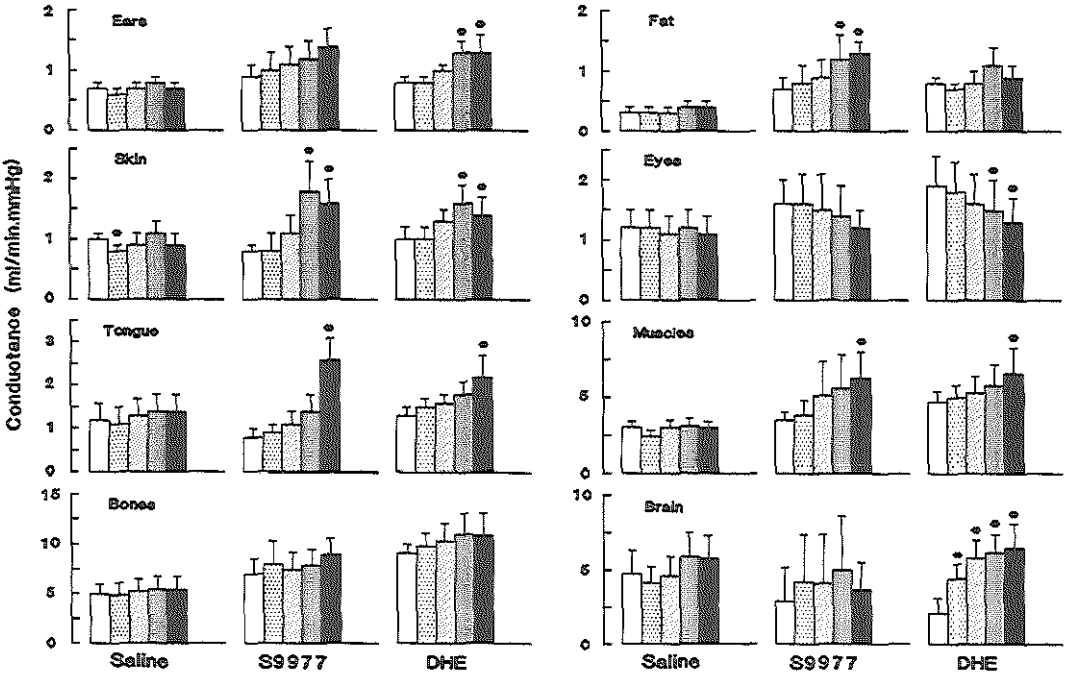

Figure 6. Effects of bolus injections of physiological saline, S9977 and dihydroergotamine on the regional (tissue) distribution of carotid artery vascular conductance in anaesthetized pigs. The five columns in each of the three panels represent, sequentially, baseline values, and values after the four i.v. bolus injections of saline, S9977 (0.3, 1, 3 and 10 mg/kg) or dihydroergotamine (DHE, 3, 10, 30 and 100 μg/kg). *, $P<0.05$ vs. baseline.

DISCUSSION

Systemic Haemodynamics

In the saline-treated animals both mean arterial blood pressure and heart rate gradually decreased to some extent during the course of the experiment. However, the changes in the drug-treated groups were more marked; dihydroergotamine increased arterial blood pressure and S9977 decreased it; nevertheless, both drugs caused bradycardia. The pressor and bradycardic action of dihydroergotamine may be, at least partly, due to its partial agonist action at α-adrenoceptors and/or dopamine$_2$ receptors (22). It is known that dihydroergotamine and ergotamine decrease heart rate in rats by an agonist

action at the presynaptic α_2-adrenoceptors located on the cardiac sympathetic nerve terminals (23). In the cat, however, the ergotamine-induced bradycardia is mediated by presynaptic dopaminergic receptors (24). The mechanism of the hypotensive and bradycardic actions of S9977 may involve a muscarinic cholinergic action, since in anaesthetized dog atropine antagonizes such effects of S9977 (A. Sassine, unpublished).

Carotid haemodynamics

The present results confirm the observations that a large fraction of 15 μm microspheres, injected into the carotid circulation of anaesthetized animals, is not trapped in the arteriolar bed of the cephalic tissues; these spheres pass via arteriovenous anastomoses and veins to the right side of the heart to be sieved by the pulmonary capillary bed (4,20,25). The simultaneous use of microspheres of four different diameters (10, 15, 25 and 35 μm) has shown that the large arteriovenous anastomoses in the cephalic circulation are mainly present in the skin and ears (26), but such 'shunt' vessels have also been located in the nasal mucosa (27), tongue (28), dura mater (29) and rete mirabile (30).

In the saline-treated group there were no important changes in the carotid blood flow or its fractionation into nutrient (arteriolar; tissue) and non-nutrient (arteriovenous anastomotic) parts. Dihydroergotamine (3, 10, 30 and 100 μg/kg) dose-dependently decreased carotid blood flow (and conductance) and yet redistributed blood in favour of the nutrient compartment at the expense of the arteriovenous shunting. The decrease in arteriovenous shunting is also reflected by the decrease in jugular venous pO_2. Since arterial pO_2 did not change, dihydroergotamine increased arterio-jugular venous O_2 difference. These results are in agreement with earlier findings; dihydroergotamine has been reported to decrease the arteriovenous anastomotic fraction of the common carotid blood flow and the jugular venous O_2 content in the cat (5) and increase the arterio-venous O_2 difference, with relief of pain in migraine patients (3).

In contrast to dihydroergotamine, the carotid haemodynamic effects of S9977, used in 100 times higher concentrations (0.3, 1, 3 and 10 mg/kg), were less marked. Though S9977 did decrease carotid (two highest doses) and arteriovenous anastomotic (highest dose) blood flows, there was no concomitant decrease in the vascular conductances. Therefore, the above effects of S9977 seem to be related to a decrease in arterial blood pressure and not to an active vasoconstriction of the arteriovenous anastomoses. The mechanisms underlying the vasodilator effects of dihydroergotamine and S9977 on the nutrient fraction are difficult to explain from the present experiments.

Possible clinical implications

Over the years we have shown that a number of drugs that are effective in the treatment of migraine, for example ergotamine, dihydroergotamine, methysergide and isomethep-tene (5,6, present results), powerfully reduce cephalic arteriovenous anastomotic shunting, though, as suggested for ergotamine (25,31), a reduction in cephalic arteriovenous anastomotic blood flow does not necessarily has to be mediated by α-adrenoceptors or 5-HT receptors. However, two new highly selective agonists at 5-HT_1-like receptors, namely AH25086 and sumatriptan (GR43175), have recently been shown to constrict cephalic arteries in several species and to diminish both feline and porcine carotid arteriovenous anastomotic blood flow (6,8-10,32). Furthermore, these new drugs do not seem to cross the blood brain barrier or have an anti-nociceptive effect even after intrathecal administration (9,10), yet they show a remarkable clinical effectiveness in acute migraine attacks (33-35).

On the basis of the above findings one can suggest that drugs which constrict cephalic arteries and arteriovenous anastomoses should be effective in the treatment of migraine (see 36). A lack of *active* vasoconstriction in the carotid vascular bed observed in this investigation with S9977, therefore, does not allow us to predict positive clinical results with this drug in the treatment of migraine. Nevertheless, it is important to highlight that, in contrast to the other antimigraine drugs which act as (partial) agonists at 5-HT_1-like and other receptors (6,8-10,32,36), S9977 is active in another potential animal model for migraine (16,17) and antagonizes the increased adenosine A_1 binding, electroencephalographic abnormalities (16) and the vascular dopaminergic hypersensitiv-ity (17) during food sensitization in the rat. Despite the evidence that stimulation of dopaminergic (11,12) and purinergic (13) neurones appear to cause a selective increase in arteriovenous anastomotic blood flow, the ineffectiveness of S9977 indicates that, under the present experimental circumstances, neither of these neurons is physiologically active. In migraine patients, such neurons may become active to open up arteriovenous anastomoses; indeed, Burnstock (37) has suggested that the events accompanying the headache phase of migraine could be explained by the neurogenic release of ATP during vasodilatation following cerebral spasm.

REFERENCES

1. Lance JW. Mechanism and management of headache. London: Butterworth, 1982.
2. Lauritzen M, Olesen J. Regional cerebral blood flow during migraine attacks by Xenon-133 inhalation and emission tomography. Brain 1984;107:447-61.

3. Heyck H. Pathogenesis of migraine. Res Clin Stud Headache 1969;2:1-28.
4. Saxena PR, Verdouw PD. Redistribution by 5-hydroxytryptamine of carotid arterial blood at the expense of arteriovenous blood flow. J Physiol (London) 1982;332:501-20.
5. Saxena PR. Arteriovenous shunting and migraine. Res Clin Stud Headache 1978; 6:89-102.
6. Saxena PR. Arteriovenous anastomoses and veins in migraine research. In: Blau JN, ed. Migraine, Clinical, Therapeutic, Conceptual and Research Aspects. Chapman and Hall Medical, London, 1987: 581-96.
7. Saxena PR, Ferrari MD. 5-HT_1-like receptor agonists and the pathophysiology of migraine. Trends Pharmacol Sci 1989;10:200-4.
8. Humphrey PPA, Feniuk W, Perren MJ, Connor HE, Oxford AW. The pharmacology of the novel 5-HT_1-like receptor agonist GR43175. Cephalalgia 1989;9 (Suppl.9):23-33.
9. Humphrey PPA, Feniuk W, Perren MJ. A rational approach to identifying a fundamentally new drug in the treatment of migraine. In: Saxena PR, Wallis DI, Wouters W, Bevan P, eds. Cardiovascular Pharmacology of 5-hydroxytryptamine, prospective therapeutic applications. Dordrecht: Kluwer Academic Publishers, 1990:417-31.
10. Den Boer MO, Villalón CM, Heiligers JPC, Humphrey PPA, Saxena PR. Role of 5-HT1-like receptors in the reduction of porcine cranial arteriovenous anastomotic shunting by sumatriptan. Br J Pharmacol 1991;102:323-330.
11. Bell C, Lang WJ, Laska F. Dopamine-containing axons supplying the arteriovenous anastomoses of the canine paw pad. J Neurochem 1978;31:1329-33.
12. Bell C, Lang WJ. Evidence for dopaminergic vasodilator innervation of the canine paw pad. Br J Pharmacol 1979;67:337-43.
13. Molyneux GS, Harmon B. Innervation of the arteriovenous anastomoses in the web of the foot of the domestic duck, Anas platyrhynchos, structural evidence for the presence of non-adrenergic non-cholinergic nerve. Am J Anat 1982;135:119-28.
14. Sicuteri F. Dopamine, the second putative protagonist in headache. Headache 1977;17:129-31.
15. Bès A, Dupui P, Güell A, Bessoles G, Géraud G. Pharmacological exploration of dopamine hypersensitivity in migraine patients. Int J Clin Pharmacol Res 1986;6:189-92.

16. Della Zuana O, Lejeune F, Tisserand F, Duhault J. Chronic food sensitization alters neuronal activity and the binding of adenosine receptor agonists to hippocampal cells in the rat. In: Clifford Rose, ed. New advances in headache research; Proceedings of the 8th Migraine Trust International Symposium, London, In press.
17. Duhault J, Della Zuana O, Deheeger C., Lejeune F. The pirbidil test as a predictive tool for screening of new antimigraine drugs in a food sensitized animal model. In: Clifford Rose, ed. New advances in headache research; Proceedings of the 8th Migraine Trust International Symposium, London, In press.
18. Heiligers JPC, Villalón CM, Bom AH, Saxena PR. Effects of S9977, a potential antimigraine drug, on arteriovenous anastomoses in the carotid vascular bed of the anaesthetized pig. Eur J Pharmacol 1990;183:1279-80.
19. Saxena PR, Schamhardt HC, Forsyth RP, Loeve J. Computer programs for the radioactive microsphere technique. Determination of regional blood flows and other haemodynamic variable in different experimental circumstances. Comp Progr Biomed 1980;12:63-84.
20. Johnston BM, Saxena PR. The effect of ergotamine on tissue blood flow and the arteriovenous shunting of radioactive microspheres in the head. Br J Pharmacol 1978; 63:541-49.
21. Steel RGD, Torrie JH. Principles and Procedures of Statistics, A Biomedical Approach, 2nd edn., Tokyo: McGraw-Hill Kogakusha Ltd. 1980.
22. Müller-Schweinitzer E, Weidmann H. Basic pharmacological properties. In: Berde B, Schild HO, eds. Ergot alkaloids and related compounds, Handbook of experimental pharmacology, Vol. 49, Heidelberg: Springer Verlag, 1978:87-232.
23. Roquebert J, Grenié B. α_2-Adrenergic agonist and α_1-adrenergic antagonist activity of ergotamine and dihydroergotamine in rats. Arch Int Pharmacodyn Thér 1986;284:30-7.
24. Saxena PR, Cairo-Rawlins WI. Presynaptic inhibition by ergotamine of the responses to cardioaccelerator nerve stimulation in the cat. Eur J Pharmacol 1979; 58:305-12.
25. Saxena PR, Koedam NA, Heiligers J, Hof RP. Ergotamine-induced constriction of cranial arteriovenous anastomoses in dogs pretreated with phentolamine and pizotifen. Cephalalgia 1983;3:71-81.
26. Saxena PR, Verdouw PD. Tissue blood flow and localization of arteriovenous anastomoses in pigs with microspheres of four different sizes. Pflügers Arch 1985; 403:128-35.

27. Änggård A. Capillary and shunt blood flow in the nasal mucosa of the cat. Acta Otolaryngology 1974;78:418-22.
28. Krönert H, Wurster RD, Pierau FRK, Pleschka K. Vasodilatory response of arteriovenous anastomoses to local cold stimuli in the dog's tongue. Pflügers Arch 1980;388:17-9.
29. Rowbotham GF, Little E. New concepts on the aetiology and vascularization of meningiomata: the mechanism of migraine; the chemical process of cerebrospinal fluid; and the formation of collections of blood or fluid in the subdural space. Brit J Surg 1965;52:21-4.
30. Gillilan LA, Markesbery WR. Arteriovenous shunts in the blood supply to the brain of some common laboratory animals — with special reference to the rete mirabile conjugatum in the cat. J Comp Neurol 1963;121:305-11.
31. Bom AH, Heiligers JPC, Saxena PR, Verdouw PD. Reduction of cephalic arteriovenous shunting by ergotamine is not mediated by 5-HT_1-like or 5-HT_2 receptors. Brit J Pharmacol 1989;97:383-90.
32. Perren MJ, Feniuk W, Humphrey PPA. The selective closure of feline carotid arteriovenous anastomoses (AVAs) by GR43175. Cephalalgia 1989;9 **(Suppl.9)**:41-6.
33. Brand J, Hadoke M, Perrin VL. Placebo controlled study of a selective 5-HT_1-like agonist, AH25086B, in relief of acute migraine. Cephalalgia 1987;7:402-3.
34. Doenicke A, Brandt J, Perrin VL. Possible benefit of GR43175, a novel 5-HT_1-like receptor agonist, for the acute treatment of severe migraine. Lancet 1988;1:1309-11.
35. Perrin VL, Färkkilä M, Goasguen J, Doenicke A, Brand J, Tfelt-Hansen P. Overview of initial clinical studies with intravenous and oral GR43175 in acute migraine. Cephalalgia 1989;9 **(Suppl.9)**:63-72.
36. Saxena P.R., Den Boer MO. Pharmacology of antimigraine drugs. J Neurol 1991;238 **(Suppl.1)**:S28-S35.
37. Burnstock G. Pathophysiology of migraine: A new hypothesis. Lancet 1981;27:1397-99.

PART III

CHAPTER 12

GENERAL DISCUSSION AND CONCLUSIONS

CHAPTER 12

GENERAL DISCUSSION AND CONCLUSIONS

12.1 EVIDENCE SUPPORTING THE EXTENSION OF THE CLASSIFICATION OF 5-HT RECEPTORS

When Gaddum and Picarelli (1957) described the existence of two types of "tryptamine" receptor, called M and D, they certainly did not anticipate how complicated and confusing the 5-HT receptor field would be three decades later, when an international committee (Bradley et al., 1986) subsequently attempted to reconcile the different nomenclatures taking into account functional and pharmacological criteria, and proposed three main types of 5-HT receptors, namely 5-HT_1-like, 5-HT_2 and 5-HT_3 receptors. In this connection, it is important to keep in mind several facts. Firstly, the separation between 5-HT_1-like and 5-HT_2 receptors is not absolute as most antagonists for 5-HT_1-like receptors (methiothepin, methysergide, mesulergine, metergoline, etc.) display similar or higher affinities for 5-HT_2 receptors (Hoyer, 1988a; Saxena and Villalón, 1990a). Secondly, amongst the 5-HT_1-like and 5-HT_2 receptor antagonists, methysergide and metergoline may act as partial agonists depending on the experimental model (Hoyer, 1988a; Schoeffter et al.,1988; Saxena and Villalón, 1990a). Thirdly, 5-HT_{1C} and 5-HT_2 receptors share many features, including second-messenger systems, pharmacological profile and structure (see Hoyer, 1988b; Hartig, 1989). Lastly, few if any 5-HT_1-like and 5-HT_2 receptor agonists and antagonists display high affinity and/or intrinsic activity for 5-HT_3 receptors (Fozard, 1990; Hoyer, 1990; Saxena and Villalón, 1990a). Significantly, in this context, 5-methoxytryptamine is inactive at 5-HT_3 receptors, but a potent agonist at the putative 5-HT_4 receptor (see Fozard, 1990; Saxena and Villalón, 1990a), which makes this compound an interesting tool.

It should nevertheless be realized that the international committee's proposal was meant to be a framework, flexible enough to adapt to and to incorporate new findings (Bradley et al., 1986). Thus, Dumuis et al. (1988, 1989) suggested the existence of a novel 5-HT receptor type described as the putative 5-HT_4 receptor, which is positively coupled to the adenylate cyclase system in mouse embryo colliculi neurons. This novel receptor site is stimulated by 5-methoxytryptamine and some benzamide derivatives including renzapride and zacopride, and is sensitive to blockade only by high concentrations of the 5-HT_3 receptor antagonist ICS 205-930, but not by similar

concentrations of other 5-HT receptor antagonists (Dumuis et al., 1988, 1989). These findings have been confirmed in peripheral tissues of other species, where the putative 5-HT_4 receptor mediates either directly or indirectly, contractions of the guinea-pig isolated ileum (Craig and Clarke, 1990) and ascending colon (Elswood et al., 1991), right human atria (Kaumann et al., 1990a,b) and porcine myocardium (Villalón et al., 1991c; this thesis) as well as relaxation of the rat oesophagus (Baxter and Clarke, 1990) and stimulation of the pig sinoatrial pacemaker (Kaumann, 1990c; Villalón et al., 1990b, 1991a,b).

Although the term 5-HT_4 has not been officially accepted by the nomenclature committee of the 5-HT club, the involvement of this novel 5-HT receptor in the above functional responses argues in favour of the extension of the 5-HT receptor classification to include the 5-HT_4 receptor.

12.2 CHARACTERIZATION OF THE 5-HT RECEPTORS MEDIATING THE DECREASE IN CEPHALIC ARTERIOVENOUS ANASTOMOTIC BLOOD FLOW IN THE PIG

The experimental evidence available up to date suggests that the receptors involved in 5-HT-induced decrease in porcine cephalic arteriovenous anastomotic blood flow belong to the 5-HT_1-like type. In this context, 5-HT (Saxena and Verdouw, 1982, 1984; Verdouw et al., 1984; Saxena et al., 1986a), 5-CT (Saxena and Verdouw, 1985a), methysergide (Saxena and Verdouw, 1984), BEA 1654 (Verdouw et al., 1985), 8-OH-DPAT (Bom et al., 1989a), RU 24969 (Bom et al., 1989b), sumatriptan (Den Boer et al., 1991) and indorenate (Villalón et al., 1990a) decrease porcine arteriovenous anastomotic blood flow, but only 5-HT and 5-CT increase arteriolar blood flow prominently (see Table 7). These results clearly indicate that the 5-HT_1-like receptors involved in constricting arteriovenous anastomoses are different from those mediating arteriolar dilatation.

Table 7: 5-HT receptors and drug effect on arterioles and arteriovenous anastomoses (AVAs) in the porcine carotid artery bed.

Drug	Arteri-oles	AVAs	Antagonism by	Resistance to	Receptor type
5-HT	----	++++	Methiothepin	Cyproheptadine Ketanserin WAL 1307 MDL 72222	5-HT_1-like
5-CT	----	++++		Cyproheptadine	5-HT_1-like
Methysergide	-	+++			5-HT_1-like
BEA 1654	--	+++		Ketanserin	5-HT_1-like
8-OH-DPAT	-	++++	Methiothepin	Ketanserin	5-HT_1-like
Ipsapirone	0	0			
RU 24969	-	++++	Methiothepin	Ketanserin (±)Pindolol	5-HT_1-like
Sumatriptan	0/-	++++	Methiothepin	Ketanserin	5-HT_1-like
Indorenate	-	++++	Methiothepin	Ketanserin Metergoline	5-HT_1-like

-, dilatation; +, contraction; 0, no effect. The number of - and + indicate the magnitude of effect.

As shown in Table 7, only methiothepin, but not other 5-HT receptor antagonists, effectively blocked the effects of the above 5-HT_1-like receptor agonists. However, three implicit drawbacks must be taken into consideration. Firstly, in addition to its high potency to block 5-HT_1-like and 5-HT_2 receptors, methiothepin has other

actions, including noradrenaline uptake inhibition and high affinity for α-adrenoceptors, histamine$_1$ (H_1) and dopamine$_2$ (D_2) receptors (see Leysen, 1985). Secondly, the high degree of heterogeneity of the 5-HT$_1$ binding site has been revealed from radioligand binding studies performed in cerebral tissue membranes. And lastly, all research concerning functional studies has been based on the use of these 5-HT receptor ligands, but due to the lack of selective antagonists for these putative 5-HT$_1$-like receptor subtypes it is still impossible to correlate the 5-HT$_1$ binding site subtypes demonstrated in cerebral tissue with the functional subtypes of 5-HT$_1$-like receptors present in the cardiovascular system.

In this connection, the 5-HT$_1$-like receptors present in the porcine cranial AVAs (arteriovenous anastomoses) seem to be unrelated to the 5-HT$_1$ binding site subtypes (5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1C}$, 5-HT$_{1D}$ and 5-HT$_{1E}$) for several reasons, namely: i) the 5-HT$_{1A}$ ligand ipsapirone (Dompert et al., 1985; Hoyer, 1988a) had no effect on the porcine cranial AVA blood flow (Bom et al., 1988b); ii) pindolol, which displays high affinities for both 5-HT$_{1A}$ and 5-HT$_{1B}$ binding sites (Hoyer, 1988a), did not antagonize the effects of RU 24969 (Bom et al., 1989b), which also has high affinity for the same binding sites (Hoyer, 1988a); and iii) metergoline, which has been shown to display higher affinities for 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1C}$ and 5-HT$_{1D}$ binding sites than methiothepin (Hoyer, 1988a), did not antagonize the effects of indorenate (Villalón et al., 1990a), which has high affinity for the 5-HT$_{1A}$ binding site, and appreciable affinities for the 5-HT$_{1B}$, 5-HT$_{1C}$ and 5-HT$_{1D}$ binding sites (Hoyer et al., 1985; Hoyer, personal communication). These -and other- findings give support to our view that compounds with high affinity for certain 5-HT recognition sites in brain tissue membranes and without agonistic action on peripheral 5-HT receptors do not necessarily have to behave as 5-HT receptor antagonists in pharmacological functional studies (i.e. effect of 5-HT$_1$-like receptor agonists and antagonists on porcine cranial AVA blood flow). For this reason, Saxena and Villalón (1990a) have coined this receptor 5-HT$_{1x}$, until more selective antagonists be available.

The implication of the present findings is two-fold. One the one hand, it is evident that binding data are useful for the screening of new compounds; on the other hand, however, by no means is it valid to predict from radioligand binding studies drug effects in functional models; for this purpose, the "classical" pharmacological approach should have priority. This is of particular relevance when trying to develop drugs with potential therapeutical usefulness in the treatment of some pathologies (e.g. migraine, hypertension, etc.)

12.3 THERAPEUTICAL AND PHARMACOLOGICAL APPROACHES FOR ANTIMIGRAINE ACTION

A number of new and established antimigraine drugs interact with 5-HT receptors (Saxena and Villalón, 1990a; Feniuk et al., 1991; Saxena and Den Boer, 1991). In this respect, selective agonists at the 5-HT_1-like receptor subtype mediating contractions of cephalic vessels (AH25086, sumatriptan), which do not penetrate into the central nervous system, are effective in the treatment of acute migraine attacks (see Perrin et al., 1989; Saxena and Ferrari, 1989; Humphrey et al., 1990; Tfelt-Hanssen and Nielsen, 1990). In addition, some antimigraine drugs behave as antagonists at 5-HT_2 (methysergide, pizotifen, ergotamine, dihydroergotamine) and/or 5-HT_1-like (methysergide, propranolol) receptors, but many 5-HT_2 and/or 5-HT_1-like receptor antagonists (ketanserin, ritanserin, cyproheptadine, mianserin, methiothepin, metergoline) have not found much use in migraine therapy. It therefore seems likely that additional properties of such antimigraine drugs, for example, the vasoconstriction in the extracerebral cephalic circulation with methysergide, ergotamine and dihydroergotamine and the antidepressant action with pizotifen, may be involved in their therapeutic action (Saxena, 1990). In agreement with these findings, dihydroergotamine, which displays high affinities for 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, 5-HT_{1D} and 5-HT_2 binding sites (Hoyer, 1988a) potently decreased porcine cranial AVA blood flow (Villalón et al., 1991d). However, other actions of dihydroergotamine such as its ability to interact with α_1 and α_2 adrenoceptors as well as D_1 and D_2 receptors (Peroutka, 1990) cannot be excluded from its therapeutical efficacy.

With respect to the pharmacological approaches to include 5-HT_3 receptor antagonists in antimigraine therapy, it is of particular interest the finding that MDL 72222 (Loisy et al., 1985) and granisetron (Ferrari, 1991) seem to be effective in the acute treatment of migraine. In contrast, ICS 205-930 is not at all effective in the acute treatment of this pathology (Lataste et al., 1989; Ferrari, 1991). Unfortunately, all the 5-HT_3 receptor antagonists tested in migraine to date have proved to be oncogenic in animals on chronic administration, precluding any follow-up studies (Ferrari, 1991). Therefore, within the bounds of serotonergic mechanisms, the antimigraine action of the above drugs seems to depend mainly upon agonist action at the 5-HT_1-like receptor subtype (5-HT_{1x}; Table 1) that mediates craniovascular contraction (Saxena and Ferrari, 1989; Saxena and Villalón, 1990a; Saxena and Den Boer, 1991).

Notwithstanding, other mechanisms underlying the decrease in cranial AVA blood flow cannot be excluded. For instance, it is interesting to note that apart from the

use of 5-HT_1-like receptor agonists, a new approach for decreasing arteriovenous anastomotic shunting may be via antagonism towards dopamine and/or purinergic mechanisms. In this connection, there is evidence that arteriovenous anastomoses are innervated by both dopaminergic (Bell et al., 1978; Bell and Lang, 1979) and purinergic (Molyneux and Harmon, 1982) neurons which, upon stimulation, appear to cause a selective increase in arteriovenous anastomotic blood flow. Since hypersensitivity to dopamine-like drugs has been reported in migraine patients (Sicuteri, 1977; Bès et al., 1986), it is tempting to suggest that drugs able to antagonize dopaminergic hypersensitivity and/or purinergic-mediated effects could have potential therapeutic usefulness in the treatment of migraine. Indeed, the novel trimethylxanthine derivative S9977 displays such properties (Della Zuana et al., 1991; Duhault et al., 1991). In our hands, however, S9977 did not induce an active vasoconstriction of the porcine cranial AVAs (Villalón et al., 1991d), which might reflect the fact that under the experimental circumstances of our model dopaminergic and/or purinergic neurons are not physiologically active. Nevertheless, it should be emphasized that there seems to be other potential animal models to predict antimigraine action, such as the blockade of vascular dopaminergic hypersensitivity and electroencephalographic abnormalities during food sensitization in the rat (Della Zuana et al., 1991; Duhault et al., 1991); in this model, S9977 is effective.

Likewise, several antimigraine drugs shown to decrease cranial AVA blood flow (including ergotamine, dihydroergotamine, methysergide and sumatriptan) are also able to antagonize neurogenic inflammation in rat dura mater (which occurs via the release of neuropeptide pro-inflammatory mediators such as substance P and neurokinin A from perivascular sensory axons) induced either by electrical stimulation of trigeminal ganglia or by capsaicin (Moskowitz and Buzzi, 1991). Interestingly, all these drugs elicit also a potent vasoconstriction of the dura mater blood vessels (Feniuk et al., 1991), emphasizing the possibility that neurogenic inflammation and vasodilatation of blood vessels (including AVAs) in the dura mater may be interrelated in the pathophysiology of migraine. In this context, some attention should be drawn to the fact that dura mater has an extensive network of arteriovenous anastomoses (see, for example, Rowbotham and Little, 1965; Kerber and Newton, 1973). Therefore, studies to determine the effect of antimigraine drugs on the fractionation of blood flow within the dura mater would give additional weight to the role of AVAs in the pathogenesis of migraine.

REFERENCES

CHAPTERS 1-6 and 12

REFERENCES (Chapters 1-6 and 12)

Antonaccio, M.J. and Cote, D. (1976) Centrally mediated antihypertensive and bradycardic effects of methysergide in spontaneously hypertensive rats. Eur. J. Pharmacol., 36, 451-454.

Apperley, E., Humphrey, P.P.A. and Levy, G.P. (1976) Receptors for 5-hydroxytryptamine and noradrenaline in rabbit isolated ear artery and aorta. Br. J. Pharmacol., 58, 211-221.

Apperley, E., Feniuk, W., Humphrey, P.P.A. and Levy, G.P. (1980) Evidence for two types of excitatory receptors for 5-hydroxytryptamine in dog isolated vasculature. Br. J. Pharmacol., 68, 215-224.

Armstrong, D.J., Kay, I.S. and Russel, N.J.W. (1986a) The pulmonary chemoreflexes evoked by phenyldiguanide, diguanide and guanide. J. Physiol., 371, 115P.

Armstrong, D.J., Kay, I.S. and Russel, N.J.W. (1986b) MDL 72222 antagonizes the reflex tachypneic response to miliary pulmonary embolism in anaesthetized rabbits. J. Physiol., 381, 13P.

Baxter, G.S. and Clarke, D.E. (1990) Putative 5-HT_4 receptors mediate relaxation of rat oesophagus. Proceedings of the 2nd IUPHAR Satellite Meeting on Serotonin, Basel, July 11-13, 1990. Abstract No. P85.

Bell, C. and Lang, W.J. (1979) Evidence for dopaminergic vasodilator innervation of the canine paw pad. Br. J. Pharmacol., 67, 337-343.

Bell, C., Lang, W.J. and Laska, F. (1978) Dopamine-containing axons supplying the arteriovenous anastomoses of the canine paw pad. J. Neurochem., 31, 1329-3333.

Bennett, J.P. and Aghajanian, G.K. (1974) d-LSD binding to brain homogenates: possible relationship to serotonin receptors. Life Sci., 15, 1935-1944.

Bennett, J.P. and Snyder, S.H. (1976) Serotonin and lysergic acid diethylamide binding in rat brain membranes: relationship to postsynaptic serotonin receptors. Molec. Pharmacol., 12, 373-389.

Berthold, H., Scholtysik, G. and Engel, G. (1989) Inhibition of the 5-HT-induced cardiogenic hypertensive chemoreflex by the selective 5-HT_3 receptor antagonist ICS 205-930. Naunyn-Schmiedeberg's Arch. Pharmacol., 339, 259-262.

Bès, A., Dupui, P., Güell, A., Bessoles, G. and Géraud, G. (1986) Pharmacological exploration of dopamine hypersensitivity in migraine patients. Int. J. Clin. Pharmacol. Res., 6, 189-192.

Bhargava, K.P. and Tangri, K.K. (1959) The central vasomotor effects of 5-hydroxytryptamine. Br. J. Pharmacol., 14, 411-414.

Black, A.M.S., Comroe, J.H., Jr. and Jacobs, L. (1952) Species difference in carotid body response to intravenous serotonin in rats. J. Pharmacol. Exp. Ther., 105, 58-73.

Black, J.L., French, R.J. and Mylecharane, E.J. (1981) Receptor mechanisms for 5-hydroxytryptamine in rabbit arteries. Br. J. Pharmacol., 74, 619-626.

Blackshear, J.L., Orlandi, C., Garnic, J.D. and Hollenberg, N.K. (1985) Differential large and small vessel responses to serotonin in the dog hind limb *in vivo*: role of the 5-HT_2 receptor. J. Cardiovasc. Pharmacol., 7, 42-49.

Blauw, G.J., Van Brummelen, P. and Van Zwieten, P.A. (1988) Serotonin-induced vasodilatation in the human forearm is antagonized by the selective 5-HT_3 receptor antagonist ICS 205-930. Life Sci., 43, 1441-1449.

Bockaert, J., Sebben, M. and Dumuis, A. (1990) Pharmacological characterization of 5-hydroxytryptamine$_4$ (5-HT_4) receptors positively coupled to adenylate cyclase in adult guinea-pig hippocampal membranes: effect of substituted benzamide derivatives. Mol. Pharmacol., 37, 408-411.

Boddeke, H.W.G.M. and Kalkman, H.O. (1990) Zacopride and BRL 24924 induce an increase in EEC-energy in rats. Br. J. Pharmacol., 101, 281-284.

Bolt, G.R. and Saxena, P.R. (1985) Cardiovascular profile and hypotensive mechanism of ketanserin in conscious renal hypertensive rabbits. Hypertension, 7, 499-506.

Bom, A.H., Duncker, D.J., Saxena, P.R. and Verdouw, P.D. (1988a) 5-HT-induced tachycardia in the pig: possible involvement of a new type of 5-HT receptor. Br. J. Pharmacol., 93, 663-671.

Bom, A.H., Saxena, P.R. and Verdouw, P.D. (1988b) Further characterization of the 5-HT_1-like receptors in the carotid circulation of the pig. Br. J. Pharmacol., 94, 327P.

Bom, A.H., Verdouw, P.D. and Saxena, P.R. (1989a) Carotid haemodynamics in pigs during infusions of 8-OH-DPAT: Reduction in arteriovenous shunting is mediated by 5-HT_1-like receptors. Br. J. Pharmacol., 96, 125-132.

Bom, A.H., Villalón, C.M., Verdouw, P.D. and Saxena, P.R. (1989b) The 5-HT_1-like receptor mediating reduction of porcine carotid arteriovenous shunting by RU 24969 is not related to either the 5-HT_{1A} or the 5-HT_{1B} subtype. Eur. J. Pharmacol., 171, 87-96.

Bouhelal, R., Smounya, L. and Bockaert, J. (1988) 5-HT_{1B} receptors are negatively coupled with adenylate cyclase in rat substantia nigra. Eur. J. Pharmacol., 151, 189-196.

Bradley, P.B., Engel, G., Feniuk, W., Fozard, J.R., Humphrey, P.P.A., Middlemiss, D. N., Mylecharane, E.J., Richardson, B.P. and Saxena, P.R. (1986) Proposals for the classification and nomenclature of functional receptors for 5-hydroxytryptamine. Neuropharmacol., 25, 563-576.

Brodie, T.G. (1900) The immediate action of an intravenous injection of blood-serum. J. Physiol. (London), 26, 48-71.

Cadogan, A. and Humphrey, P.P.A. (1991) Classification of the 5-HT receptor mediating cardio-excitation in the snail, Helix aspersa. Br. J. Pharmacol., 102, 97P.

Catravas, J.D. and Gillis, C.N. (1983) Pulmonary clearance of ^{14}C-5-HT and ^{3}H-norepinephrine in vivo. Effects of pretreatment with imipramine or cocaine. J. Pharmacol. Exp. Ther., 224, 28-33.

Chang, J.Y. (1987) Serotonergic neuro-receptor mechanisms in brain vessels. Doctors' Thesis, University of Lund.

Chang, J.Y. and Owman Ch. (1989a) Cerebrovascular serotonergic receptors mediating vasoconstriction: further evidence for the existence of 5-HT_2 receptors in rats and 5-HT_1-like receptors in guinea-pig basilar arteries. Acta Physiol. Scand., 136, 59-67.

Chang, J.Y. and Owman, Ch. (1989b) Serotonin potentiates noradrenaline-induced vasoconstriction through 5-HT_1 type receptors in guinea-pig basilar artery. J. Cereb. Blood. Flow Metab., 9, 713-716.

Charlton, K.G., Bond, B.A. and Clarke, D.E. (1986) An inhibitory prejunctional 5-HT_1-like receptor in the isolated perfused rat kidney. Naunyn-Schmiedeberg's Arch. Pharmacol., 332, 8-15.

Chiba, S. (1977) Pharmacologic analysis of chronotropic and inotropic responses to 5-hydroxytryptamine in the dog heart. Jap. J. Pharmacol., 27, 727-734.

Chiba, S., Kobayashi, M. and Furukawa, Y. (1978) Potentiation of tyramine action of 5-hydroxytryptamine in the dog heart. Jap. J. Pharmacol., 28, 160-162.

Clancy, B.M. and Maayani, S. (1985) 5-Hydroxytryptamine receptor in isolated rabbit aorta: characterization with tryptamine analogues. J. Pharmacol. Exp. Ther., 233, 761-769.

Clarke, D.E., Craig, D.A. and Fozard, J.R. (1989) The 5-HT_4 receptor: naughty but nice. Trends Pharmacol. Sci., 10, 385-386.

Cocks, T.M. and Angus, J.A. (1983) Endothelium-dependent relaxation of coronary arteries by noradrenaline and serotonin. Nature, 305, 627-630.

Cohen, R.A. (1985) Serotonergic prejunctional inhibition of canine coronary adrenergic nerves. J. Pharmacol. Exp. Ther., 235, 76-80.

Cohen, M.L., Fuller, R.W. and Wiley, S. (1981) Evidence for 5-HT_2 receptors mediating contraction in vascular smooth muscle. J. Pharmacol. Exp. Ther., 218, 421-425.

Cohen, R.A., Shepherd, J.T. and Vanhoutte, P.M. (1983) Inhibitory role of the endothelium in the response of isolated coronary arteries to platelets. Science, 221, 273-274.

Comroe, J.H., Van Lingen, B., Stroud, R.C. and Roncoroni, A. (1953) Reflex and direct cardiopulmonary effects of 5-OH-Tryptamine (Serotonin). Their possible role in pulmonary embolism and coronary thrombosis. Am. J. Physiol., 173, 379-386.

Conn, J.P. and Sanders-Bush, E. (1986) Regulation of serotonin-stimulated phosphoinositide hydrolysis: Relation to the serotonin 5-HT_2 binding site. J. Neurosci., 6, 3669-3675.

Conn, J.P., Sanders-Bush, E., Hoffman, B.J. and Hartig, P.R. (1986) A unique serotonin receptor in choroid plexus is linked to phosphatidylinositol turnover. Proc. Natl. Acad. Sci., 83, 4086-4088

Connor, H.E., Feniuk, W. and Humphrey, P.P.A. (1989) Characterization of 5-HT receptors mediating contraction of canine and primate basilar artery by use of GR43175, a selective 5-HT_1-like receptor agonist. Br. J. Pharmacol., 96, 379-387.

Connor, H.E., Feniuk, W., Perren, M.J. and Humphrey, P.P.A. (1986) 5-Carboxamidotryptamine is a selective agonist at 5-hydroxytryptamine receptors mediating vasodilatation and tachycardia in anaesthetized cats. Br. J. Pharmacol., 87, 417-426.

Cooper, S.M., McClelland, M., McRitchie, B. and Turner, D.H. (1986) BRL 24924: a new and potent gastric motility stimulant. Br. J. Pharmacol., 88, 383P.

Coote, J.H. (1990) The central antihypertensive action of 5-hydroxytryptamine: the location of site of action. In: Saxena, P.R., Wallis, D.I., Wouters, W. and Bevan, P. eds. Cardiovascular Pharmacology of 5-Hydroxytryptamine, Prospective Therapeutic Applications. Dordrecht: Kluwer Academic Publishers, pp.259-270.

Craig, D.A. and Clarke, D.E. (1990) Pharmacological characterization of a neuronal receptor for 5-hydroxytryptamine in guinea-pig ileum with properties similar to the 5-hydroxytryptamine$_4$ receptor. J. Pharmacol. Exp. Ther., 252, 1378-1386.

Creite, A. (1869) Versuche über die Wirkung des Serumeiweisses nach Injection in das Blut. Zeitschrift für rationelle Medicin, B. 36, 90.

Dabiré, H., Chaouche-Teyara, K., Cherqui, C., Fournier, B., Laubie, M. and Schmitt, H. (1989a) Characterization of DOI, a putative 5-HT_2 receptor agonist in the rat. Eur. J. Pharmacol., 168, 369-374.

Dabiré, H., Chaouche-Teyara, K., Cherqui, C., Fournier, B. and Schmitt, H. (1989b) DOI is a mixed agonist-antagonist at postjunctional 5-HT_2 receptors in the pithed rat. Eur. J. Pharmacol., 170, 109-111.

Dabiré, H., Cherqui, C., Fournier, B. and Schmitt, H. (1987) Comparison of effects of some 5-HT_1 agonists on blood pressure and heart rate of normotensive anaesthetized rats. Eur. J. Pharmacol., 140, 259-266.

Dalton, D.W. (1986) The cardiovascular effects of centrally administered 5-hydroxytryptamine in the conscious normotensive and hypertensive rat. J. Autonom. Pharmacol., 6, 67-75.

Dalton, D.W., Feniuk, W. and Humphrey, P.P.A. (1986) An investigation into the mechanisms of the cardiovascular effects of 5-hydroxytryptamine in conscious normotensive and DOCA-salt hypertensive rats. J. Autonom. Pharmacol., 6, 219-228.

Davies, M., Wilkinson, L.S. and Roberts, M.H.T. (1988) Evidence for excitatory 5-HT_2 receptors on rat brain stem neurons. Br. J. Pharmacol., 94, 483-491.

Davies, M.F., Deisz, R.A., Prince, D.A. and Peroutka, S.J. (1987) Physiological and pharmacological characterization of the response of guinea pig neocortical neurons to serotonin. Soc. Neurosci. Abstracts, 13, 801.

Dawes, G.S. and Mott, J.C. (1950) Circulatory and respiratory reflexes caused by aromatic guanidines. Br. J. Pharmacol., 5, 65-76.

De Clerk, F., Xhonneux, B., Leysen, J. and Janssen, P.A.J. (1984) Evidence for functional 5-HT_2 receptor sites on human blood platelets. Biochem. Pharmacol., 33, 2807-2811.

Della Zuana, O., Lejeune, F., Tisserand, F. and Duhault, J. (1991) Chronic food sensitization alters neuronal activity and the binding of adenosine receptor agonists to hippocampal cells in the rat. In: Clifford Rose, ed. New advances in headache research; Proceedings of the 8th Migraine Trust International Symposium, London, In press.

Den Boer, M.O., Villalón, C.M., Heiligers, J.P.C., Humphrey, P.P.A. and Saxena, P.R. (1991) Role of 5-HT_1-like receptors in the reduction of porcine cranial arteriovenous anastomotic shunting by sumatriptan. Br. J. Pharmacol., 102, 323-330.

Derkach, V., Surprenant, A. and North, R.A. (1989) 5-HT_3 receptors are membrane ion channels. Nature, 339, 706-709.

Dhasmana, K.M., Banerjee, A.K. and Saxena, P.R. (1989) Hypotension and bradycardia after intrathecal ketanserin and phentolamine in the rat. Eur. J. Pharmacol., 168, 257-260.

Dhasmana, K.M., De Boer, H.J., Banerjee, A.K. and Saxena, P.R. (1988) Analysis of the tachycardiac response to 5-hydroxytryptamine in the spinal guinea-pig. Eur. J. Pharmacol., 145, 67-73.

Docherty, J.R. (1988) Investigation of cardiovascular 5-hydroxytryptamine receptor subtypes in the rat. Naunyn-Schmiedeberg's Arch. Pharmacol., 337, 1-8.

Doenicke, A., Brand, J., and Perrin, V.L. (1988) Possible benefit of GR43175, a novel 5-HT_1-like receptor agonist for the treatment of severe migraine. Lancet, 1, 1309-1311.

Dompert, W.V., Glaser, T. and Traber, J. (1985) ^{3}H-TVX Q 7821: Identification of 5-HT_1 binding sites as target for a novel putative anxiolytic. Naunyn-Schmiedeberg's Arch. Pharmacol., 328, 467-470.

Doods, H.N., Boddeke, H.W.G.M., Kalkman, H.O., Hoyer, D., Mathy, M.J. and Van Zwieten, P.A. (1988) Central 5-HT_{1A} receptors and the mechanism of the hypotensive effect of (+)8-OH-DPAT, DP-5-CT, R28935, and urapidil. J. Cardiovasc. Pharmacol., 11, 432-437.

Douglas, W.W. (1985) 5-Hydroxytryptamine (serotonin). In: Goodman and Gilman's The Pharmacological Basis of Therapeutics, 7th Edition, A. Goodman Gilman, L.S. Goodman, T.W. Rall, F. Murad (editors). McMillan Publishing Company, New York, pp. 628-638.

Dreteler, G.H., Wouters, W. and Saxena, P.R. (1989) Systemic and regional haemodynamic effects of the putative 5-HT_{1A} receptor agonist flesinoxan in the cat. J. Cardiovasc. Pharmacol., 14, 770-776.

Drummond, P.D. and Lance, J.W. (1983) Extracranial vascular changes and the source of pain in migraine headache. Ann. Neurol., 13, 32-37.

Duhault, J., Della Zuana, O., Deheeger, C. and Lejeune, F. (1991) The pirbidil test as a predictive tool for screening of new antimigraine drugs in a food sensitized animal model. In: Clifford Rose, ed. New advances in headache research; Proceedings of the 8th Migraine Trust International Symposium, London, In press.

Dumuis, A., Bouhelal, R., Sebben, M. and Bockaert, J. (1988) A 5-HT receptor in the central nervous system, positively coupled with adenyl cyclase, is antagonized by ICS 205-930. Eur. J. Pharmacol., 146, 187-188.

Dumuis, A., Sebben, M. and Bockaert, J. (1989) The gastrointestinal prokinetic benzamide derivatives are agonists at the non-classical 5-HT receptor (5-HT_4) positively coupled to adenylate cyclase in neurons. Naunyn Schmiedeberg's Arch. Pharmacol., 340, 403-410.

Duncker, D.J., Saxena, P.R. and Verdouw, P.D. (1985) 5-Hydroxytryptamine causes tachycardia in pigs by acting on receptors unrelated to 5-HT_1, 5-HT_2 or M-type. Br. J. Pharmacol., 86, 596P.

Edmeads, J. (1991) What is migraine ? Controversy and stalemate in migraine pathophysiology. J. Neurol., 238 (Suppl.1), S2-S5.

Eglen, R.M. and Whiting, R.L. (1989) Comparison of the positive chronotropic responses to 5-hydroxytryptamine with ß-adrenoceptor agonists on the guinea pig atria in vitro. J. Cardiovasc. Pharmacol., 13, 45-51.

Elswood, C.J., Bunce, K.T. and Humphrey, P.P.A. (1991) Identification of putative 5-HT_4 receptors in guinea-pig ascending colon. Eur. J. Pharmacol., 196, 149-155.

Engel, G., Göthert, M., Hoyer, D., Schlicker, E. and Hillebrand, K. (1986) Identity of inhibitory presynaptic 5-hydroxytryptamine (5-HT) autoreceptors in the rat brain cortex with 5-HT_{1B} binding sites. Naunyn-Schmiedeberg's Arch. Pharmacol., 332, 1-7.

Engel, G., Göthert, M., Müller-Schweinitzer, E., Schlicker, E., Sistonen, L. and Stadler, P.A. (1983) Evidence for common pharmacological properties of [^{3}H]5-hydroxytryptamine binding sites, presynaptic 5-hydroxytryptamine autoreceptors in CNS and inhibitory presynaptic 5-hydroxytryptamine receptors on sympathetic nerves. Naunyn-Schmiedeberg's Arch. Pharmacol., 324, 116-124.

Erspamer, V. (1966) Peripheral, physiological and pharmacological actions of indole alkylamines. In: Handbook of Experimental Pharmacology, Vol. 14, V. Erspamer (editor). Springer-Verlag, Berlin, pp 245-359.

Erspamer, V. and Asero, B. (1952) Identification of enteramine, the specific hormone of the enterochromaffin cell system, as 5-hydroxytryptamine. Nature, 169, 800-801.

Fastier, F.N., McDowall, M.A. and Waal, H. (1959) Pharmacological properties of phenyldiguanide and other amidine derivatives in relation to those of 5-hydroxytryptamine. Br. J. Pharmacol., 14, 527-535.

Feniuk, W. and Humphrey, P.P.A. (1990) Putative agonists and antagonists at 5-HT_1-like receptors. In: Saxena, P.R., Wallis, D.I., Wouters, W. and Bevan, P. eds. Cardiovascular Pharmacology of 5-Hydroxytryptamine: Prospective Therapeutic Applications. Dordrecht: Kluwer Academic Publishers, pp.69-80.

Feniuk, W., Hare, J. and Humphrey, P.P.A. (1981) Analysis of the mechanism of 5-hydroxytryptamine-induced vasopressor responses in ganglion-blocked anaesthetized dogs. J. Pharm. Pharmacol., 33, 155-160.

Feniuk, W., Humphrey, P.P.A. and Watts, A.D. (1983) 5-Hydroxytryptamine-induced relaxation of isolated mammalian smooth muscle. Eur. J. Pharmacol., 96, 71-78.

Feniuk, W., Humphrey, P.P.A., Perren, M.J., Connor, H.E. and Whalley, E.T. (1991) Rationale for the use of 5-HT_1-like agonists in the treatment of migraine. J. Neurol., 238(Suppl.1), S57-S61.

Ferrari, M.D. (1991) 5-HT_3 receptor antagonists and migraine therapy. J. Neurol., 238(Suppl.1), S53-S56.

Fillion, G.M.B., Lluch, S. and Uvnas, B. (1971) Release of noradrenaline from the dog heart in situ after intravenous and intracoronary administration of 5-hydroxytryptamine. Acta Physiol. Scand., 83, 115-123.

Fillion, G.M.B.,Rouselle, J-C.,Fillion, M-P.,Beaudoin, D.M.,Goiny, M.R.,Deniau, J-M. and Jacob, J.J. (1978) High affinity binding of [^{3}H]-5-hydroxytryptamine to brain synaptosomal membranes: comparison with [^{3}H]-lysergic acid diethylamide binding. Molec. Pharmacol., 14, 50-59.

Fozard, J.R. (1984a) Neuronal 5-HT receptors in the periphery. Neuropharmacology, 23, 1473-1486.

Fozard, J.R. (1984b) MDL 72222, a potent and highly selective antagonist at neuronal 5-hydroxytryptamine receptors. Naunyn-Schmiedeberg's Arch. Pharmacol., 326, 36-44.

Fozard, J.R. (1990) Agonists and antagonists at 5-HT_3 receptors. In: Saxena, P.R., Wallis, D.I., Wouters, W. and Bevan, P. eds. Cardiovascular Pharmacology of 5-Hydroxytryptamine: Prospective Therapeutic Applications. Dordrecht: Kluwer Academic Publishers, pp.101-116.

Fozard, J.R. and Mwaluko, G.M.P. (1976) Mechanism of the indirect sympathomimetic effect of 5-hydroxytryptamine on the isolated heart of the rabbit. Br. J. Pharmacol., 57, 115-125.

Fozard, J.R., Mir, A.K. and Middlemiss, D.N. (1987) Cardiovascular response to 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) in the rat: site of action and pharmacological analysis. J. Cardiovasc. Pharmacol., 9, 328-347.

Frenken, M. and Kaumann, A.J. (1984) Interaction of ketanserin and its metabolite ketanserinol with 5-HT_2 receptors in pulmonary and coronary arteries of calf. Naunyn-Schmiedeberg's Arch. Pharmacol., 326, 334-339.

Gaddum, J.H. and Picarelli, Z.P. (1957) Two kinds of tryptamine receptors. Br. J. Pharmacol., 12, 323-328.

Galzin, A.M., Blier, P., Chodkiewicz, J.P., Poirier, M.F., Loo, H., Roux, F.X., Redondo, A., Lista, A., Ramdine, R. and Langer, S.Z. (1988) Pharmacological characterization of the serotonin 5-HT autoreceptor modulating the electrically evoked release of ^{3}H-5-HT from slices of human frontal cortex. Soc. Neurosci. Abstr., 14, 129.7.

Gershon, M.D., Dreyfus, C.F., Pickel, V.M., Joh, T.H. and Reis, D.J. (1977). Serotonergic neurons in the peripheral nervous system. Identification in gut by immunohistochemical localization of tryptophan hydroxylase. Proc. Natl. Acad. Sci. U.S.A., 74, 3086-3089.

Gershon, M.D., Mawe, G.M. and Branchek, T.A. (1990) 5-Hydroxytryptamine neurotransmission in the gut. In: Saxena, P.R., Wallis, D.I., Wouters, W. and Bevan, P., eds. Cardiovascular Pharmacology of 5-Hydroxytryptamine: Prospective Therapeutic Applications. Dordrecht: Kluwer Academic Publishers, pp.191-210.

Gillis, R.A., Kellar, K.J., Quest, J.A., Namath, I.J., Martino-Barrows, H.K., Gatti, P.J. and Dretchen, K. (1988) Experimental studies on the neurocardiovascular effects of urapidil. Drugs, 35 (Suppl. 6), 20-33.

Ginzel, K.H. (1958) The effects of 5-hydroxytryptamine on peripheral receptors of cardiovascular and respiratory reflexes. In: Lewis, G.P., ed. 5-Hydroxytryptamine. London: Pergamon, pp.131-135.

Glennon, R.A. (1990) Serotonin receptors: Clinical implications. Neurosci. Biobehav. Rev., 14, 35-47.

Glusa, E. and Markwardt F. (1984) Studies on 5-hydroxytryptamine receptors on isolated human femoral veins and arteries and the influence of dihydroergotamine. Pharmacology, 29, 336-342.

González, R. and García, M. (1977) Sobre el mecanismo de acción de la serotonina en la aurícula aislada de hamster. I. Serotonina: amina de acción mixta. Acta Physiol. Lat. Am., 27, 239-247.

González, R. and García, M. (1978) Lack of α-adrenergic component in the chronotropic action of serotonin on isolated atrium of hamster. Pol. J. Pharmacol., 30, 425-429.

Göthert, M. and Dührsen, U. (1979) Effects of 5-hydroxytryptamine and related compounds on the sympathetic nerves of the rabbit heart. Naunyn-Schmiedeberg's Arch. Pharmacol., 308, 9-18.

Göthert, M. and Schlicker, E. (1990) Identification and classification of 5-HT$_1$ receptor subtypes. J. Cardiovasc. Pharmacol., 15 (Suppl. 7), S1-S7, 1990.

Göthert, M., Fink, K., Molderings, G. and Schlicker, E. (1990) 5-Hydroxytryptamine and related drugs and transmitter release from autonomic nerves in the cardiovascular system. In: Saxena, P.R., Wallis, D. Wouters, W. and Bevan, P., eds. Cardiovascular Pharmacology of 5-Hydroxytryptamine. Prospective Therapeutic Applications. Dordrecht: Kluwer Academic Publishers. pp. 285-294.

Göthert, M., Schlicker, E. and Kollecker, P. (1986) Receptor-mediated effects of serotonin and 5-methoxytryptamine on noradrenaline release in the vena cava and in the heart of the pithed rat. Naunyn-Schmiedeberg's Arch. Pharmacol., 332, 124-130.

Gradin, K., Petterson, A., Hedner, T. and Persson, B. (1985a) Acute administration of 8-hydroxy-2-(di-N-propylamino)-tetralin (8-OH-DPAT), a selective 5-HT receptor agonist, causes a biphasic blood pressure response and a bradycardia in the normotensive Sprague-Dawley rat and in the spontaneously hypertensive rat. J. Neural. Transm., 62, 305-319.

Gradin, K., Petterson, A., Hjorth, S., Hedner, T., Arvidsson, L.E. and Persson B. (1985b) Cardiovascular effects in the Sprague-Dawley rat of 8-hydroxy-2(di-N-propylamino) tetralin, a selective 5-hydroxytryptamine receptor agonist. J. Pharm. Pharmacol., 37, 263-265.

Graham, J.R. and Wolff, H.G. (1938) Mechanism of migraine headache and action of ergotamine tartrate. Arch. Neurol. Psychiatry, 39, 737-763.

Grimson, B.S., Robinson, S.C., Danford, E.T., Tindall, G.T. and Greenfield, J.C. (1969) Effect of serotonin on internal and external carotid artery blood flow in the baboon. Am. J. Physiol., 216, 50-55.

Hageman, G.R., Urthaler, F. and James, T.N. (1977) Cyproheptadine blockade of a cardiogenic hypertensive chemoreflex. Proc. Soc. Exp. Biol. Med., 154, 578-581.

Hageman, G.R., Urthaler, F., Schwatzell, R.H. and James, T.N. (1980) Analysis of sympathetic discharges during cardiogenic hypertensive chemoreflex. Am. J. Physiol., 238, H61-H65.

Ham, A.W. (1974) Pancreas, liver and gallbladder. In: Histology, 7th edition, A.W. Ham, (editor). J.B. Lippincott Company Philadelphia, 681-716.

Hamlin, K.E. and Fischer, F.E. (1951) The synthesis of 5-hydroxytryptamine. J. Am. Chem. Soc., 73, 5007-5008.

Harris, P., Fritts, H.W. and Cournand A. (1960) Some circulatory effects of 5-hydroxytryptamine in man. Circulation, 21, 1134-1139.

Hartig, P.R. (1989) Molecular biology of 5-HT receptors. Trends Pharmacol. Sci., 10, 64-69.

Heninger, G.R., Charney, D.S. and Smith, A. (1987) Effects of serotonin receptor agonists and antagonists on neuroendocrine function in rhesus monkeys. Soc. Neurosci. Abstracts, 13, 801.

Heuring, R.E. and Peroutka, S.J. (1987) Characterization of a novel [^{3}H]5-hydroxytryptamine binding site subtype in bovine brain membranes. J. Neurosci., 7, 894-903.

Heyck, H. (1969) Pathogenesis of migraine. Res. Clin. Stud. Headache, 2, 1-28.

Higgins, G.A., Kilpatrick, G.J., Bunce, K.T., Jones, B.J. and Tyers, M.B. (1989) 5-HT_3 receptor antagonists injected into the area postrema inhibit cisplastin-induced emesis in the ferret. Br. J. Pharmacol., 97, 247-255.

Hirose, K. (1918) Relation between the platelet count of human blood and its vasoconstrictor action after clotting. Arch. Int. Med., 21, 604-612.

Hof, R.P. and Fozard, J.R. (1989) 8-OH-DPAT, flesinoxan and guanfacine: systemic and regional haemodynamic effects of centrally acting antihypertensive agents in anaesthetized rabbit. Brit. J. Pharmacol., 96, 864-871.

Hollander, W., Michelson, A.L. and Wilkins, R.W. (1957) Serotonin and antiserotonins. I. Their circulatory, respiratory and renal effects in man. Circulation, 16, 246-255.

Holt, S.E., Cooper, M. and Wyllie, J.H. (1986) On the nature of the receptor mediating the action of 5-hydroxytryptamine in potentiating responses of the mouse urinary bladder to electrical stimulation. Naunyn-Schmiedeberg's Arch. Pharmacol., 334, 333-340.

Hong, E. and Villalón, C.M. (1988) External carotid vasodilatation induced by serotonin and indorenate. Proc. West Pharmacol. Soc., 31, 99-101.

Houston, D.S. and Vanhoutte, P.M. (1986) Serotonin and the cardiovascular system. Role in health and disease, and implications for therapy. Drugs, 31, 149-163.

Houston, D.S. and, Vanhoutte, P.M. (1988) Comparison of serotonergic receptor subtypes on the smooth muscle and endothelium of the canine coronary artery. J. Pharmacol. Exp. Ther., 244, 1-10.

Hoyer, D. (1988a) Functional correlates of serotonin 5-HT_1 recognition sites. J. Receptor Res., 8, 59-81.

Hoyer, D. (1988b) Molecular pharmacology and biology of 5-HT_{1C} receptors. Trends Pharmacol. Sci., 9, 89-94

Hoyer, D. (1990) Serotonin 5-HT_3, 5-HT_4, and 5-HT-M receptors. Neuropsychopharmacology, 3, 371-383.

Hoyer, D., Engel, G. and Kalkman, H.O. (1985) Molecular pharmacology of 5-HT_1 and 5-HT_2 recognition sites in rat and pig brain membranes. Radioligand binding studies with[^{3}H]-5-HT, [^{3}H]-8-OH-DPAT, (-)[^{125}I]iodo-cyanopindolol, [^{3}H]-mesulergineand [^{3}H]-ketanserin. Eur. J. Pharmacol., 118, 13-23.

Hoyer, D., Pazos, A., Probst, A. and Palacios, J.M. (1986a) Serotonin receptors in human brain. I. Characterization and autoradiographic localization of 5-HT_{1A} recognition sites. Apparent absence of 5-HT_{1B} recognition sites. Brain Res., 376, 85-96.

Hoyer, D., Pazos, A., Probst, A. and Palacios, J.M. (1986b) Serotonin receptors in the human brain. II. Characterization and autoradiographic localization of 5-HT_{1C} and 5-HT_2 recognition sites. Brain Res., 376, 97-107.

Humphrey, P.P.A. and Feniuk, W. (1989) Pharmacological characterization of functional neuronal receptors for 5-hydroxytryptamine. In: Nobin, A., Owman, C. and Arneklo-Nobin, B., eds. Neuronal Messengers in Vascular Function. Amsterdam: Elsevier Science Publishers, pp.3-19.

Humphrey, P.P.A., Apperley, E., Feniuk, W. and Perren, M.J. (1990) A rational approach to identifying a fundamentally new drug for the treatment of migraine. In: Saxena, P.R., Wallis, D.I., Wouters, W. and Bevan, P., eds. Cardiovascular Pharmacology of 5-Hydroxytryptamine: Prospective Therapeutic Applications. Dordrecht: Kluwer Academic Publishers, pp.417-431.

Humphrey, P.P.A., Feniuk, W., Perren, M.J., Connor, H.E., Oxford, A. W., Coates, I.H. and Butina, D. (1988) GR 43175, a selective agonist for the 5-HT_1-like receptor in dog isolated saphenous vein. Br. J. Pharmacol., 94, 1123-1132.

Imaizumi, Y., Baba, M., Imaizumi, Y. and Watanabe, M. (1984) Involvement of the endothelium in the relaxation of isolated chick jugular vein by 5-hydroxytryptamine. Eur. J. Pharmacol., 97, 335-336.

Ireland, S.J. and Tyers, M.B. (1987) Pharmacological characterization of 5-hydroxytryptamine-induced depolarization of the rat isolated vagus nerve. Br. J. Pharmacol., 90, 229-238.

James, T.N. (1964) The chronotropic action of serotonin studied by direct perfusion of the sinus node. J. Pharmacol. Exp. Ther., 146, 209-214.

James, T.N., Isobe, J.H. and Urthaler, F. (1975) Analysis of components in a cardiogenic hypertensive chemoreflex. Circulation, 52, 179-192.

Janeway, T.C., Richardson, H.B. and Park, E.A. (1918) Experiments on the vasoconstrictor action of blood serum. Arch. Int. Med., 21, 565-603.

Kalkman, H.O., Boddeke, H.W.G.M., Doods, H.N., Timmermans, P.B.M.W.M. and Van Zwieten, P.A. (1983) Hypotensive activity of serotonin receptors agonists in rats is related to their affinity for 5-HT_1 receptors. Eur. J. Pharmacol., 91, 155-156.

Kalkman, H.O., Engel, G. and Hoyer, D. (1984) Three distinct types of serotonergic receptors mediate the triphasic blood pressure response to serotonin in rats. J. Hypertension, 6 (Suppl.2), S421-S428.

Kaumann, A.J. (1985) Two classes of myocardial 5-hydroxytryptamine receptors that are neither 5-HT_1 nor 5-HT_2. J. Cardiovasc. Pharmacol., 7 (Suppl. 7), S76-S78.

Kaumann, A.J., Murray, K.J., Brown, A.M., Frampton, J.E., Sanders, L. and Brown, M.J. (1990a) Heart 5-HT receptors. A novel 5-HT receptor in human atrium. In: Serotonin: From Cell Biology to Pharmacology and Therapeutics., Eds. Paoletti, R., Vanhoutte, P.M., Brunello, N., and Maggi, F.M., Dordrecht: Kluwer Academic Publishers, 347-354.

Kaumann, A.J., Sanders, L., Brown, A.M., Murray, K.J. and Brown, M.J. (1990b) A 5-hydroxytryptamine receptor in human atrium. Br. J. Pharmacol., 100, 879-885.

Kaumann, A.J. (1990c) Piglet sinoatrial 5-HT receptors resemble human atrial 5-HT_4-like receptors. Naunyn-Schmiedeberg's Arch. Pharmacol., 342, 619-622

Kerber, C.W. and Newton, T.H. (1973) The macro and microvasculature of the dura mater. Neuroradiology, 6, 175-179.

Kilpatrick, G.J., Jones, B.J. and Tyers, M.B. (1987) Identification and distribution of 5-HT_3 receptors in rat brain using radioligand binding. Nature, 330, 746-748.

Kimura, T. and Satoh, S. (1983) Presynaptic inhibition by serotonin of cardiac sympathetic transmission in dogs. Clin. Exp. Pharmacol. Physiol., 10, 535-542.

Koenig, J.I., Gudelsky, G.A. and Meltzer, H.Y. (1987) Stimulation of corticosterone and ß-endorphin secretion in the rat by selective 5-HT receptor subtype activation. Eur. J. Pharmacol., 137, 1-8.

Kolassa, N., Beller, K.D. and Sanders, K.H. (1989) Evidence for the interaction of urapidil with 5-HT_{1A} receptors in the brain leading to a decrease in blood pressure. Am. J. Cardiol., 63, 36C-39C.

Kottegoda, S.R. and Mott, J.C. (1955) Cardiovascular and respiratory actions of 5-hydroxytryptamine in the cat. Br. J. Pharmacol., 10, 66-72.

Krstic', M.K. (1985) Central serotonergic and tryptaminergic regulation of the cardiovascular system. Period. Biol., 87, 131-140.

Krstic', M.K. and Katusic, Z.S. (1982) Divergent effects of cyproheptadine and R 50656, a 5-HT_2 antagonist, on the cardiovascular responses to 5-hydroxytryptamine in rats. Eur. J. Pharmacol., 85, 225-227.

Kuntz, A. (1953) The autonomic nervous system. Lea and Febiger, Philadelphia.

Lamping, K.G., Marcus, M.L. and Dole, W.P. (1985) Removal of the endothelium potentiates canine large coronary artery constrictor responses to 5-hydroxytryptamine *in vivo*. Circ. Res., 57, 46-54.

Lance, J.W. (1982) Mechanism and management of headache. London: Butterworth.

Lance, J.W., Anthony, M. and Gonski, A. (1967) Serotonin, the carotid body, and cranial vessels in migraine. Arch. Neurol., 16, 553-558.

Lance, J.W., Lambert, G.A., Goadsby, P.J. and Zagami, A.S. (1989) 5-Hydroxytryptamine and its aetiological involvement in migraine. Cephalalgia, 9(Suppl.9), 7-13.

Langer, S.Z. (1980) Presynaptic regulation of the release of catecholamines. Pharmacol. Rev., 32, 337-362.

Lataste, X., Ferrari, M.D., Hirt, D., Notter, M. and Wilkinson, M. (1989) Efficacy and tolerability of ICS 205-930, a 5-HT_3 receptor antagonist, in migraine prophylaxis. Cephalalgia, 9 (Suppl. 10), 346-347.

Leff, P., Martin, G.R. and Morse, J.M. (1987) Differential classification of vascular smooth muscle and endothelial cell 5-HT receptors by use of tryptamine analogues. Br. J. Pharmacol., 91, 321-331.

Lemberger, H.F., Mason, N. and Cohen, M.L. (1984) 5-HT_2 receptors in the rat portal vein: desensitization following cumulative serotonin addition. Life Sci., 35, 71-77.

LeMessurier, D.H., Schwartz, C.J. and Whelan, R.F. (1959) Cardiovascular effects of intravenous infusions of 5-hydroxytryptamine in man. Br. J. Pharmacol., 14, 246-250.

Leonhardt, S., Herrick-Davies, K. and Titeler, M. (1989) Detection of a novel serotonin receptor subtype (5-HT_{1E}) in human brain: interaction with a GTP-binding protein. J. Neurochem., 53, 465-471.

Lexchin, J.L., Cude-Simpson, K.D. and Stancer, H.C. (1977) Brain and blood indole metabolites after peripheral administration of ^{14}C-5-HT in rat. Neurochem. Res. 2, 39-50.

Leysen, J.E. (1981) Serotonergic receptors in brain tissue: properties and identification of various ^{3}H-ligand binding sites *in vitro*. J. Physiol. (Paris), 77, 351-362.

Leysen, J.E. (1985) Serotonergic binding sites. In: Serotonin and the cardiovascular system. Ed. P.M. Vanhoutte, Raven Press, New York. pp 43-62.

Loisy, C., Beorchia, S., Centzone, V., Fozard, J.R., Schechter, P.J. and Tell, G.P. (1985) Effects on migraine headache of MDL 72222, an antagonist at neuronal 5-HT receptors. Double-blind, placebo-controlled study. Cephalalgia, 5, 79-82.

Lorenz, R.R. and Vanhoutte, P.M. (1985) Prejunctional adrenergic inhibition by aggregating platelets in canine blood vessels. Am. J. Physiol., 249, H685-H689.

Lowther, S., De Paermentier, F., Crompton, M.R. and Horton, R.W. (1991) 5-HT_{1D} and 5-HT_{1E} binding sites in post-mortem human brain. Br. J. Pharmacol., 102, 246P.

Lucki, I., Nobler, M.S. and Frazer, A. (1984) Differential actions of serotonin antagonists on two behavioral models of serotonin receptor activation in the rat. J. Pharmacol. Exp. Ther., 228, 133-139.

Lucot, J.B. (1990) RU 24969-induced emesis in the cat: 5-HT_1 sites other than 5-HT_{1A}, 5-HT_{1B} or 5-HT_{1C} implicated. Eur. J. Pharmacol., 180, 193-199.

Main, B.W., Smith, T. and Kurz, K.D. (1984) Cardiovascular activity of 8-hydroxy-2-(di-n-propylamino)-tetralin (8-OH-DPAT) in SHR. Fed. Proc., 43, 556.

Martin, G.E. and Lis, E.V. (1985) Hypertensive action of 8-hydroxy-2-(di-N-propylamino) tetralin (8-OH-DPAT) in spontaneously hypertensive rats. Arch. Int. Pharmacodyn., 273, 251-261.

Martin, G.R., Leff, P., Cambridge, D. and Barrett, V.J. (1987) Comparative analysis of two types of 5-hydroxytryptamine receptor mediating vasorelaxation: differential classification using tryptamines. Naunyn-Schmiedeberg's Arch. Pharmacol., 336, 365-373.

Maxwell, G.M., Castillo, C.A., Clifford, J.E., Crumpton, C.W. and Rowe, G.G. (1959) Effects of serotonin (5-hydroxytryptamine) on the systemic and coronary vascular bed of the dog. Am. J. Physiol., 197, 736-738.

McCall, R.B., Patel, B.N. and Harris L.T. (1987) Effects of $serotonin_1$ and $serotonin_2$ receptor agonists and antagonists on blood pressure, heart rate and sympathetic nerve activity. J. Pharmacol. Exp. Ther., 242, 1152-1159.

McGrath, M.A. (1977) 5-Hydroxytryptamine and neurotransmitter release in canine blood vessels, inhibition by low and augmentation by high concentrations. Circ. Res., 41, 428-435.

McGrath, J.C., MacLennan, S.J. and Stuart-Smith, K. (1985) Characterization of the receptor mediating contraction of human umbilical artery by 5-hydroxytryptamine. Br. J. Pharmacol., 84, 199-202.

McQueen, D.S. (1990) Cardiovascular reflexes and 5-hydroxytryptamine. In: Saxena, P.R., Wallis, D.I., Wouters, W. and Bevan, P. eds. Cardiovascular Pharmacology of 5-Hydroxytryptamine: Prospective Therapeutic Applications. Dordrecht: Kluwer Academic Publishers, pp.233-245.

McQueen, D.S., Ungar and A. (1971) On the direct and crossed components of reflex responses to stimulation of the carotid body chemoreceptors in the dog. J. Physiol., 219, 1-16.

Mena, M.A. and Vidrio, H. (1979) Reversal of serotonin vasodilatation in the dog external carotid bed by sympathetic denervation. J. Cardiovasc. Pharmacol., 1, 149-154.

Mir, A.K. and Fozard, J.R. (1990) 5-Hydroxytryptamine in central cardiovascular regulation. In: Saxena, P.R., Wallis, D.I., Wouters, W. and Bevan, P. eds. Cardiovascular Pharmacology of 5-Hydroxytryptamine: Prospective Therapeutic Applications. Dordrecht: Kluwer Academic Publishers, pp.101-116.

Mohr, B., Bom, A.H., Kaumann, A.J. and Thämer, V. (1987) Reflex inhibition of efferent renal sympathetic nerve activity by 5-hydroxytryptamine and nicotine is elicited by different epicardial receptors. Pflügers, Arch., 409, 145-151.

Molderings, G.J., Fink, K., Schlicker, E. and Göthert, M. (1987) Inhibition of noradrenaline release via presynaptic 5-HT_{1B} receptors of the rat vena cava. Naunyn-Schmiedeberg's Arch. Pharmacol., 336, 245-250.

Molderings, G.J., Göthert, M., Fink, K., Roth, E. and Schlicker, E. (1989) Inhibition of noradrenaline release in the pig coronary artery via a novel serotonin receptor. Eur. J. Pharmacol., 164, 213-222.

Molyneux, G.S. and Harmon, B. (1982) Innervation of the arteriovenous anastomoses in the web of the foot of the domestic duck, Anas platyrhynchos, structural evidence for the presence of non-adrenergic non-cholinergic nerve. Am. J. Anat., 135, 119-128.

Moskowitz, M.A. and Buzzi, M.G. (1991) Neuroeffector functions of sensory fibers: implications for headache mechanisms and drug actions. J. Neurol., 238(Suppl.1), S18-S22.

Müller-Schweinitzer, E. and Engel, G. (1983) Evidence for mediation by 5-HT_2 receptors of 5-hydroxytryptamine-induced contraction of canine basilar artery. Naunyn-Schmiedeberg's Arch. Pharmacol., 324, 287-292.

Mylecharane. E.J. (1990) Agonists and antagonists at 5-HT_2 receptors. In: Saxena, P.R., Wallis, D.I., Wouters, W. and Bevan, P. eds. Cardiovascular Pharmacology of 5-Hydroxytryptamine: Prospective Therapeutic Applications. Dordrecht: Kluwer Academic Publishers, pp.81-100.

Mylecharane, E.J. and Phillips, C.A. (1989) Pre-synaptic sympathetic inhibition and 5-hydroxytryptamine-induced vasodilatation. In: Mylecharane, E.J., Angus, J.A., De la Lande, I.S. and Humphrey, P.P.A. eds. Serotonin: Actions, Receptors, Pathophysiology. London: Macmillan Press, pp.136-145.

Noble, J.G. and Nanson, E.M. (1959) An investigation of the effects of 5-hydroxytryptamine (serotonin) on the cardiovascular system of the dog. Ann. Neurosurg., 15, 150, 846-853.

Paintal, A.S. (1977) Effects of drugs on chemoreceptors, pulmonary and cardiovascular receptors. Pharmacol. Ther. Bull., 3, 41-63.

Page, I.H. (1958) Serotonin (5-hydroxytryptamine); the last four years. Physiol. Rev., 38, 227-335.

Page, I.H. and McCubbin, J.W. (1953) Modification of vascular responses to serotonin. Am. J. Physiol., 174, 436-440.

Parsons, A.A., Whalley, E.T., Feniuk, W., Connor, H.E. and Humphrey, P.P.A. (1989) 5-HT_1-like receptors mediate 5-hydroxytryptamine-induced contraction of human isolated basilar artery. Br. J. Pharmacol., 96, 434-440.

Pazos, A., Hoyer, D. and Palacios, J.M. (1985) The binding of serotonergic ligands to the porcine choroid plexus: Characterization of a new type of serotonin recognition site. Eur. J. Pharmacol., 106, 539-546.

Pedigo, B.W., Yamamura, H.I. and Nelson, D.L. (1981) Discrimination of multiple serotonin binding sites by the neuroleptic spiperone in rat brain. J. Neurochem., 36, 220-226.

Peroutka, S.J. (1986) Pharmacological identification of 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1C} binding sites in rat frontal cortex. J. Neurochem., 47, 529-540.

Peroutka, S.J. (1990) The pharmacology of current anti-migraine drugs. Headache, 30, 5-11.

Peroutka, S.J. and Kuhar, M.J. (1984) Autoradiographic localization of 5-HT_1 receptors to human and canine basilar arteries. Brain. Res., 310, 193-196.

Peroutka, S.J. and Snyder, S.H. (1979) Multiple serotonin receptors: differential binding of [^{3}H]-5-hydroxytryptamine, [^{3}H]-lysergic acid diethylamide and [^{3}H]-spiroperidol. Molec. Pharmacol., 16, 687-699.

Peroutka, S.J., Noguchi, M., Tolner, D.J. and Allen, G.A. (1983) Serotonin-induced contraction of canine basilar artery: Mediation by 5-HT_1 receptors. Brain. Res., 259, 327-330.

Perren, M.J., Feniuk, W. and Humphrey, P.P.A. (1989) The selective closure of feline carotid arteriovenous anastomoses by GR43175. Cephalalgia, 9 (Suppl. 9), 41-46.

Perren, M.J., Feniuk, W. and Humphrey, P.P.A. (1991) Vascular 5-HT_1-like receptors that mediate contraction of the dog isolated saphenous vein and carotid arterial vasoconstriction in anaesthetized dogs are not of the 5-HT_{1A} or 5-HT_{1D} subtype. Br. J. Pharmacol., 102, 191-197.

Perrin, V.L., Färkkilä, M., Goasguen, J., Doenicke, A., Brand, J. and Tfelt-Hansen, P. (1989) Overview of initial clinical studies with intravenous and oral GR43175 in acute migraine. Cephalalgia, 9 (Suppl. 9), 63-72.

Ramage, A. (1985a) Effects of ritanserin and cinanserin on the cardiovascular system of the cat. Br. J. Pharmacol., 86, 598P.

Ramage, A. (1985b) The effects of ketanserin, methysergide and LY 53857 on sympathetic nerve activity. Eur. J. Pharmacol., 113, 295-303.

Ramage, A.G. (1986) A comparison of the effects of doxazosin and alfuzosin with those of urapidil on preganglionic nerve activity in anaesthetized cats. Eur. J. Pharmacol., 129, 307-314.

Ramage, A.G. and Fozard, J.R. (1987) Evidence that the putative 5-HT_{1A} receptor agonists, 8-OH-DPAT and ipsapirone, have a central hypotensive action that differs from that of clonidine in anaesthetized cats. Eur. J. Pharmacol., 38, 179-191.

Ramage, A.G. and Wilkinson, S.J. (1988) Evidence for differentiation of sympathoinhibitory action of 8-OH-DPAT and clonidine in anaesthetized cats. Br. J. Pharmacol., 93, 121P.

Ramage, A.G., Wouters, W. and Bevan, P. (1988) Evidence that the novel antihypertensive agent flesinoxan causes differential sympathoinhibition and also increases vagal tone by a central action. Eur. J. Pharmacol., 151, 373-379.

Rapport, M.M. (1949) Serum vasoconstrictor (serotonin). V. The presence of creatinine in the complex: a proposed study of the vasoconstrictor principle. J. Biol. Chem., 180, 961-969.

Rapport, M.M., Green, A.A. and Page, I.H. (1948a) Partial purification of the vasoconstrictor in beef serum. J. Biol. Chem., 174, 735-741.

Rapport, M.M., Green, A.A. and Page, I.H. (1948b) Serum vasoconstrictor (serotonin). IV. Isolation and characterization. J. Biol. Chem., 176, 1243-1251.

Reid, G. (1952) Circulatory effects of 5-hydroxytryptamine. J. Physiol., 118, 435-453.

Richardson, B.P., Engel, G., Donatsch, P. and Stadler, P.A. (1985) Identification of serotonin M-receptor subtypes and their specific blockade by a new class of drugs. Nature, 316, 126-131.

Roth, R.A. and Gillis, C.N. (1975) Multiple forms of amino oxidase in perfused rabbit lung. J. Pharmacol. Exp. Ther., 194, 537-544.

Rowbotham, G.F. and Little E. (1965) New concepts on the aetiology and vascularization of meningiomata: the mechanism of migraine; the chemical process of cerebrospinal fluid; and the formation of collections of blood or fluid in the subdural space. Brit. J. Surg., 52, 21-24.

Rummo, G. and Bordoni, L. (1889) Toxicité du sérum du sang de l'home et des animaux à l'état normal et dans les maladies par infection. Archiv de Biologie Italiene, Bd. XII, XLVI-XLVII.

Saito, A., Kimura, S. and Goto, K. (1986) Calcitonin gene-related peptide as potential neurotransmitter in guinea pig right atrium. Am. J. Physiol., 250, H693-H698.

Sanders, K.H., Beller, K.D., Bischler, P. and Kolassa, N. (1988) Interaction of urapidil with brain serotonin$_{1A}$ receptors increases the blood pressure reduction due to peripheral alpha-adrenoceptor inhibition. J. Hypertension Suppl., 6, S65-S68.

Sawada, M., Ichinose, M., Ito, I., Maeno, T. and McAdoo D.J. (1984) Effects of 5-hydroxytryptamine on membrane potential, contractility, accumulation of cyclic AMP, and Ca^{2+} movements in anterior aorta and ventricle of aplysia. J. Neurophysiol., 51, 361-374.

Saxena, P.R. (1972) The effects of antimigraine drugs on the vascular responses by 5-hydroxytryptamine and related biogenic substances on the external carotid bed of dogs: Possible pharmacological implications to their antimigraine action. Headache, 12, 44-54.

Saxena, P.R. (1978) Arteriovenous shunting and migraine. Res. Clin. Stud. Headache, 6, 89-102.

Saxena, P.R. (1986) Nature of the 5-hydroxytryptamine receptors in mammalian heart. Progr. Pharmacol., 6, 173-185.

Saxena, P.R. (1987) The arteriovenous anastomoses and veins in migraine research. In: Migraine. Therapeutic, Conceptual and Research Aspects. Ed. J.N. Blau. (Elsevier, Amsterdam), p.581-596.

Saxena, P.R. (1988) Further characterization of 5-hydroxytryptamine$_1$-like receptors mediating tachycardia in the cat: No apparent relationship to known subtypes of the 5-hydroxytryptamine$_1$ binding site. Drug Dev. Res., 13, 245-258.

Saxena, P.R. (1989) Cardiovascular effects from stimulation of 5-hydroxytryptamine receptors. Fundam. Clin. Pharmacol., 3, 245-265.

Saxena, P.R. (1990) 5-Hydroxytryptamine and migraine. In: Saxena, P.R., Wallis, D.I., Wouters, W. and Bevan, P. eds. Cardiovascular Pharmacology of 5-Hydroxytryptamine: Prospective Therapeutic Applications. Dordrecht: Kluwer Academic Publishers, pp.407-416.

Saxena, P.R. (1991) 5-HT in migraine -and introduction. J. Neurol., 238(Suppl.1), S36-S37.

Saxena, P.R. and De Vlaam-Schluter, G.M. (1974) Role of some biogenic substances in migraine and relevant mechanism in antimigraine action of ergotamine -studies in an experimental model for migraine. Headache, 13, 142-162.

Saxena, P.R. and Den Boer, M.O. (1991) Pharmacology of antimigraine drugs. J. Neurol., 238(Suppl.1), S28-S35.

Saxena, P.R. and Ferrari, M.D. (1989) 5-Hydroxytryptamine$_1$-like receptor agonists and the pathophysiology of migraine. Trends Pharmacol. Sci., 10, 200-204.

Saxena, P.R. and Lawang, A. (1985) A comparison of cardiovascular and smooth muscle effects of 5-hydroxytryptamine and 5-carboxamidotryptamine, a selective agonist of 5-HT_1 receptors. Arch. Int. Pharmacodyn., 277, 235-252.

Saxena, P.R. and Verdouw, P.D. (1982) Redistribution by 5-hydroxytryptamine of carotid arterial blood at the expense of arteriovenous blood flow. J. Physiol. (London), 332, 501-520.

Saxena, P.R. and Verdouw, P.D. (1983) Reduction of the arteriovenous anastomotic fraction of carotid blood flow by 5-hydroxytryptamine. In: Bevan, J.A. et al., ed. Vascular Neuroeffector Mechanisms: 4th International Symposium. New York: Raven Press, pp. 265-271.

Saxena, P.R. and Verdouw, P.D. (1984) Effects of methysergide and 5-hydroxytryptamine on carotid blood flow distribution in pigs: further evidence for the presence of atypical 5-HT receptors. Br. J. Pharmacol., 82, 817-826.

Saxena, P.R. and Verdouw, P.D. (1985a) 5-Carboxamide tryptamine, a compound with high affinity for 5-hydroxytryptamine$_1$ binding sites, dilates arterioles and constricts arteriovenous anastomoses. Br. J. Pharmacol., 84, 533-544.

Saxena, P.R. and Verdouw, P.D. (1985b) Tissue blood flow and localization of arteriovenous anastomoses in pigs with microspheres of four different sizes. Pflügers Arch., 403, 128-135.

Saxena, P.R. and Villalón, C.M. (1990a) Cardiovascular effects of serotonin agonists and antagonists. J. Cardiovasc. Pharmacol., 15 (Suppl. 7), S17-S34.

Saxena, P.R. and Villalón, C.M. (1990b) 5-HT$_{1A}$ receptor agonism: a novel mechanism for antihypertensive action. Trends Pharmacol. Sci., 11, 95-96.

Saxena, P.R. and Villalón, C.M. (1991) 5-Hydroxytryptamine, a chameleon in the heart. Trends Pharmacol. Sci., 12, 223-227.

Saxena, P.R., Duncker, D.J., Bom, A.H., Heiligers, J. and Verdouw, P.D. (1986a) Effects of MDL 72222 and methiothepin on carotid vascular responses to 5-hydroxytryptamine in the pig: evidence for the presence of vascular 5-hydroxytryptamine$_1$-like receptors. Naunyn-Schmiedeberg's Arch. Pharmacol., 333, 198-204.

Saxena, P.R., Forsyth, R.P., Johnston, B.M. and De Werk, A. (1978) Regional and systemic haemodynamic changes evoked by 5-hydroxytryptamine in awake and anaesthetized rabbits. Eur. J. Pharmacol., 50, 61-68.

Saxena, P.R., Heiligers, J., Mylecharane, E.J. and Tio, R. (1985a) Excitatory 5-hydroxytryptamine receptors in the cat urinary bladder are of the M- and 5-HT$_2$-type. J. Autonom. Pharmacol., 5, 101-107.

Saxena, P.R., Mylecharane, E.J. and Heiligers, J. (1985b) Analysis of the heart rate effects of 5-hydroxytryptamine in the cat; mediation by '5-HT$_1$-like' receptors. Naunyn-Schmiedeberg's Arch. Pharmacol., 330, 121-129.

Saxena, P.R., Koedam, N.A., Heiligers, J. and Hof, R.P. (1983) Ergotamine-induced constriction of cranial arteriovenous anastomoses in dogs pretreated with phentolamine and pizotifen. Cephalalgia, 3, 71-81.

Saxena, P.R., Richardson, B., Mylecharane, E.J., Middlemiss, D.N., Humphrey, P.P.A., Fozard, J.R., Feniuk, W., Engel, G. and Bradley, P.B. (1986b) Functional receptors for 5-hydroxytryptamine. Trends Pharmacol. Sci., 7 (7), Centerfold.

Saxena, P.R., Van Houwelingen, P. and Bonta, I.L. (1971) The effects of myanserin hydrochloride on the vascular responses evoked by 5-hydroxytryptamine and related vasoactive substances. Eur. J. Pharmacol., 13, 295-305.

Schipper, J. and Tulp, M.T.M. (1988) Serotonin autoreceptors in guinea-pig cortex slices resemble the 5-HT_{1D} binding site. Soc. Neurosci. Abstr. 14, 221.8.

Schlicker, E., Fink, K., Göthert, M., Hoyer, D., Molderings, G., Roschke, I. and Schoeffter, P. (1989) The pharmacological properties of the presynaptic serotonin autoreceptor in the pig brain cortex conform to the 5-HT_{1D} receptor subtype. Naunyn-Schmiedeberg's Arch. Pharmacol., 340, 45-51.

Schmidt, A. W. and Peroutka, S.J. (1989) 5-Hydroxytryptamine receptor "families". The FASEB Journal, 3, 2242-2249.

Schneider, J.A. and Yonkman, F.F. (1954) Species differences in the respiratory and cardiovascular response to serotonin (5-hydroxytryptamine). J. Pharmacol. Exp. Ther., 111, 84-98.

Schoeffter, P. and Hoyer, D. (1990) 5-Hydroxytryptamine (5-HT)-induced endothelium-dependent relaxation of pig coronary arteries is mediated by 5-HT receptors similar to the 5-HT_{1D} receptor subtype. J. Pharmacol. Exp. Ther., 252, 387-395.

Schoeffter, P., Waeber, C., Palacios, J.M. and Hoyer, D. (1988) The 5-hydroxytryptamine 5-HT_{1D} receptor subtype is negatively coupled to adenylate cyclase in calf substantia nigra. Naunyn-Schmiedeberg's Arch. Pharmacol., 337, 602-608.

Sicuteri, F. (1977) Dopamine, the second putative protagonist in headache. Headache, 17, 129-131.

Skinner, S.L. and Whelan, R.F. (1962) Carotid body stimulation by 5-hydroxytryptamine in man. J. Physiol., 162, 35-43.

Smit, J.F.M., Essen, H., Struyker-Boudier, H.A.J. (1978) Serotonin-mediated cardiovascular responses to electrical stimulation of raphe nuclei in the rat. Life Sci., 23, 173-178.

Sukamoto, T.T., Yamamoto, S., Watanabe, S. and Ueki, S. (1984) Cardiovascular responses to centrally administered serotonin in conscious normotensive and spontaneously hypertensive rats. Eur. J. Pharmacol., 100, 173-179.

Takacs, L. and Vajda, V. (1963) Effects of serotonin on cardiac output and organ blood flow of rats. Am. J. Physiol., 204, 301-303.

Taylor, E.W., Duckles, S.P. and Nelson, D.L. (1986) Dissociation constants of serotonin agonists in the canine basilar artery correlate to K_i values at 5-HT_{1A} binding site. J. Pharmacol. Exp. Ther., 236, 118-125

Tfelt-Hanssen, P. and Nielsen, T.H. (1990) Treatment of migraine with drugs related to 5-hydroxytryptamine. In: Saxena, P.R., Wallis, D.I., Wouter, W. and Bevan, P. eds. Cardiovascular Pharmacology of 5-hydroxytryptamine: Prospective Therapeutic Applications. Dordrecht: Kluwer Academic Publications, pp.433-441.

Thompson, J.A., Wei, E.P. and Kontos, H.A. (1984) Inhibition by ketanserin of serotonin-induced cerebral arteriolar vasoconstriction. Stroke, 15, 1021-1024.

Thoren, P. (1979) Role of cardiac vagal C-fibres in cardiovascular control. Rev. Physiol. Biochem. Pharmacol., 86, 1-94.

Trendelenburg, U. (1960) The action of histamine and 5-hydroxytryptamine on isolated mammalian atria. J. Pharmacol. Exp. Ther., 130, 450-460.

Trevethik, M.A., Feniuk, W. and Humphrey, P.P.A. (1984) 5-Hydroxytryptamine-induced relaxation of neonatal porcine vena cava *in vitro*. Life Sci., 35, 477-486.

Tyers, M.B., Costall, B., Domeney, A.M., Jones, B.J., Kelly, M.E., Naylor, R.J. and Oakley, N.R. (1987) The anxiolytic activities of 5-HT_3 antagonists in laboratory animals. Neurosci. Lett., Suppl. 29, S68.

Vane, J.R. (1969). The release and fate of vasoactive hormones in the circulation. Br. J. Pharmacol., 35, 1989; 209-242.

Van Heuven-Nolsen, D., Tysse Klasen, T.H.M., Luo, Q. and Saxena, P.R. (1990) 5-HT_1-like receptors mediate contractions in the rabbit saphenous vein. Eur. J. Pharmacol., 191, 375-382.

Vanhoutte, P.M. (1990) The endothelium and the role of 5-hydroxytryptamine in vascular disease. In: Saxena, P.R., Wallis, D.I., Wouters, W. and Bevan, P. eds. Cardiovascular Pharmacology of 5-Hydroxytryptamine, Prospective Therapeutic Applications. Dordrecht: Kluwer Academic Publishers, pp. 379-390.

Vanhoutte, P.M., Verbeuren, T.J. and Webb, R.C. (1981) Local modulation of the neuroeffector interaction in the blood vessel wall. Physiol. Rev., 61, 151-247.

Van Nueten, J.M. (1983) 5-Hydroxytryptamine and precapillary vessels. Fed. Proc., 42, 223-227.

Van Nueten, J.M., Leysen, J.E., De Clerck, F. and Vanhoutte, P.M. (1984) Serotonergic receptor subtypes and vascular reactivity. J. Cardiovasc. Pharmacol., 6, S564-S574

Verdouw, P.D., Jennewein, H.M., Heiligers, J., Duncker, D.J. and Saxena, P.R. (1984) Redistribution of carotid artery blood flow by 5-HT: effects of the 5-HT_2 receptor antagonists ketanserin and WAL 1307. Eur. J. Pharmacol., 102, 499-509.

Verdouw, P.D., Jennewein, H.M., Mierau, J. and Saxena, P.R. (1985) N-(3-acetylaminophenil) piperazine hydrochloride (BEA 1654), a putative 5-HT_1 agonist, causes constriction of arteriovenous anastomoses and dilatation of arterioles. Eur. J. Pharmacol., 107, 337-346.

Vidrio, H. and Hong, E. (1976) Vascular tone and reactivity to serotonin in the internal and external carotid vascular beds of the dog. J. Pharmacol. Exp. Ther., 197, 49-56.

Villalón, C.M. and Hong, E. (1988) Inhibitory effect of pelanserin and ketanserin on the external carotid bed vasodilatation induced by serotonin. Proceedings of the International Congress on Cardiovascular Pharmacology of 5-HT. Amsterdam, October 4-7, 1988. Abstract No. 65.

Villalón, C.M., Bom, A.H., Heiligers, J., Den Boer, M.O. and Saxena, P.R. (1990a) Constriction of porcine arteriovenous anastomoses by indorenate is unrelated to 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} or 5-HT_{1D} receptor subtype. Eur. J. Pharmacol., 190, 167-176.

Villalón, C.M., Den Boer, M.O., Heiligers, J.P.C. and Saxena, P.R. (1990b) Mediation of 5-hydroxytryptamine-induced tachycardia in the pig by the putative 5-HT_4 receptor. Br. J. Pharmacol., 100, 665-668.

Villalón, C.M., Den Boer, M.O., Heiligers, J.P.C. and Saxena, P.R. (1991a) Further characterization, using tryptamine and benzamide derivatives, of the putative 5-HT_4 receptor mediating tachycardia in the pig. Br. J. Pharmacol., 102, 107-112.

Villalón, C.M., Den Boer, M.O., Heiligers, J.P.C. and Saxena, P.R. (1991b) The 5-HT_4 receptor mediating tachycardia in the pig. In: Serotonin: Molecular Biology, Receptors and Function. ed. Fozard, J.R. and Saxena, P.R. Basel: Birkhauser, pp. 253-263.

Villalón, C.M., Den Boer, M.O., Heiligers, J.P.C., Verdouw, P.D., Duncker, D.J.G.M. and Saxena, P.R. (1991c) Role of the putative 5-HT_4 receptor in positive inotropic effect of 5-HT in the pig. Fund. Clin. Pharmacol., (In Press).

Villalón, C.M., Bom, A.H., Den Boer, M.O., Heiligers, J.P.C. and Saxena, P.R. (1991d) Effects of S9977 and dihydroergotamine in an experimental model for migraine. Pharmacol. Res., (In Press).

Waeber, C., Schoeffter, P., Palacios, J.M. and Hoyer, D. (1988) Molecular pharmacology of 5-HT_{1D} recognition sites: Radioligand binding studies in human, pig and calf brain membranes. Naunyn-Schmiedeberg's Arch. Pharmacol., 337, 595-601.

Walker, R.J. (1985) The pharmacology of serotonin receptors in invertebrates. In: Green, A.R., ed. Neuropharmacology of Serotonin. Oxford: Oxford University Press, pp.366-408.

Wallis, D.I. and North, R.A. (1978) The action of 5-hydroxytryptamine on single neurons of the rabbit cervical ganglion. Neuropharmacol., 17, 1023-1028.

Weiss, O. (1896) Ueber die Wirkungen von Blutserum-Injectionen ins Blut. Archiv für die Gesamte Physiologie des Menschen und der Thiere, LXV, 215-230.

Wilson, H. Coffman, W.J. and Cohen, M.L. (1990) 5-Hydroxytryptamine$_3$ receptors mediate tachycardia in conscious instrumented dogs. J. Pharmacol. Exp. Ther., 252, 683-688.

Wolf, W.A., Kuhn, D.M. and Lovenberg, W. (1985) Serotonin and central regulation of arterial blood pressure. In: Vanhoutte, P.M., ed. Serotonin and the Cardiovascular System. New York: Raven Press, pp.63-73.

Wolff, H.S. (1963) Headache and other head pain. Oxford University Press, Oxford.

Wouters, W., Hartog, J. and Bevan, P. (1988a) Flesinoxan. Cardiovasc. Drug Rev., 6, 71-83.

Wouters, W., Tulp, M.Th. M. and Bevan, P. (1988b) Flesinoxan lowers blood pressure and heart rate in cats via 5-HT$_{1A}$ receptors. Eur. J. Pharmacol., 149, 213-223.

Wright, C.E. and Angus, J.A. (1989) 5-Carboxamidotryptamine elicits 5-HT$_2$ and 5-HT$_3$ receptor-mediated cardiovascular responses in the conscious rabbit: evidence for 5-HT release from platelets. J. Cardiovasc. Pharmacol., 13, 557-564.

Yagaloff, K.A. and Hartig, P.R. (1985) ^{125}I-Lysergic acid diethylamide binds to a novel serotonergic site on rat choroid plexus epithelial cells. J. Neurosci., 5, 3178-3183.

Young, A.R., Hamel, E., MacKenzie, E.T. (1986) Recent studies on the serotonergic innervation of the cerebral circulation. In: Owman, C., Hardebo, J.E., eds. Neural regulation of brain circulation. Amsterdam: Elsevier Science Publishers, B.V., pp.195-217.

Yusof, A.P.M. and Coote, J.H. (1988) Excitatory and inhibitory actions of intrathecally administered 5-hydroxytryptamine on sympathetic nerve activity in the rat. J. Autonom. Nerv. Syst., 22, 229-236.

Zakusov, V.V. (1962) Principles of pharmacological influence on coronary chemoreflexes. Arch. Int. Pharmacodyn., 140, 646-654.

Zinner, M.J., Kasher, F. and Jaffe, B.M. (1983) The haemodynamic effect of intravenous infusions of serotonin in conscious dogs. J. Surg. Res., 34, 171-178.

SUMMARY

AND

SAMENVATTING

SUMMARY

The biogenic amine 5-hydroxytryptamine (5-HT; serotonin) seems to play a role in several (patho)physiological processes (Chapter 1). The present thesis describes the effects of 5-HT-related drugs on heart rate, cardiac contractility and carotid blood flow distribution in anaesthetized pigs. Using "selective" agonists and antagonists the 5-HT receptor (sub)types involved in the 5-HT-induced changes in the above haemodynamic variables were characterized, taking into account the most recent advances in the classification of 5-HT receptors (Chapter 2).

After delving into the literature about the general cardiovascular effects of 5-HT and related drugs (Chapter 3) and their effects on heart rate and cardiac contractility in several species (Chapter 4), the receptors involved in 5-HT-induced positive chronotropic (Chapters 7 and 8) and positive inotropic effects (Chapter 9) were delineated in the anaesthetized pig. Intravenous (i.v.) bolus injections of 5-HT induced dose-dependent increases in heart rate, which were mimicked (in order of potency) by the tryptamine derivatives 5-methoxytryptamine and α-methyl-5-HT, and by the substituted benzamide derivatives renzapride, zacopride, cisapride, metoclopramide and dazopride. These benzamide derivatives behaved as partial agonists, antagonizing the increases in heart rate by 5-HT and 5-methoxytryptamine. Likewise, the selective 5-HT_3 receptor antagonists MDL 72222 and granisetron as well as the selective D_2 receptor antagonist domperidone (3 mg/kg each) all failed to antagonize the tachycardiac responses induced by 5-HT and 5-methoxytryptamine. In marked contrast, the increases in heart rate induced by either 5-HT, tryptamine- or benzamide derivatives were clearly antagonized by high doses (1-3 mg/kg, i.v.) of ICS 205-930, without affecting the tachycardiac responses to isoprenaline. These findings suggest that 5-HT and the tryptamine- and benzamide derivatives increase porcine heart rate by a direct action on the cardiac pacemaker, via the activation of a putative 5-HT_4 receptor. Similarly, 5-HT (i.v.) induced also an increase in the maximum rate of rise in left ventricular pressure ($LVdP/dt_{max}$) and cardiac output (CO) either at the animal's own sinus rhythm or during atrial pacing (150 beats/min), suggesting a positive inotropic effect, which was mimicked by 5-methoxytryptamine and, to a lesser extent, by renzapride. As previously described, renzapride behaved as a partial agonist, antagonizing the increases in $LVdP/dt_{max}$ and CO induced by 5-HT and 5-methoxytryptamine, but not those by isoprenaline, suggesting the involvement of the putative 5-HT_4 receptor. The pharmacological profile of this porcine myocardial receptor is similar to that of the putative 5-HT_4 receptor present in neurons from mouse embryo colliculi, guinea-pig cholinergic neurons and human heart.

Chapter 5 deals with the effects of 5-HT and related drugs in the carotid circulation and its distribution into nutrient (arteriolar) and non-nutrient (arteriovenous anastomotic; AVA) fractions. On the basis of these findings, an attempt was made to further delineate the putative subtype of 5-HT_1-like receptor in the porcine carotid circulation, analyzing the effects of the 5-HT_{1A} receptor agonist indorenate (Chapter 10). Intracarotid infusions of indorenate resulted in dose-related decreases in total common carotid artery blood flow, which were almost exclusively due to a reduction in the cephalic AVA blood flow. These effects of indorenate were not appreciably modified after treatment with the 5-HT_2 receptor antagonist ketanserin, but were markedly reduced after treatment with methiothepin, which antagonizes not only 5-HT_2 receptors, but also the putative 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} and 5-HT_{1D} subtypes of 5-HT_1-like receptors. Nevertheless, metergoline, a drug with higher affinities than methiothepin for the foregoing 5-HT_1 receptor subtypes, failed to significantly modify the responses to indorenate. Therefore, the above effects of indorenate are mediated by 5-HT_1-like receptors unrelated to either 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} or 5-HT_{1D} binding site subtypes.

Based on the proposal that dilatation of cephalic arteries and arteriovenous anastomoses (AVA's) probably plays a role in the pathophysiology of migraine, and that a number of effective antimigraine drugs, including ergotamine, methysergide, isometheptene and sumatriptan, have a selective carotid vasoconstrictor action which is mainly confined to cephalic AVA's, one might propose the porcine fractionation of carotid arterial blood flow into AVA and arteriolar parts as an experimental model for the screening and development of drugs with potential antimigraine action. With this suggestion in mind, in Chapter 11 we set out to study the effects of a novel trimethylxanthine derivative, S9977, which antagonizes the dopaminergic hypersensitivity and the increase in adenosine receptors induced by food sensitization in the rat (another animal experimental model for migraine), on the fractionation of total common carotid blood flow in anaesthetized pigs. Furthermore, the effects of dihydroergotamine, a clinically established antimigraine drug, were analyzed for comparison. Dihydroergotamine elicited a dose-dependent reduction in AVA blood flow and conductance, and an increase in the arteriolar blood flow and conductance. In marked contrast, S9977 decreased carotid (two highest doses) and AVA (highest dose) blood flow, but there was no concomitant decrease in the vascular conductances. These findings indicate that the effects of S9977 seem to be related to a decrease in arterial blood pressure and not to an active vasoconstriction of AVA's. Therefore, in line with the "shunt theory" of migraine, these results do not predict an antimigraine action of S9977. However, on the basis of other pharmacological effects of the drug, it still remains a possibility.

SAMENVATTING

De biogene amine 5-hydroxytryptamine (5-HT; serotonine) lijkt een rol te spelen in verscheidene (patho)fysiologische processen (Hoofdstuk 1). Dit proefschrift beschrijft de effecten van aan 5-HT gerelateerde stoffen op de hartfrequentie, cardiale contractiliteit en de bloedstroomverdeling over het carotide vaatbed in genarcotiseerde varkens. De 5-HT-receptor (sub)typen betrokken bij 5-HT-geïnduceerde veranderingen in bovenstaande haemodynamische variabelen werden, met behulp van relatief selectieve agonisten en antagonisten gekarakteriseerd. Hierbij werd met de meest recente ontwikkelingen op het gebied van 5-HT-receptor classificatie rekening gehouden (Hoofdstuk 2).

Na een diepgaand literatuur onderzoek naar de algemene cardiovasculaire effecten van 5-HT en gerelateerde stoffen (Hoofdstuk 3) en hun effecten op hartfrequentie en cardiale contractiliteit in verschillende species (Hoofdstuk 4), werden de receptoren welke betrokken zijn bij de door 5-HT-geïnduceerde positief chronotrope (Hoofdstuk 7 en 8) en positief inotrope effecten (Hoofdstuk 9) beschreven in het genarcotiseerde varken. Intraveneuze bolus injecties met 5-HT induceerde een dosis afhankelijke toename van de hartfrequentie. Overeenkomstige responsen werden waargenomen (in volgorde van potentie) met de tryptamine derivaten 5-methoxytryptamine en α-methyl-5-HT, en met de gesubstitueerde benzamide derivaten renzapride, zacopride, cisapride, metoclopramide en dazopride. Deze benzamide derivaten gedroegen zich als partiële agonisten, omdat ze de toename in hartfrequentie geïnduceerd door 5-HT en 5-methoxytryptamine antagoneerden. Daarentegen werden de tachycarde responsen geïnduceerd door 5-HT en 5-methoxytryptamine noch door de selectieve 5-HT_3-receptor antagonisten MDL 72222 en granisetron, noch door de selectieve D_2-receptor antagonist domperidon (elk 3 mg/kg) geantagoneerd. Echter, de toename in hartfrequentie geinduceerd door 5-HT, tryptamine- of benzamide-derivaten werd duidelijk geantagoneerd door hoge doses (1-3 mg/kg) ICS 205-930, zonder dat het de tachycarde responsen van isoprenaline beinvloed werden. Deze bevindingen zijn in overeeenstemming met het concept dat 5-HT en de tryptamine- en benzamide-derivaten de hartfrequentie van varkens verhogen door een directe werking op de cardiale pacemakercellen, via activering van een mogelijke 5-HT_4-receptor.

Intraveneuze bolus injecties met 5-HT induceerden eveneens een toename in $LVdP/dt_{max}$ en CO, zowel gedurende een spontaan sinus ritme als ook gedurende stimulatie van het atrium (150 slagen/ min), hetgeen een positief inotroop effect doet vermoeden. Een overeenkomstig effect werd gezien met 5-methoxytryptamine en in mindere mate met renzapride. Zoals hierboven beschreven, gedroeg renzapride zich

opnieuw als een partieel agonist, doordat het de toename in $LVdP/dt_{max}$ en CO geïnduceerd door 5-HT en 5-methoxytryptamine, maar niet door isoprenaline antagoneerde. Dit suggereert de betrokkenheid van een gepostuleerde 5-HT_4-receptor.

Het farmacologisch profiel van deze nieuwe receptor in het myocard van het varken komt overeen met dat van de gepostuleerde 5-HT_4-receptor aanwezig in neuronen van muis embryo colliculi, cavia cholinerge neuronen en het humane hart.

In hoofdstuk 5 worden de effecten van 5-HT en verwante stoffen op de carotide circulatie en de verdeling in nutrient (arteriolar) en non-nutrient (arterioveneuze anastomosen) beschreven. Op basis van deze resultaten, is een poging ondernomen het gepostuleerde subtype van de 5-HT_1-achtige receptor te beschrijven door de effecten van de 5-HT_{1A}-agonist indorenate te analyseren (Hoofdstuk 10). Infusies met indorenate in de a. carotis communis van genarcotiseerde varkens na vagosympathectomie, resulteerde in een dosis afhankelijke afname van de bloed flow door de a. carotis communis, welke bijna uitsluitend het gevolg was van een reductie van de flow door arterioveneuze anastomosen. Deze effecten van indorenate werden nauwelijks beinvloed door voorbehandeling met de 5-HT_2-receptor antagonist ketanserine, maar werden duidelijk gereduceerd door behandeling met methiothepine, hetgeen niet alleen 5-HT_2-receptoren antagoneert, maar ook de gepostuleerde 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} of 5-HT_{1D} subtypen van 5-HT_1-achtige receptoren. Echter, metergoline, een stof met een hogere affiniteit voor 5-HT_1-achtige receptor-subtypen dan methiothepine, bleek niet instaat de responsen van indorenate te beinvloeden. Er kan dan ook geconcludeerd worden dat indorenate, overeenkomstig 8-OH-DPAT en RU 24969, zowel de flow door het carotus vaatbed als ook de cephale arterioveneuze anastomosen vermindert in het varken via stimulatie van 5-HT_1-achtige receptoren. Hoewel deze receptoren niet overeen lijken te komen met 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} of 5-HT_{1D} bindingsplaatsen.

Uitgaande van het concept dat dilatatie van cephale arteriën en AVAs waarschijnlijk een rol speelt in de pathofysiologie van migraine en dat een aantal effectieve antimigraine farmaca, waaronder ergotamine, methysergide, isomethepteen en sumatriptan, een selectieve constrictoire werking op het carotis vaatbed vertonen welke voornamelijk het gevolg is van een constrictie van cephale arterioveneuze anastomosen, lijkt een onderverdeling van de arteriële carotis bloeddoorstroming in een arterioveneus anastomotisch en een arterieël gedeelte, in varkens, als experimenteel model voor screening en ontwikkeling van farmaca met potentieel antimigraine werking mogelijk. Vanuit deze achtergrond, is in hoofdstuk 11 de studie naar de effecten van een nieuw trimethylxanthine-derivaat, S9977, op de verdeling van de totale carotide doorbloeding in arterioveneuze-en arteriolaire-bloeddoorstroming in genarcotiseerde varkens beschreven. S9977 antagoneert de dopaminerge overgevoeligheid en de toename in

adenosine receptoren geinduceerd door voedingsgevoeligheid (dierexperimenteel model voor migraine).

Ter vergelijking werden de effecten van dihydroergotamine, een klinisch toegepast antimigraine geneesmiddel, geanalyseerd. Dihydroergotamine vertoonde een dosis afhankelijke reductie van de artrioveneuze doorbloeding en geleiding, en een toename in nutrient (arteriolair) fractie en conductance. In duidelijk contrast hiermee is de bevinding dat S9977 de carotis (twee hoogste doses) en de arterioveneuze doorbloeding (hoogste dosis) verminderde, zonder gelijktijdige afname in vasculaire conductance. Dus lijken de effecten van S9977 gerelateerd aan een afname in arteriële bloeddruk en niet aan een actieve vasoconstrictie van arterioveneuze anastomosen. Deze resultaten voorspellen danook geen antimigraine werking voor S9977, indien wordt uitgegaan van de "shunt theorie". Echter op basis van andere farmacologische effecten van deze stof blijft het wel mogelijk.

LIST OF PUBLICATIONS
AND
CURRICULUM VITAE

LIST OF PUBLICATIONS

ABSTRACTS

1. Hong, E. and Villalón, C.M., External carotid vasodilatation induced by serotonin and indorenate. Proc. West. Pharmacol. Soc., 31, 99-101, 1988.

2. Hong, E. and Villalón, C.M., Potential therapeutic use for 5-HT_{1A} and ß-blockers in migraine. Proceedings of the International Congress on Cardiovascular Pharmacology of 5-HT. Amsterdam, October 4-7, 1988. Abstract No. 64.

3. Villalón, C.M. and Hong, E., Inhibitory effect of pelanserin and ketanserin on the external carotid bed vasodilatation induced by serotonin. Proceedings of the International Congress on Cardiovascular Pharmacology of 5-HT. Amsterdam, October 4-7, 1988. Abstract No. 65.

4. Hong, E., Villalón, C.M. and de León, H., Pharmacological evidences indicating that indorenate is a S_{1A} agonist. The Pharmacologist, 31, 3, 1989.

5. Saxena, P.R. and Villalón, C.M., Cardiovascular effects of serotonin agonists and antagonists. Proceedings of the International Symposium on 5-HT_{1A} Receptors in the Brain: A subtype of serotonin receptors involved in blood pressure reduction. Bonn, Federal Republic of Germany. November 18, 1989. Abstract No. 3.

6. Villalón, C.M., Bom, A.H., Heiligers, J., den Boer, M. and Saxena, P.R., Reduction of porcine carotid arteriovenous shunting by indorenate is unrelated to 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, or 5-HT_{1D} receptor subtype. Br. J. Pharmacol., 99, 36P, 1990.

7. Den Boer, M., Villalón, C.M., Heiligers, J., Saxena, P.R. and Humphrey, P.P.A., The craniovascular effects of sumatriptan in the pig. Br. J. Pharmacol., 99, 37P, 1990.

8. Villalón, C.M., Heiligers, J., den Boer, M.O. and Saxena, P.R., Putative 5-HT_4 receptor mediates 5-HT-induced tachycardia in the anaesthetized pig. Eur. J. Pharmacol., 183, 1160, 1990.

9. Heiligers, J.P.C., Villalón, C.M., Bom, A.H. and Saxena, P.R., Effects of S9977, a potential antimigraine drug, on arteriovenous anastomoses in the carotid vascular bed of the anaesthetized pig. Eur. J. Pharmacol., 183, 1279-1280, 1990.

10. Den Boer, M.O., Villalón, C.M., Heiligers, J.P.C., Saxena, P.R. and Humphrey, P.P.A., The effects of sumatriptan on the carotid circulation of the pig. Eur. J. Pharmacol., 183, 1275-1276, 1990.

11. Saxena, P.R., Villalón, C.M. and van Heuven, D., 5-HT_1-like receptors unrelated to the known binding sites ? Proceedings of the 2nd IUPHAR satellite meeting on serotonin. Basel, Switzerland. July 11-13, 1990. Abstract No. 17.

12. Villalón, C.M., den Boer, M., Heiligers, J.P.C. and Saxena, P.R., 5-HT_4 receptors mediate tachycardia in the pig. Proceedings of the 2nd IUPHAR satellite meeting on serotonin. Basel, Switzerland. July 11-13, 1990. Abstract No. 67.

13. Den Boer, M., Villalón, C.M., Heiligers, J.P.C., Saxena, P.R. and Humphrey, P.P.A., The effects of sumatriptan on the carotid circulation of the pig. Proceedings of the 2nd IUPHAR satellite meeting on serotonin. Basel, Switzerland. July 11-13, 1990. Abstract No. P147.

14. Villalón, C.M.,DenBoer, M.O.,Heiligers, J.P.C.,Verdouw, P.D.,Duncker, D.J.G.M. and Saxena, P.R., Role of the putative 5-HT_4 receptor in the positive inotropic effect of 5-HT in the pig. Fundament. Clin. Pharmacol., 1991. In Press.

FULL PAPERS

1. Bom, A.H., Villalón, C.M., Verdouw, P. and Saxena, P.R., The 5-HT_1-like receptor mediating reduction of porcine carotid arteriovenous shunting by RU 24969 is unrelated to either 5-HT_{1A} or 5-HT_{1B} subtype. Eur. J. Pharmacol., 171, 87-96, 1989.

2. Saxena, P.R. and Villalón, C.M., Cardiovascular effects of serotonin agonists and antagonists. J. Cardiovasc. Pharmacol., 15 (Suppl. 7), S17-S34, 1990.

3. Villalón, C.M., Den Boer, M.O., Heiligers, J.P.C. and Saxena, P.R., Mediation of 5-hydroxytryptamine-induced tachycardia in the pig by the putative 5-HT_4 receptor. **Special Report.** Br. J. Pharmacol., 100, 665-667, 1990.

4. Saxena, P.R. and Villalón, C.M., Brain 5-HT_{1A} receptor agonism: A novel mechanism for antihypertensive action. Trends Pharmacol. Sci., 11, 95-96, 1990.

5. Villalón, C.M., Bom, A.H., Heiligers, J.P.C., den Boer, M.O. and Saxena, P.R., Porcine arteriovenous anastomotic constriction by indorenate is unrelated to 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} or 5-HT_{1D} receptor subtype. Eur. J. Pharmacol., 190, 167-176, 1990.

6. Villalón, C.M., Den Boer, M.O., Heiligers, J.P.C. and Saxena, P.R., Further characterization, using tryptamine and benzamide derivatives, of the putative 5-HT_4 receptor mediating tachycardia in the pig. Br. J. Pharmacol., 102, 107-112, 1991.

7. Den Boer, M.O., Villalón, C.M., Heiligers, J.P.C., Humphrey, P.P.A. and Saxena, P.R., Reduction of porcine cranial arteriovenous anastomotic shunting by the antimigraine drug sumatriptan is mediated by a 5-HT_1-like receptor. Br. J. Pharmacol., 102, 323-330, 1991.

8. Saxena, P.R. and Villalón, C.M., 5-Hydroxytryptamine, a chameleon in the heart. Trends Pharmacol. Sci., 12, 223-227, 1991.

9. Van Heuven, D., Villalón, C.M., den Boer, M.O. and Saxena, P.R., 5-HT_1-like receptors unrelated to the known binding sites ? In: Serotonin: Molecular Biology, Receptors and Function. Ed. Fozard, J.R. and Saxena, P.R., 1991, Basel, Birkhauser, (in press).

10. Villalón, C.M., Den Boer, M.O., Heiligers, J.P.C. and Saxena, P.R., The 5-HT_4 receptor mediating tachycardia in the pig. In: Serotonin: Molecular Biology, Receptors and Function. Ed. Fozard, J.R. and Saxena, P.R., 1991. Basel, Birkhauser, pp. 253-263.

11. Villalón, C.M., Bom, A.H., Den Boer, M.O. Heiligers, J.P.C. and Saxena, P.R., Effects of S9977, a potential antimigraine drug, and dihydroergotamine on carotid blood flow fractionation in the pig. Pharmacol. Res., 1991 (in press).

12. Villalón, C.M., Heiligers, J.P.C., Verdouw, P.D., Duncker, D.J.G.M. and Saxena, P.R., Role of the putative 5-HT_4 receptor in the positive inotropic effect of 5-HT in the pig. **To be submitted.**

13. Dhasmana, K.M., Villalón, C.M., Zhu, Y.N. and Saxena, P.R., Role of 5-hydroxytryptamine$_1$-like receptors in the 5-HT-induced increases in rat intragastric pressure. **To be submitted.**

CURRICULUM VITAE

Carlos M. Villalón was born on November 2, 1959 in Mexico City, Mexico. After secundary school education (concluded in 1978), he studied pharmacy from 1978 to 1983 at the National School of Biological Sciences (E.N.C.B., Mexico City). Two years later, he obtained the degree of Pharmacist. From 1983 to 1986 he was enrolled in the Master Sciences Programme in Pharmacology at the Centre for Research and Advanced Studies (CINVESTAV, Mexico City), obtaining the M.Sc. degree in Pharmacology on April 1986. Subsequently, he was accepted to continue his studies in the Doctor Sciences Programme in Pharmacology at the same Research Centre from 1986 to 1988, obtaining the D.Sc. degree in Pharmacology on September 1988, under the supervision of Prof. Dr. Enrique Hong. Immediatly afterwards, he continued his postdoctoral training at the Institute of Pharmacology of the Erasmus University of Rotterdam, under the supervision of Prof. Dr. Pramod R. Saxena, for which he has been awarded postdoctoral grants from CONACyT (Mexico), the Ministry of Education and Science (the Netherlands), the European Economic Community and the Department of Pharmacology, Erasmus University Rotterdam.

He has also worked as a teacher of Chemistry and Biology at secondary school from 1981 to 1984, and as a lecturer of Physiology and Pharmacology at the Department of Physiology of the E.N.C.B. (head: Prof. Dr. Mauricio Russek) from 1985 to 1986.

ACKNOWLEDGEMENTS

ACKNOWLEDGEMENTS

My gratitude to all the people who have contributed to the completion of this thesis cannot be adequately expressed here. Nevertheless, may I show clearly that two people have been particularly influential in my scientific development, namely:

- Prof. Dr. Pramod R. Saxena (Department of Pharmacology, Erasmus University, Rotterdam). Thank you for giving me the opportunity to interact in an international atmosphere, for providing me with the essential enthusiasm to do scientific research in spite of the inherent limitations when abroad, and for doing your best to improve many skills, including my understanding of pharmacology of 5-HT and my ability to write scientific papers in the English language.

- Prof. Dr. Enrique Hong (Sección de Terapéutica Experimental, CINVESTAV, Mexico City). I am equally indebted to you for your wise teaching, not only in the personal context, but also scientifically. You were my first inspiration to do research in cardiovascular pharmacology.

I also wish to thank several people from the Department of Pharmacology, Erasmus University Rotterdam, who made my stay pleasant. To Mr. Jan P.C. Heiligers, for his honesty, excellent technical assistance and contribution to a friendly atmosphere. To Drs. Marien den Boer, for his help with the computer drawings. To Drs. Marcel van Gelderen, for making the Dutch translation of the summary of this thesis. To Drs. Shreekanth Tadipatri, for his patience, comradeship and lively talks. To Dr. Anton H. Bom, for his in depth discussions on scientific subjects and comradeship while in this department. The secretarial assistance of Mrs. Magda Busscher-Lauw is also acknowledged.

Dr. K. Mohan Dhasmana (Department of Anesthesiology, Erasmus University Rotterdam). I am grateful to you for your personal support and confidence in my scientific abilities.

I must also thank the members of the "Promotiecommissie": Prof. Dr. P.R. Saxena, Prof. Dr. A.J. Man in't Veld, Prof. Dr. P.D. Verdouw and Prof. Dr. P.A. van Zwieten for their constructive criticisms.

Para finalizar, pero no por eso menos importante, deseo hacer patente mi sincero agradecimiento al Dr. Carlos Hoyo-Vadillo por su constante apoyo moral y su auténtica amistad.

LIST OF ABBREVIATIONS

ADP	adenosine diphosphate
AVA's	arteriovenous anastomoses
cAMP	cyclyc adenosine monophosphate
5-CT	5-carboxamidotryptamine
5-CH_3O-T	5-methoxytryptamine
mCPP	1-(3-chlorophenyl)piperazine
D	dopamine
DOI	1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane
EDRF	endothelium-derived relaxing factor
μg	microgram
mg	milligram
cGMP	cyclic guanosine monophosphate
GTP	guanosine triphosphate
5-HIAA	5-hydroxyindole acetic acid
HR	heart rate
5-HT	5-hydroxytryptamine; serotonin
5-HTP	5-hydroxytryptophan
i.m.	intramuscular administration
i.v.	intravenous administration
Kg	kilogram
LSD	lysergic acid diethylamide
$LVdP/dt_{max}$	maximum rate of rise in left ventricular pressure
M	molar
mM	millimolar
μM	micromolar
fM	fentomolar
MAO	monoamine oxidase
8-MeO-ClEPAT	8-methoxy-2-(N-2-chloroethyl-N-n-propylamino)tetralin
α-methyl-5-HT	alpha-methyl-5-hydroxytryptamine
2-methyl-5-HT	2-methyl-5-hydroxytryptamine
min	minute
8-OH-DPAT	8-hydroxy-2-(di-N-propylamino)tetralin
PI	phosphoinositide
SNC	central nervous system
TFMPP	1-(3-trifluoromethyl)piperazine