CHARACTERIZATION OF 5-HYDROXYTRYPTAMINE RECEPTORS IN THE CARDIOVASCULAR SYSTEM

KARAKTERISERING VAN 5-HYDROXYTRYPTAMINE RECEPTOREN IN HET CARDIOVASCULAIRE STELSEL

PROEFSCHRIFT

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Chapter 1: INTRODUCTION

At the beginning of this century it was already known to physiologists that injection of blood serum causes vasoconstriction (Janeway et al., 1918) and a vagally-mediated reflex resulting in bradycardia (Brodie, 1900), whereas injection of blood plasma did not show these effects. Page, who was searching for a humoral agent responsible for hypertension, was intrigued by this phenomenon. In cooperation with Green and Rapport this humoral agent was isolated, cristallized, its structure characterized and named serotonin (Rapport et al., 1948). These investigators were unaware of the experiments done by Erspamer and Vially, who demonstrated that extracts of the stomach and intestine caused constriction of the uterus. The active agent in this extract was called enteramine and it was shown later that enteramine was identical to serotonin (5-hydroxytryptamine; 5-HT) (Erspamer & Asero, 1952).
In the intestines and the brain 5-HT is synthetized from the essential amino-acid L-tryptophan. 5-HT can be released from the intestines into the blood, where 5-HT is either taken up by thrombocytes or removed from the blood by the liver or endothelial cells in the lungs, mainly resulting in the formation of 5-hydroxyindolacetic acid (5-HIAA). Since 5-HT does not cross the blood-brain barrier, there exists a clear distinction between its central and its peripheral function. In the central nervous system 5-HT is involved in appetite, sleep, memory, thermoregulation, sexual behaviour, hallucinations, anxiety and depression. In the periphery 5-HT plays a role in the aggregration of thrombocytes, constriction of smooth muscle cells, presynaptic inhibition, and stimulation of nerve fibers. In 1957 Gaddum & Picarelli showed that 5-HT can act on different subtypes of 5-HT receptors within the same tissue. The development of novel 5-HT agonists and antagonists and the use of radioligand-binding techniques allows us nowadays to even further discriminate between the different subtypes of receptors, as will be discussed in Chapter 2.

Aim of the thesis

Until recently, mainly rats, guinea pigs, rabbits, cats and dogs were used to study the cardiovascular effects of 5-HT. From these studies it was clear that for some effects of 5-HT (e.g. 5-HT-induced tachycardia) there exists a large heterogeneity with respect to receptor subtype(s) and mechanism(s) (see Saxena, 1986). Therefore, the first part of the thesis deals with the mechanism and receptor type involved in the 5-HT-induced heart rate changes in the cat and the pig.
In the second part of the thesis the effect of 5-HT and other agonists at the 5-HT receptors on total carotid blood flow and its distribution are described. 5-HT_2 receptors have been shown to play a minor role in carotid blood flow changes (Verdouw et al., 1984). Therefore, the role of 5-HT_1-like and 5-HT_3 receptors has been determined.

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Chapter 2: 5-HT RECEPTOR CLASSIFICATION

Gaddum & Picarelli (1957) suggested the existence of two types of 5-HT receptors based on the study 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-bromo-lysergic acid diethylamide and dihydroergotamine antagonized the effect of 5-HT. They concluded that 5-HT must act by two different mechanisms and receptors: a M-receptor located on nervous tissue, mediating the release of acetylcholine from nerve endings, and a D-receptor, located on the smooth muscles. However, neither morphine nor dibenzyline are specific 5-HT receptor antagonists.

In the following two decades no important progress in the classification of 5-HT receptors was made, until Bennett & Aghajanian (1974) 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 & Snyder, 1976; Fillion et al., 1978). However, 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 a high affinity for [^{3}H]-5-HT and a 5-HT_2 recognition site with a high affinity for [^{3}H]-spiperone (Peroutka & Snyder, 1979). More recently, 5-HT_1 recognition sites have been shown to be heterogeneous: 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} and 5-HT_{1D} subtypes have been described (Pedigo et al., 1981; Schnellman et al., 1984; Asarch et al., 1985; Hoyer et al., 1985; Heuring & Peroutka, 1987). The development of ketanserin, a compound with a high affinity for the 5-HT_2 recognition sites (Leysen et al., 1981), made it possible to correlate 5-HT_2 recognition sites with functional D-receptors, which are renamed as 5-HT_2 receptors. The effects mediated by the M-

receptor can be blocked by selective antagonists, like MDL 72222 (Fozard, 1984) and ICS 205-930 (Donatsch, 1984; Richardson et al., 1985). Since both compounds did not label recognition sites in the brain, these receptors were called 5-HT_3 receptors. However, more recently, 5-HT_3 recognition sites have been described in the brain by Kilpatrick et al. (1987). Since 5-HT also has a high affinity for 5-HT_1 recognition sites and some effects of 5-HT cannot be blocked by 5-HT_2 or 5-HT_3 receptor antagonists, an international committee proposed the presence of 5-HT_1-like receptors (Bradley et al., 1986). According to their criteria 5-HT_1-like receptors are not blocked by ketanserin, cyproheptadine, MDL 72222 or ICS 205-930, but can be stimulated by 5-carboxamido-tryptamine. Furthermore, methiothepin, a compound with affinity for both 5-HT_1 and 5-HT_2 recognition sites, must block the effects of 5-hydroxytryptamine and 5-carboxamidotryptamine. Unfortunately, no selective 5-HT_1 receptor antagonist is available yet.

The committee (Bradley et al., 1986) already indicated that further research would lead to the discovery of 5-HT receptors that cannot be classified as 5-HT_1-like, 5-HT_2 or 5-HT_3 receptors, and warned for the defining of further 5-HT_x receptors, without 'controlled studies with potent, selective, competitively acting receptor blocking drugs'.

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Chapter 3: 5-HYDROXYTRYPTAMINE-INDUCED HEART RATE CHANGES IN MAMMALS.

Among the many cardiovascular effects of 5-hydroxytryptamine (5-HT), changes in heart rate have been demonstrated by many investigators. Both bradycardia, bradycardia followed by tachycardia, and tachycardia have been described depending on the species, anaesthesia, experimental model and way of administration. The various mechanisms by which 5-HT can induce heart rate changes are summarized in Table 1.

Table 1. Mechanisms for heart rate changes induced by 5-HT.

1. Bradycardia.

-stimulation of afferent vagal nerve fibers, inducing centrally mediated reflexes.
-presynaptic inhibition of cardiac efferent nerve fibers.
-decrease in cardiac efferent sympathetic nerve fibers and/or increase in efferent vagal nerve activity (by e.g. baroreflexes).

2. Tachycardia.

-stimulation of cardiac 5-HT receptors.
-release of noradrenaline from cardiac efferent sympathetic nerve fibers
 a) 5-HT receptor-mediated.
 b) by a tyramine-like action of 5-HT.
-release of adrenaline and noradrenaline from the adrenal medulla.
-stimulation of cardiac afferent sympathetic nerve fibers, inducing (supra)spinal reflexes.
-stimulation of cardiovascular chemoreceptors resulting in an increase in efferent sympathetic nerve activity.
-increase in cardiac efferent sympathetic nerve activity and/or decrease in efferent vagal nerve activity.

Since the mechanisms involved in 5-HT-induced heart rate changes show a large variability between species, each species will be discussed separately, followed by the results obtained in the pig (Chapter 4).

Table 2.

Bradycardia due to 5-HT receptor stimulation in different species.

Species	Condition	Receptor	Mechanism of action
Dog	Intact	5-HT_3	Cardiopulmonary afferent nerve fiber stimulation
	Intact	5-HT?	Presynaptic sympathetic inhibition
	Intact	-----	Baroreflex during cardiogenic hypertensive reflex
Cat	Intact	5-HT_3	Cardiopulmonary afferent nerve fiber stimulation
	Intact	5-HT_{1A}	Changes in autonomic outflow
Rabbit	Intact	5-HT_3	Cardiopulmonary afferent nerve fiber stimulation
	Isolated	5-HT_3	Nodose ganglion stimulation
Rat	Intact	5-HT_3	Cardiopulmonary afferent nerve fiber stimulation
		5-HT_{1A}	Changes in autonomic outflow
		$5\text{-HT}_?$	Presynaptic sympathetic inhibition
Man	Intact	5-HT_3?	von Bezold-Jarisch reflex

Table 3.

Tachycardia due to 5-HT receptor stimulation in different species.

Species	Condition	Receptor	Mechanism of action
Dog	Isolated	-----	Tyramine-like action on isolated right atrium
	Intact	-----	Tyramine-like action
	Intact	5-HT	Adrenal catecholamine release
Cat	Isolated	5-HT_1-like	Myocardial effect in right atria from reserpinized kitten
	Spinal	5-HT_1-like	Myocardial effect
Rabbit	Isolated	5-HT_3	Catecholamine release from cardiac sympathetic nerve endings
Pig	Intact	5-$HT_?$	Myocardial effect
Guinea pig	Isolated	-----	CGRP release ?
	Spinal	-----	Tyramine-like action
Rat	Intact	5-HT_2	Myocardial effect
	Ganglion-blocked	5-HT_2	Myocardial effect
	Pithed	5-HT_2	Myocardial effect
Man	Intact	----	Unknown

Bradycardia in the dog.

Injections of 5-HT into the pulmonary artery causes a bradycardia, the onset of which coincides with the increase in mean arterial blood pressure (MacCanon & Horvath, 1954). A lowering of heart rate is also observed during intra-aortic infusion of 5-HT, an effect which can be blocked by methysergide (Vyden et al., 1967). More recently, Kimura & Satoh (1983) demonstrated that 5-HT lowers heart rate during electrical stimulation of the ansa subclavia. This effect cannot be blocked by pretreatment with atropine or cyproheptadine. Their results suggest that 5-HT causes presynaptic inhibition of cardiac catecholamine release, which is not mediated by 5-HT_2 receptors. Direct infusion of 5-HT into the sinus node artery causes a dose-dependent decrease in heart rate, which is resistant to atropine and/or bilateral cervical vagotomy (James, 1964). In later studies James et al. (1975) described the cardiogenic hypertensive chemoreflex. Injections of 5-HT into the left atrium or small branches of the proximal left coronary artery produce a rapid increase in mean arterial blood pressure and a decrease in heart rate followed by an increase in heart rate, whereas 5-HT injections in the distal part of the left circumflex or left anterior descending artery produces the von Bezold-Jarisch reflex: bradycardia and a fall in blood pressure. Based on histological studies, revealing the presence of a structure resembling a chemoreceptor, it was suggested that 5-HT stimulates this chemoreceptor, resulting in a reflex increase in efferent vagal and sympathetic nerve activity. Cyproheptadine abolishes the cardiogenic hypertensive reflex in atropine-pretreated dogs, whereas methysergide or morphine are ineffective (Hageman, et al., 1977). Efferent sympathetic nerve activity increases markedly after 5-HT injections and preceeds the increase in blood pressure (Hageman et al., 1980). Urthaler et al. (1978) have demonstrated that atropine pretreatment and bilateral stellectomy, followed by occlusion of the adrenal arteries and veins, almost completely abolishes the effects of 5-HT. Based on the results of Humphrey & Feniuk (see later) the cardiogenic hypertensive reflex can also be

explained as a mixture of baroreflexes and 5-HT-induced catecholamine release from the adrenals. However, the increase in sympathetic nerve activity cannot be explained by the above-mentioned mechanisms and might be the result of spinal reflexes or stimulation of cardiovascular chemoreceptors. A possible role of 5-HT_3 receptors has been described by Berthold et al. (1989). The inhibition of the 5-HT-induced cardiogenic hypertensive chemoreflex by ICS 205-930 shows a possible mediation by 5-HT_3 receptors, but the location of the 5-HT_3 receptors remains unknown. A possible location at afferent cardiac sympathetic nerve fibers has to be considered. The state of the preparation and the anaesthesia also plays an important role in these effects as has been demonstrated by Zucker & Cornish (1980) in both conscious and anaesthetized closed chest and open chest dogs. In conscious dogs a von Bezold-Jarisch reflex is followed by a delayed tachycardia and hypertension, whereas in anaesthetized dogs the von Bezold-Jarisch reflex is markedly attenuated and the tachycardia and hypertension are enhanced, especially in open-chest dogs.

In conclusion, 5-HT-induced bradycardia can be caused by: 1) stimulation of afferent vagal nerve fibers, resulting in the von Bezold-Jarisch reflex: bradycardia and inhibition of sympathetic nerve activity, resulting in hypotension; 2) a presynaptic inhibition of cardiac sympathetic nerve activity and 3) a baroreflex or chemoreflex-mediated inhibition of efferent sympathetic nerve activity caused by the sudden increase in blood pressure or stimulation of chemoreceptors.

Tachycardia in the dog

1. Isolated right atrium.

In isolated right atrium preparation perfused via the sinus node artery with heparinized blood from a donor dog at a constant pressure, low doses of 5-HT show a slight negative chronotropic effect, whereas higher doses cause tachycardia (Chiba, 1977). The negative chronotropic effect is not reduced by atropine or methysergide. Pretreatment with propranolol completely prevents the tachycardic response to 5-HT and noradrenaline, whereas desimipramine only suppresses the effects of 5-HT. Furthermore, the responses to tyramine are potentiated during 5-HT infusion (Chiba, 1978). These results suggest that the positive chronotropic effect of 5-HT in the isolated right atrium of the dog might be caused by a tyramine-like action of 5-HT, leading to the release of catecholamines from sympathetic nerve endings.

2. Intact dog.

5-HT-induced tachycardia in anaesthetized dogs has been reported by many investigators (McCawley et al., 1952; Maxwell et al., 1959; Noble et al., 1959). This increase in heart rate coincides with a fall in mean arterial blood pressure. Therefore, it was unclear whether 5-HT was acting directly on the heart or by other mechanisms (e.g. baroreflexes). To reduce reflex effects, Fillion et al. (1971) used ganglion blocked (atropine- and chlorisondamine-pretreated) dogs. Low doses of 5-HT cause a dose-dependent increase in noradrenaline concentration of coronary sinus blood and a high dose of 5-HT also causes an increase in noradrenaline concentration of vena caval blood, which cannot be explained by noradrenaline release from the heart alone. Intracoronary injections of 5-HT also cause an increase in noradrenaline concentration of coronary sinus blood. Stimulation of the cardioaccelerator nerves causes an increase in heart rate and a release of ^{3}H-5-HT from

^{3}H-5-HT-preloaded nerve endings. This tachycardia can be blocked by propranolol, but the ^{3}H-5-HT release is unaffected. In reserpine-pretreated dogs no tachycardia can be induced by 5-HT (Jacob & Fillion, 1967). However, the 5-HT-induced tachycardia in untreated dogs can be reduced by ergotamine and methylergotamine. Furthermore, methylergotamine does not interfere with the increase in heart rate caused by electrical stimulation of the cardioaccelerator nerves.

In order to discover the extracardiac source of catecholamines released into the blood after 5-HT administration, Feniuk & Humphrey (1979) studied the effects of drugs and bilateral adrenalectomy in mecamylamine-pretreated dogs. Cyproheptadine and methysergide dose-dependently inhibit the 5-HT-induced tachycardia. Pretreatment with the catecholamine depleting agent syrosingopine reduces the tachycardia and completely blocks the release of noradrenaline and adrenaline into the blood. In adrenalectomized dogs the tachycardia is also reduced by 43% (Feniuk et al., 1981). These results are in agreement with those of Kimura and Satoh (1983) who demonstrated that 5-HT causes a dose-dependent increase in heart rate in vagotomized and cardiac decentralized dogs, which can be antagonized by cyproheptadine or bufetolol.

From this one can conclude that the 5-HT-induced tachycardia in the dog is partially mediated by catecholamine release from the adrenals, probably by an action on 5-HT_2 receptors on adrenal medullary chromaffin cells. Since bilateral adrenalectomy did not completely abolish the tachycardia, a 5-HT_2 receptor-mediated release of adrenaline and noradrenaline from other sources has to be considered. Furthermore, at higher doses a tyramine-like action of 5-HT can also contribute to the tachycardia.

However, Breuer et al. (1985) have demonstrated that intravenous infusion of 5-HT causes an increase in heart rate, which is not markedly reduced by the 5-HT_2 receptor antagonist ketanserin (100 µg kg-1 i.v.), a dose which decreases arterial blood pressure in dogs (Phillips et al., 1985).

Bradycardia in the cat.

1. Reflex-mediated bradycardia.

Rapid intravenous injections of 5-HT result in bradycardia and hypotension. This bradycardia can be blocked by procaine or bilateral vagotomy, which prevents the increase in afferent vagal nerve activity, or by atropine, which blocks the effects of reflex-mediated enhanced release of acetylcholine from efferent vagal nerve endings. 2-Methyl,3-ethyl,5-amino indole pretreatment can also antagonize the reflex effects (Comroe et al., 1953). 5-HT injections into the left ventricle also induce this reflex, but injections of 5-HT into the aortic arch are ineffective. Besides the increase in efferent vagal activity, an inhibition of sympathetic outflow is induced by 5-HT, resulting in an increase in hind limb blood flow, which cannot be blocked by atropine (Kottegoda & Mott, 1955). Intracoronary injection of 5-HT into the left coronary artery increases afferent vagal nerve activity as demonstrated by Gilev (see: Zakusov, 1962). This increase can be reduced by 2-methyl-3-methyl-5-aminoindole or by a thiopyranoindole derivative named thipindole, but not by reserpine, which has been shown to depress the von Bezold-Jarisch reflex, probably by a central action. In all the above-mentioned studies it is not completely clear which population of afferent fibers is stimulated: pulmonary vagal afferents, cardiac vagal afferents, chemoreceptors in the aortic bodies (Kirby & McQueen, 1984), or 5-HT receptors on the nodose ganglia (Jacobs & Comroe, 1971) (see also: Paintal, 1964, 1973). In order to avoid stimulation of different populations of afferent nerve fibers, 5-HT can also be injected into the pericardial sac to induce the von Bezold-Jarisch reflex. This results in a stimulation of 5-HT receptors located on vagal afferent nerve fibers in the epicardium, without affecting 5-HT receptors on afferent nerve fibers in other areas. Nicotine also elicits this reflex. The effects of 5-HT, but not of nicotine, are blocked by the selective 5-HT_3 receptor antagonists MDL 72222 (Mohr et al., 1987) and ICS 205-930 (Bom,

unpublished results), showing that the blockade is not caused by a local anaesthetic effect on the nerve fibers (see Chapter 4). The bradycardia induced by intravenous injection of 5-HT can also be blocked by MDL 72222 (Saxena et al., 1985). From this one can conclude that most of the 5-HT receptors involved in reflex bradycardia belong to the 5-HT_3 receptor subtype. Phenylbiguanide mimicks the reflex effects of 5-HT in the cat (Dawes & Mott, 1950; Dawes & Fastier, 1950; Barer & Nuesser, 1958; Fastier et al., 1959). These effects can be blocked by MDL 72222 or ICS 205-930 (Bom, unpublished results). Therefore, phenylbiguanide can be used as an agonist at these 5-HT_3 receptors.

2. Centrally-induced bradycardia.

5-HT itself cannot cross the blood brain barrier, but several drugs with a high affinity for central 5-HT receptors do. Compounds with a high affinity for 5-HT_{1A} recognition sites, like 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT), N,N dipropyl-carboxamidotryptamine (DP-5-CT), flesinoxan, ipsapirone, p-aminophenyl-ethyl-m-trifluoromethylphenyl piperazine (PAPP), 1-(2-methoxyphenyl)piperazine (2-MPP) and spiperone, lower heart rate, whereas compounds with a high affinity for 5-HT_{1B} recognition sites - 1-(3-trifluoromethyl)piperazine (TFMPP), 1-(3-chlorophenyl)piperazine (mCPP) - or 5-HT_2 recognition sites - 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), piperazinyl-6-chloropyrazine (MK 212), cinanserin, 6-methyl-1-(1-methylethyl)ergoline-8-carboxylic acid,2-hydroxy-1-methyl propyl ether(z)-butanedioate (LY 53857) - do not decrease heart rate (Doods et al., 1988; McCall et al., 1987; Ramage, 1985a,1985b; Ramage & Fozard, 1987; Wouters et al., 1988). However, ritanserin, ketanserin and methysergide cause a fall in heart rate (Antonaccio & Taylor, 1977; Ramage, 1985a,1985b; Tadepalli et al., 1979; Van Zwieten et al., 1987). Copeland & Bentley (1985) reported no significant changes in heart rate by ketanserin.

Tachycardia in the cat.

1. Isolated heart.

Using the Langendorff preparation Reid (1952) and Schneider & Yonkman (1953) demonstrated that 5-HT exerts a positive chronotropic action on the cat heart. On isolated cat atria Trendelenburg (1960) has shown that 5-HT causes an increase in contraction frequency, which is not affected by pretreatment of the animals with reserpine. The tachycardic response is attenuated by low concentrations of lysergic acid diethylamide (LSD) and high concentrations of cocaine or TM 10, a compound which prevents the release of noradrenaline from adrenergic nerve endings. Therefore the effect of low doses of 5-HT on beating atria can be explained by direct receptor stimulation. In right atria from reserpine-pretreated kitten the positive chronotropic effect of 5-HT can be blocked irreversibly by phenoxybenzamine and can be competitively antagonized by methysergide. Ketanserin, MDL 72222 and yohimbine are ineffective, suggestive for the involvement of 5-HT_1-like receptors. 8-OH-DPAT and tryptamine also cause an increase in beating rate (Kaumann, 1985).

2. Intact cat.

Intrapericardial injection of 5-HT induces an epicardial chemoreflex (bradycardia and sympathoinhibition, resulting in hypotension), followed by tachycardia, which can not be antagonized by ketanserin or MDL 72222 (Mohr et al., 1987). 5-HT also causes tachycardia in vagotomized, spinal cats (Weidmann & Cerletti, 1957). This effect can be antagonized by pizotifen, mesulergine, methiothepin, metergoline and methysergide, whereas ketanserin and ritanserin are ineffective (Saxena et al., 1985; Saxena, 1988). Adrenalectomy did not modify this action of 5-HT despite the fact that 5-HT can liberate adrenaline from the feline adrenal medulla (Reid, 1952). 5-Carboxamidotryptamine, a compound with

a high affinity for 5-HT_1 recognition sites, also induces tachycardia (Saxena et al., 1985; Connor et al., 1986). Other agonists for 5-HT_1-like receptors, like BEA 1654, 8-OH-DPAT and RU 24969 also do this, but are less potent (Saxena, 1988).
According to the data of Hoyer (1988) all the antagonists for the tachycardic effect of 5-HT share a high affinity for the 5-HT_{1c} recognition site. However, 5-carboxamidotryptamine, which is more potent than 5-HT in this effect, has a low affinity for this recognition site.
Therefore we can conclude that 5-HT_1-like receptors in the right atrium of the cat are mediating the tachycardic response, but it is still impossible to characterize the putative 5-HT_1 receptor subtype, since there is no clear relationship between the cardioaccelarator effect of selective agonists and their affinity for subtypes of 5-HT_1 recognition sites.

Bradycardia in the rabbit.

Intravenous bolus injection of 5-HT in the conscious rabbit induces the von Bezold-Jarisch reflex. The bradycardia can be blocked by methscopolamine and the fall in blood pressure by the combination of methscopolamine and propranolol. Ketanserin is ineffective, excluding the mediation by 5-HT_2 receptors (Wright & Angus, 1983). Phenyldiguanide and 5-carboxamidotryptamine also result in the von Bezold-Jarisch reflex. MDL 72222 blocks the reflex effects of all three agonists. In reserpine-pretreated rabbits, the 5-HT-induced reflex is enhanced, whereas 5-carboxamidotryptamine is ineffective. Pretreatment with fluoxetine, a 5-HT uptake inhibitor, reduces the bradycardic effect of 5-carboxamidotryptamine and even reveals a small tachycardic effect of this compound, whereas 5-HT shows a small increase and prolongation of the von Bezold-Jarisch reflex (Wright & Angus, 1989). These authors suggest that 5-carboxamidotryptamine releases 5-HT from platelets. In

anaesthetized rabbits both 5-HT and phenyldigunanide induce this reflex (Dawes & Mott, 1950). The reflex induced by both compounds can be antagonized by 2-naphtyl-guanidine and bufotenine (Fastier et al., 1959) and also by MDL 72222, showing that both drugs act on 5-HT_3 receptors (Armstrong & Kay, 1985). 5-HT also stimulates receptors which are present in the nodose ganglion (Higashi & Nishi, 1983) and in nerve fibers of the vagus (Riccioppo Neto, 1978). These receptors belong to the 5-HT_3 subtype, since they can be blocked by MDL 72222, ICS 205-930 and metoclopramide (Donatsch et al., 1984; Fozard et al., 1985)

Tachycardia in the rabbit.

1. Isolated heart.

5-HT has positive chronotropic and inotropic effects on the isolated right atrium of the rabbit which cannot be blocked by atropine, hexamethonium, and low doses of LSD (Lévy & Michel-Ber, 1956). In the Langendorff preparation 5-HT also causes an increase in beating rate which can be antagonized by propranolol (Jacob & Poite-Bevierre, 1960; Fozard & Mwaluko, 1976; Fozard and Mobarok Ali, 1978). Using the selective 5-HT_3 receptor antagonist MDL 72222 Fozard (1984) demonstrated that 5-HT increases beating rate by an action on 5-HT_3 receptors located at the terminal sympathetic nerve fibers resulting in a release of catecholamines.

2. Intact rabbits.

Initially, 5-HT induces the von Bezold-Jarisch reflex in conscious rabbits, which is later followed by tachycardia (Bolt & Saxena, 1985).

Tachycardia in the guinea pig.

1. Isolated heart.

The increase in beating rate in the isolated right atrium of the guinea pig induced by 5-HT is not modified by pretreatment with atropine, hexamethonium or LSD, but is reduced by ephedrine, a compound which releases catecholamines from nerve endings. After stimulation with nicotine, the response to 5-HT is also attenuated, suggestive for a neuronal action of 5-HT (Lévy & Michel-Ber, 1956). However, Magistretti & Valzelli (1955) have shown that LSD reduces the positive chronotropic effect of 5-HT, which has been confirmed by Trendelenburg (1960). In right atria from reserpine-pretreated guinea pigs 5-HT causes a biphasic positive chronotropic effect (Walter et al., 1984), which cannot be blocked by (-)-bupranolol, (-)-pindolol or both compounds given together. Since (-)-pindolol has a high affinity for 5-HT_{1A} and 5-HT_{1B} recognition sites (Hoyer, 1988), it is unlikely that the effect of 5-HT is mediated by the putative 5-HT_{1A} or 5-HT_{1B} receptor subtypes or by release of noradrenaline, which was confirmed by Eglen & Whiting (1989). These authors showed that 8-OH-DPAT and 5-carboxamidotryptamine are inactive, whereas 5-HT causes an uniphasic dose-response curve, in contrast to the results from Walter et al. This might be explained by the addition of normetanephrine and pargylline to the incubation solution in Eglen's study, which reduces extraneuronal uptake and MAO activity, respectively. Neither pretreatment with methysergide, metergoline, ketanserin, MDL 72222, ICS 205-930, phentolamine, hexamethonium or H_2 receptor antagonists, nor reserpinization modify the response to 5-HT. However, in this study pindolol and atenolol showed a noncompetitive antagonism.

It is still unclear whether 5-HT acts on a 5-HT receptor, which is different from the 5-HT_1-like, 5-HT_2 or 5-HT_3 receptors. The possible 5-HT-induced release of calcitonin gene-related peptide (CGRP) must also be considered, since it has been demonstrated that CGRP is present in

the guinea pig sinus node and that CGRP has a positive chronotropic action (Saito et al., 1986).

2. Intact animal

In spinal, vagosympathectomized guinea pigs 5-HT also increases heart rate dose-dependently. This is not affected by pretreatment with methiothepin, ketanserin or MDL 72222, but can be blocked by propranolol or atenolol (Dhasmana et al., 1988). The 5-HT uptake inhibitor indalpine reduces the heart rate effect of 5-HT, but from this study it is not clear whether this is caused by an increase in heart rate by indalpine itself, as observed in the pig (Bom et al., 1988), or not. In reserpinized animals the effect of tyramine is almost completely suppressed and 5-HT does not show a dose-dependent action on heart rate anymore, in contrast to the results of Eglen & Whiting (1989) in isolated atria.
In conclusion, the positive chronotropic action of 5-HT seems not to be mediated by 5-HT_1-like, 5-HT_2 or 5-HT_3 receptors. The mechanism(s) by which 5-HT is acting is still not clear.

Bradycardia in the rat.

1. Reflex bradycardia

In the anaesthetized rat 5-HT evokes the von Bezold-Jarisch reflex, which can be blocked by metoclopramide (Fozard & Host, 1982), MDL 72222 (Fozard, 1983) or ICS 205-930 (Richardson et al., 1985). As in the rabbit, 5-HT also depolarizes cervical vagus nerve fibers, which is mimicked by phenylbiguanide and 2-methyl-5-hydroxytryptamine. This effect can be blocked by metoclopramide, MDL 72222, ICS 205-930, quipazine, tropacaine and m-chloro-phenylpiperazine (mCPP) (Ireland & Tyers, 1987).

2. Centrally-acting drugs

8-OH-DPAT, a compound with high affinity for the 5-HT_{1A} recognition site, reduces heart rate in both normotensive Sprague-Dawley rats and spontaneous hypertensive rats (Gradin et al., 1985a,b), which can be prevented by the combination of atropine and propranolol, suggestive for a central action of 8-OH-DPAT on efferent vagal and sympathetic nerve activity. In these studies methiothepin, metergoline, cianserine and pirenperone did not antagonize the 8-OH-DPAT-induced bradycardia. However, Martin & Lis (1985) have shown that methiothepin blocks the 8-OH-DPAT-induced bradycardia in spontaneous hypertensive rats, whereas cyproheptadine and metergoline are ineffective. 8-OH-DPAT also reduces heart rate in Wistar-Kyoto, two-kidney Goldblatt hypertensive and DOCA-salt hypertensive rats. This response cannot be blocked by metergoline, methysergide or LY53857, but is attenuated by the dopamine antagonist sulpiride (Main et al., 1984). More recently, Fozard et al. (1987) have shown that 8-OH-DPAT lowers heart rate and blood pressure in spontaneous hypertensive rats, which is not affected by p-chloro-phenylalanine (pCPA), a compound which reduces the synthesis of 5-HT in the brain. In pithed animals 8-OH-DPAT did not cause marked changes in heart rate. 8-OH-DPAT did not modify the increase in heart rate during electrical stimulation of the spinal cord, excluding a possible presynaptic inhibitory action of this drug. Furthermore, when blood pressure was increased with angiotensin II, 8-OH-DPAT, no change in blood pressure or heart rate occurred. Cisternal administration of 8-methoxy-2-(N-2-chloroethyl-N-n propyl) aminotetralin (8-MeO-ClePAT) markedly reduces the effects of 8-OH-DPAT. Also metergoline, methiothepin, (±)-pindolol, (±)-cyanopindolol, buspirone, yohimbine, idazoxan and WY 26392 act as antagonists, whereas prazosin, MDL 72222 and cis-flupenthixol are ineffective. A low dose of ketanserin (0.1 mg kg^{-1}, s.c.) did not affect the response to 8-OH-DPAT, whereas a higher dose of ketanserin lowered heart rate by itself, thereby reducing the bradycardic effect of 8-OH-DPAT. In this study the doses of

metergoline and methiothepin were higher than those used by Martin & Lis (1985) or Gradin et al. (1985), which might explain the conflicting results. Fozard et al. (1987) concluded that 8-OH-DPAT lowers blood pressure and heart rate by an action on the central nervous sytem, probably by a direct action on 5-HT_{1A} receptors. Indirectly, a α_2-adrenoceptor-mediated action also plays a role, since α_2-adrenoceptor blockade also inhibited the response to 8-OH-DPAT.
Methysergide also loweres heart rate in spontaneous hypertensive rats, whereas cyproheptadine is ineffective. Since methysergide does not antagonize the responses to spinal cord stimulation or noradrenaline and tyramine a central action of this drug has been suggested (Antonaccio & Cote, 1976).
In vagotomized, pithed rats, 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, suggestive for presynaptic inhibition. However, this effect cannot be blocked by rauwolscine or low doses of methiothepin. Higher doses of methiothepin causes an increase in noradrenaline release and heart rate and therefore cannot be used for comparison. The noradrenaline-induced tachycardia can also be reduced by 5-methoxytryptamine in this model, but higher doses are necessary (Goethert et al., 1986).

Tachycardia in the rat.

1. Isolated heart.

In blood-perfused isolated hearts 5-HT causes only minor changes in heart rate, whereas LVdP/dt and coronary perfusion pressure are increased. Methysergide has no effect on these parameters, but reduces the effects of 5-HT. Propranolol does not modify the response to 5-HT (Sakai & Akima, 1979). In the Langendorff preparation 5-HT causes an

increase in heart rate, which can be blocked by methysergide and propranolol, but not by atenolol (Higgins et al., 1981).

2. Intact rat.

In pithed Wistar rats 5-HT causes tachycardia, which can be antagonized by cyproheptadine, but not by the 5-HT_2 receptor antagonist R 50656 (Krstic & Katusic, 1982). In untreated Wistar rats 5-HT does not induce tachycardia, probably due to the reflex bradycardia. However, in mecamylamine-induced ganglion-blocked rats the bradycardia is abolished and a tachycardic response revealed, which can be blocked by ketanserin or cyproheptadine (Saxena & Lawang, 1985). In pithed Wistar rats the 5-HT-induced tachycardia can be reduced by ketanserin, but at high doses 5-HT also exhibits a tyramine-like action, which can be reduced by desipramine or propanolol. Despite pretreatment with ketanserin and desipramine 5-HT still shows a positive chronotropic effect, which might be explained by a 5-HT-induced release of CGRP (see Tachycardia in the guinea pig). 5-methoxytryptamine also causes tachycardia, but this tachycardia can be blocked by ketanserin or methysergide, but not by propranolol or desipramine (Goethert et al., 1986). In pithed spontaneous hypertensive rats 5-carboxamidotryptamine, 8-OH-DPAT, ipsapirone and RU 24969 do not induce tachycardia, showing that 5-HT_1-like receptors are not involved, whereas the 5-HT-induced tachycardia can be reduced by ketanserin, LY 53857, methysergide or propranolol (Docherty, 1988). These results suggest that 5-HT_2 receptors are mediating the tachycardia in the rat, but at higher doses a tyramine-like action and another mechanism (CGRP ?) can also contribute to this effect.

Tachycardia in the isolated atrium of the hamster.

In this species the 5-HT-induced tachycardia is partly mediated by receptor stimulation and partly by a tyramine-like action of 5-HT (Gonzalez & Garcia, 1977). The dose-response curve is neither modified by pretreatment with a combination of phentolamine and propranolol, nor by pretreatment with phenoxybenzamine (Gonzalez & Garcia, 1978).

Tachycardia in non-human primates.

Infusions of 5-HT into the external carotid artery induces tachycardia in the baboon (African papiopapiogera), whereas blood pressure remains unchanged. However, infusion of 5-HT in the internal carotid artery does not cause any change in heart rate (Grimson et al., 1969). This might be explained by a high amount of arteriovenous anastomotic shunting in the tissues supplied by the external carotid artery, allowing a large fraction of the blood flow to go directly to the heart. The amount of unbound 5-HT in the blood reaching the heart will therefore be higher during external carotid infusions.

Heart rate changes in man.

Many investigators have shown that slow intravenous injection or infusions of 5-HT cause a dose-dependent increase in heart rate (Hollander et al., 1957; LeMessurier et al., 1959; Parks et al., 1959). Harris et al. (1960) demonstrated that rapid injection of 5-HT into the right atrium induces an asystole and a drop in blood pressure followed by bradycardia (resembling the von Bezold-Jarisch reflex observed in animals). The bradycardia in this patient was later followed by a period of tachycardia.

Conclusion

As shown in the above-mentioned studies 5-HT induces heart rate changes by a whole spectrum of mechanisms and receptors. Therefore we studied the epicardial chemoreflex in cats (Chapter 4) and the tachycardic response to 5-HT in the pig (Chapter 5) in order to find by which mechanisms and receptor-subtypes 5-HT was acting.

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Chapter 4

REFLEX INHIBITION OF EFFERENT RENAL SYMPATHETIC NERVE ACTIVITY BY 5-HYDROXYTRYPTAMINE AND NICOTINE IS ELICITED BY DIFFERENT EPICARDIAL RECEPTORS.

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Reflex inhibition of efferent renal sympathetic nerve activity by 5-hydroxytryptamine and nicotine is elicited by different epicardial receptors*

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Abstract. Intrapericardial administration of 5-hydroxytryptamine (5-HT) induced reflex effects consisting in an inhibition of renal sympathetic nerve activity (RSNA), bradycardia and a fall in blood pressure. Nicotine caused the same reflex effects as 5-HT. The reflex effects of both 5-HT and nicotine were abolished by vagotomy. MDL 72222, an antagonist at 5-HT M-receptors, abolished or attenuated the decreases in RSNA, heart rate and blood pressure induced by 5-HT, leaving the reflex effects of nicotine unchanged. In the absence of MDL 72222 the reflex bradycardia partially concealed a positive chronotropic response to 5-HT. After blockade of the bradycardia response by MDL 72222, 5-HT elicited a significant tachycardia, which was not altered by propranolol and phentolamine, but was prevented by phenoxybenzamine. 5-HT probably reaches the sinoatrial node and activates 5-HT receptors that mediate directly the increase in heart rate. The nicotine receptor antagonist hexamethonium selectively abolished or attenuated the reflex effects of nicotine without interfering with those of 5-HT. We conclude that 5-HT and nicotine elicit similar reflex effects in epicardial vagal nerve endings by stimulation of M-receptors or nicotine receptors, respectively.

Key words: Renal nerve activity – Heart rate – 5-Hydroxytryptamine – Nicotine – MDL 72222 – Hexamethonium

Introduction

The cardiovascular changes that follow an intravenous injection of 5-hydroxytryptamine (5-HT) can be regarded as net result of direct and reflex effects on heart and blood vessels. In most species the response to 5-HT is triphasic: an initial depressor effect, a short lasting pressor effect and finally a prolonged depressor effect (Douglas 1965). The initial component is due to a reflex bradycardia (Comroe et al. 1953; Mott and Paintal 1953; Schneider and Yonkman 1954); the second component is probably related to the sum of positive inotropic effects, tachycardia and arterial constriction; the late component is due to arterial vasodilation (Kalkman et al. 1983).

The changes in blood pressure and heart rate which are reflexly mediated may result from excitation of sensory vagal afferents in different organs, e.g. type J pulmonary receptors (Paintal 1955), cardiac receptors (Zakusov 1962) and carotid body chemoreceptors (Black et al. 1972; Kirby and McQueen 1984). Injection of 5-HT into the blood stream may additionally induce a depressor response mediated by stimulation of 5-HT receptors in the central nervous system (Takahashi 1985). The purpose of the present work was to analyse the reflex changes elicited by exclusive stimulation of epicardial sensory afferents by 5-HT. To avoid the complex mosaic of effects due to activation of multiple mechanism by i.v. administration, we administered 5-HT intrapericardially (i.pc.). To investigate whether an inhibition of efferent sympathetic nerve activity is part of the reflex effects, as described for nicotine (Öberg and Thoren 1973), we measured renal sympathetic nerve activity (RSNA) during i.pc. administration of 5-HT. MDL 72222 blocks the reflex bradycardia of 5-HT in the rat (Fozard 1984) by competing with 5-HT for M-receptors (Gaddum and Picarelli 1957; recently reclassified as 5-HT_3-receptors by Bradley et al. 1986) on the afferent vagal fibres. Hexamethonium prevents the action of nicotine on nonmedullated vagal fibres (Volle and Koelle 1975).

In order to find out whether the reflex effects of 5-HT and nicotine are induced through nonspecific or specific epicardial receptors we used the M-receptor antagonist MDL 72222 (1αH, 3α,5αH-tropan-3-yl-3,5-dichlorobenzoate) and the nicotine receptor antagonist hexamethonium. The results show that 5-HT causes the Bezold-Jarisch reflex and a decrease of RSNA through epicardial M-receptors located on afferent vagal fibers.

Methods

The experiments were performed on 29 cats weighing 1.9–4.9 kg anaesthetized with chloralose (60 mg/kg i.v.). A tracheal cannula was inserted and the cats were ventilated artificially with air supplemented with oxygen. The arterial pCO_2 and pH were measured periodically and the values were maintained within the physiological range by adjusting either the tidal volume or the respiratory frequency. The arterial pO_2 exceeded 100 mm Hg. The arterial pCO_2 was maintained between 25 and 30 mm Hg and pH between 7.35 and 7.45. Body temperature was kept between 37° C and 39° C by external heating.

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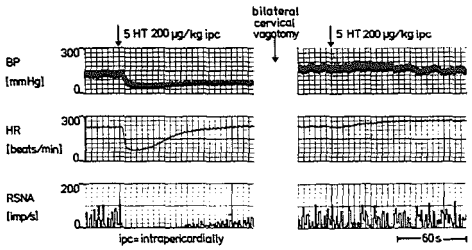

Fig. 1. Effect of intrapericardial administration of 5-HT on blood pressure (BP), heart rate (HR) and renal sympathetic nerve activity (RSNA) before and after vagotomy

Surgical procedure. After a leftsided thoracotomy the pericardium was opened. For administration of drugs a silastic catheter was introduced into the pericardial sac and sutured to the pericardium in such a way that its tip lay over the apical portion of the left ventricular surface.

Haemodynamic measurements. Blood pressure was measured in a femoral artery through a catheter connected to a Statham P23 Gb transducer. Heart rate was measured with a cardiotachometer (Gould, Oxnard, CA, USA) which was triggered by the arterial pressure wave.

Recording of nerve activity. A branch of the renal sympathetic nerve was dissected free from the surrounding tissue and cut distally. The nerve was covered with mineral oil and placed on bipolar platinum electrodes. Nerve activity was recorded as previously described by Vogt and Thämer (1980).

Experimental protocol. The protocol was started 30 min after completing the surgical preparation.

For stimulation of epicardial receptors 5-HT or nicotine were injected i.pc. The drugs were diluted in 0.9% NaCl at room temperature and the injected volume was always 1 ml. Injection of 1 ml of 0.9% NaCl at room temperature did not elicit reflex changes. In six preliminary experiments we found that no tachyphylaxis occurred following repeated administration of 5-HT and nicotine and the reproducibility of each type of stimulation was good.

We used doses of 5-HT and nicotine which produced a decrease of at least 30% in either heart rate or RSNA. Two minutes after administration of 5-HT or nicotine i.pc. the drugs were washed out with 20 ml 0.9% NaCl warmed to 37° C. The interval between two injections was 30 min. The antagonists MDL 72222 and hexamethonium were applied i.pc. 2 min before application of 5-HT or nicotine. The dose of MDL 72222 was increased (0.1 – 10 µg/kg) until it reduced or abolished the reflex effects of 5-HT. 30 min later this blocking dose of MDL 72222 was injected i.pc. again and its effect on the reflex changes of nicotine investigated. In another series of experiments the dose of hexamethonium was increased (10 – 40 µg/kg) until the reflex effects of nicotine were abolished or greatly diminished. After 30 min this blocking dose of hexamethonium was applied i.pc. again and its effect on the reflex effects of 5-HT was studied.

Statistical analysis. Data are given as mean values ± standard error of the mean. Student's *t*-test for paired observation was used to assess the significance of differences of mean values.

Table 1. Intrapericardial doses of 5-HT needed to elicit a decrease of at least 30% in either heart rate or RSNA and the corresponding intrapericardial doses of MDL 72222, which blocked the reflex

No. of animal	Dose of 5-HT (µg/kg)	Dose of MDL 72222 (µg/kg)
1	20	1
2	30	0.1
3	60	1
4	60	1
5	70	1
6	80	0.1
7	80	0.5
8	80	1
9	80	10
10	100	1
11	130	1
12	200	0.5
13	300	1

In cats 14 – 19 the blocking agent was hexamethonium and results are shown in Fig. 6

Results

Effects of 5-HT

5-HT was administered i.pc. in 29 cats, which showed a variable sensitivity for 5-HT: in 10 of the 29 cats 5-HT failed to produce reflex effects to doses up to 300 µg/kg. It cannot be excluded that higher doses would have initiated a reflex. Nevertheless in all 10 cats pronounced reflex responses could be elicited when 5-HT was injected i.v. in doses from 15 to 300 µg/kg.

In the remaining 19 cats 3 different types of reflex effects were observed: a decrease in RSNA, bradycardia and a fall in blood pressure (Figs. 1 – 3 and 6). The dose of 5-HT required to cause at least a 30% decrease in heart rate or RSNA varied from 20 to 300 µg/kg. To assure that the observed reflex effects were mediated via vagal afferent fibres, vagotomy was performed in 6 animals. After this procedure the 5-HT-induced decreases in RSNA, heart rate and blood pressure were abolished and 5-HT caused tachycardia (Fig. 1, Table 2).

In 3 of the vagotomized cats propranolol (0.5 mg/kg) and phentolamine (1 mg/kg) were administered jointly i.v. This lowered mean arterial blood pressure from 108 ± 22 mm Hg to 70 ± 25 mm Hg and decreased heart rate, but the 5-HT-induced increase in heart rate persisted (Table 2).

In the other 3 vagotomized cats i.v. administration of phenoxybenzamine (5 mg/kg) led to a fall in mean arterial blood pressure from 150 ± 15 mm Hg to 77 ± 6 mm Hg, while heart rate increased (Table 2). The pretreatment with phenoxybenzamine abolished the 5-HT mediated tachycardia (Table 2).

Effects of nicotine

Nicotine in a dose of 10 or 20 µg/kg consistently elicited decreases of RSNA, heart rate and blood pressure in each animal (Figs. 4, 5).

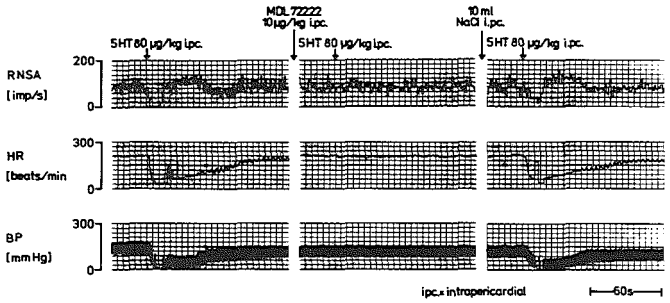

Fig. 2
Reversible blockade of the reflex effect of 5-HT by MDL 72222. (BP) arterial pressure; (HR) heart rate; (RSNA) renal sympathetic nerve activity

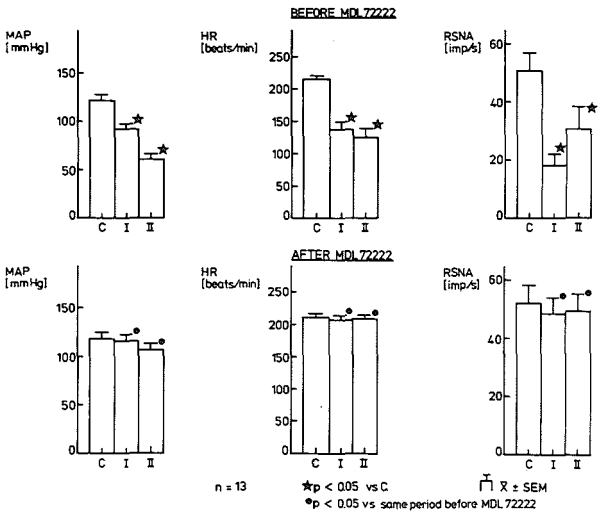

Fig. 3
Selective blockade of the reflex effects of 5-HT by MDL 72222. (MAP) mean arterial pressure, (HR) heart rate, (RSNA) renal sympathetic nerve activity. *C* control; *I* average value for the period of 1 to 15 s after the administration of 5-HT; *II* average value for the period of 16 to 30 s after the administration of 5-HT; n = number of cats

Blockade by MDL 72222 of the reflex effects of 5-HT

I.pc. administration of MDL 72222 did not change RSNA, heart rate and blood pressure, but greatly diminished or abolished the response in RSNA, heart rate and blood pressure to 5-HT (Figs. 2, 3). With the exception of one experiment (cat no. 9 in Table 1) we found that 1 µg/kg MDL 72222 was sufficient to block the reflex effects of 5-HT; sometimes a blockade could be obtained with even lower dosages (Table 1). The inhibitory effect of MDL 72222 was reversible; after washing the pericardial sac with 0.9% NaCl the reflex could be induced again with 5-HT. Reversibility of blockade was even observed when the concentration of MDL 72222 was unusually high (Fig. 2).

The response in heart rate to 5-HT was biphasic in the absence of MDL 72222; following the bradycardia there was a trend to a positive chronotropic response. Heart rate fell from 215 ± 6 to a minimum of 125 ± 14 beats/min and reached a value of 227 ± 6 beats/min after 2 min. This late tachycardia became significant when the bradycardia was abolished by MDL 72222; heart rate significantly increased from 210 ± 7 to 235 ± 4 beats/min after 2 min ($p < 0.05$, $n = 13$).

Lack of effect of MDL 72222 on the nicotine-induced reflex

The dose of MDL 72222, (0.1 – 10 µg/kg) which blocked the reflex effects of 5-HT did not alter the reflex response to nicotine (Fig. 4).

Effect of hexamethonium on the reflex effects of nicotine

I.pc. administration of hexamethonium did not cause significant changes in RSNA, heart rate and blood pressure. A dose of 10 to 40 µg/kg hexamethonium greatly diminished the nicotine-induced bradycardia, decrease in RSNA and fall in blood pressure (Fig. 5).

Hexamethonium does not block the 5-HT induced reflex

The dose of hexamethonium, which diminished the reflex response to nicotine did not change the fall in heart rate and

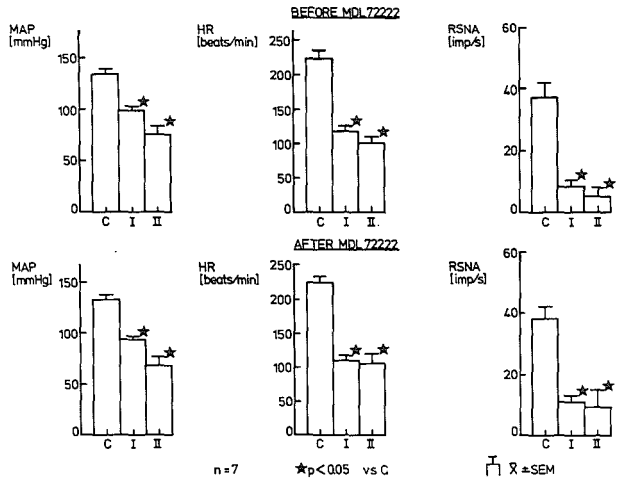

Fig. 4
Lack of blockade of the reflex effects of nicotine by MDL 72222. Details were equivalent to those of Fig. 3

Table 2
Effect of vagotomy and combinations with propranolol, phentolamine and phenoxybenzamine on the positive chronotropic response to 5-HT

	n	Heart rate (beats/min)		
		Control	Vagotomy	Vagotomy + 0.5 mg/kg i.v. propranolol + 1 mg/kg i.v. phentolamine
Before 5-HT	3	218 ± 14	217 ± 9	185 ± 9
2 min after 5-HT		248 ± 8	248 ± 11	230 ± 25
	n	Heart rate (beats/min)		
		Control	Vagotomy	Vagotomy + 5 mg/kg i.v. phenoxybenzamine
Before 5-HT	3	217 ± 16	227 ± 9	250 ± 18
2 min after 5-HT		235 ± 46	262 ± 11	245 ± 15

blood pressure and the inhibition of RSNA induced by 5-HT (Fig. 6).

Discussion

Our experiments demonstrate that 5-HT can induce pronounced reflex effects by stimulation of epicardial receptors. The reflex response includes a bradycardia as well as an inhibition of RSNA and a fall in blood pressure. All 3 responses were abolished by vagotomy, indicating that the decrease in RSNA is also mediated by excitation of sensory vagal afferents, as already known for the bradycardia and early hypotension elicited by i.v. administration of 5-HT (Comroe et al. 1953; Schneider and Yonkman 1954; Kottegoda and Mott 1955).

We assume that sympathetic inhibition is not restricted to the renal bed because: (1) the reflex pattern of other chemical agents like nicotine includes a strong vasodilatation in the skeletal muscle (Öberg and Thorén 1973) and (2) Kottegoda and Mott (1955) described an increase in blood flow in the cat's hind limb after i.v. injection of 5-HT, which persisted after administration of atropine but was abolished by vagotomy. We also found that ip.c. 5-HT reduced efferent nerve activity in cardiac nerves (Bom et al. 1985). These findings suggest that sympathetic inhibition as well as bradycardia contribute to the early fall in blood pressure induced by 5-HT.

Page (1952) and Comroe et al. (1953) observed a considerable variability in the sensitivity of cats to 5-HT injected i.v. Our data also show that the sensitivity of cats to 5-HT administered i.pc. was very variable: in 10 of 29 cats no reflex effects could be induced, while the remaining 19 cats required doses from 20 to 300 μg/kg to show clear reflex responses. In all the cats without reflex response to 5-HT i.pc., marked reflex responses were obtained when 5-HT was administered i.v. A general lack of receptors sensitive to 5-HT is therefore unlikely.

One explanation for the variable sensitivity for 5-HT might be a quite different number and distribution of 5-HT receptors on the epicardial surface of individual cats.

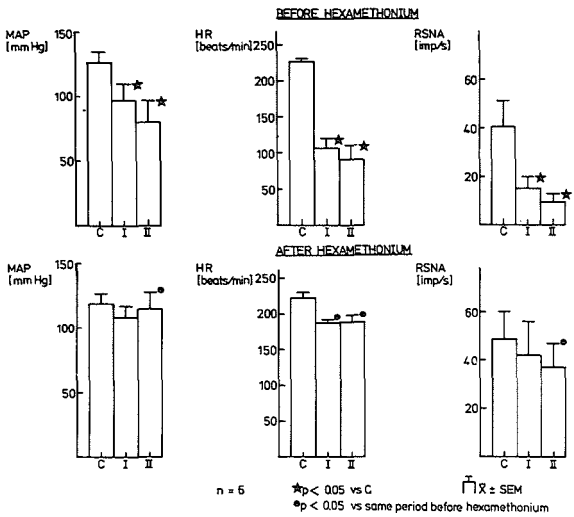

Fig. 5
Blockade of the reflex effects of nicotine by hexamethonium. Details were equivalent to those of Fig. 3

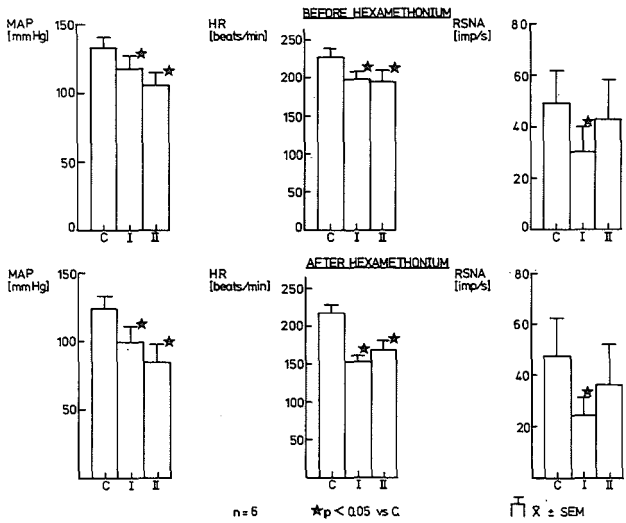

Fig. 6
Lack of blockade of the reflex effects of 5-HT by hexamethonium. Details were equivalent to those of Fig. 3

Although we know the total dose of 5-HT injected i.pc., variability in actual 5-HT concentrations due to differences in the quantity of pericardial fluid in the respective heart regions is possible. These factors could also account for the variability of the reflex responses. These explanations, however, appear unlikely because cats should have shown the same variability in sensitivity to the blocking agent MDL 72222 and this was not observed. Another explanation could be an interaction of a vagal depressor response and a counteracting sympathetic reflex. Reimann and Weaver (1980) reported that epicardial application of bradykinin, which is able to stimulate vagal as well as sympathetic afferent fibers, leads to variable reflex responses in cats. They observed excitatory responses, inhibitory responses or no changes in blood pressure and RSNA, whereas the response was always inhibitory after thoracic sympathectomy and always excitatory after vagotomy. Therefore they assumed that the varying responses of individual cats depended on the relative density of sympathetic and vagal receptors accessible to the chemical or the relative excitability of the two afferent

groups. The same could be true for 5-HT because this substance does not only stimulate vagal afferent fibres but also cardiac afferent sympathetic fibres (Uchida et al. 1969). However, the participation of afferent sympathetic fibres appears unlikely for 2 reasons: (1) we never saw an excitatory response in the intact animal and (2) even after vagotomy none of the six cats showed an increase in blood pressure and RSNA. Zimpfer et al. (1981) described a depression of chemoreceptor reflex responses with the anaesthetic α-chloralose in dogs. Although there was no difference regarding doses of α-chloralose among cats in our experiments, a different sensitivity to α-chloralose leading to varying reflex responses might be an explanation for the variability in the sensitivity to 5-HT.

To block the reflex effects of 5-HT we used the 5-HT antagonist MDL 72222, which has been shown to block selectively: (1) the excitatory action of 5-HT on sympathetic nerve terminals of the rabbit heart (Fozard and Gittos 1983), (2) the rapid response of cat carotid body chemoreceptors to 5-HT (Kirby and McQueen 1984) and (3) the 5-HT induced depolarization in the nodose ganglion and the superior cervical ganglion of the rabbit (Azami et al. 1985). Fozard (1984) also reported a marked attenuation by MDL 72222 of the heart rate and blood pressure response of the rat to i.v. 5-HT.

We were able to prevent the reflex effects of 5-HT by MDL 72222, whereas the reflex effects of nicotine were unchanged. This evidence is compatible with a selective blockade of vagal 5-HT receptors of the M-type (Gaddum and Picarelli 1957; Fozzard 1984; Richardson et al. 1985) by MDL 72222.

MDL 72222 selectively inhibited the positive chronotropic response to 5-HT in the isolated rabbit heart (Fozard 1984). In contrast, we found in the cat that marginal tachycardia developed with 5-HT, which became more pronounced when the bradycardic response to 5-HT was blocked by MDL 72222 or vagotomy. Therefore, MDL 72222 is not an antagonist at the receptor site responsible for the tachycardia in the cat. This is in agreement with the earlier findings of Trendelenburg (1960), who showed striking differences in the mechanism by which 5-HT induces a positive chronotropic response in the isolated atrium of rabbit and cat.

In the rabbit 5-HT seems to act by a release of noradrenaline from sympathetic nerve endings because the effects were prevented by reserpine pretreatment, whereas in the cat reserpine had no specific effects against 5-HT. This indicates that the positive chronotropic action of 5-HT in the cat is not mediated by endogenous catecholamines. In analogy to these experiments on isolated tissues we were not able to block the 5-HT mediated tachycardia in the cat by the α-receptor antagonist phentolamine and the β-receptor antagonist propranolol. Kaumann (1983, 1985a, b) has shown that the positive chronotropic effects of 5-HT on isolated kitten atria are irreversibly blocked by phenoxybenzamine. We confirmed this in vivo in three experiments in vagotomized cats where 5-HT did not induce an increase in heart rate after administration of phenoxybenzamine.

The nature of myocardial 5-HT receptors is not completely understood. 5-HT has a relatively low affinity for cat sinoatrial receptors (Kaumann 1983, 1985a, b) suggesting involvement of 5-HT_2-receptors. However concentrations of ketanserin saturating 5-HT_2-receptors do not antagonize the positive chronotropic effects of 5-HT in kitten atria, ruling out their 5-HT_2-nature.

Surprisingly, some drugs with high affinity for 5-HT_1-receptors also do not antagonize the positive chronotropic effects of 5-HT in kitten atria. Thus it has been concluded that sinoatrial 5-HT receptors of kitten heart are neither 5-HT_1 nor 5-HT_2 (Kaumann 1985a, 1986).

The nicotinic antagonist hexamethonium selectively blocked the reflex effects of nicotine, but did not change the responses in heart rate, RSNA and blood pressure to 5-HT. Hexamethonium applied i.pc. did not change control conditions in heart rate, blood pressure and RSNA. The dosage was too low to block transmission in sympathetic ganglia if absorption would have occurred, but it might have blocked transmission in parasympathetic ganglia, which are situated in the subepicardial region. This is unlikely for 2 reasons: (1) heart rate did not rise after application of hexamethonium and (2) the bradycardia induced by 5-HT was unchanged after application of hexamethonium.

We conclude that reflex bradycardia and reflex decrease of RSNA can be elicited on epicardial sensory vagal afferents by specific activation of M-receptors with 5-HT or of nicotinic receptors with nicotine.

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Chapter 5

5-HYDROXYTRYPTAMINE-INDUCED TACHYCARDIA IN THE PIG: POSSIBLE INVOLVEMENT OF A NEW TYPE OF 5-HYDROXYTRYPTAMINE RECEPTOR

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5-Hydroxytryptamine-induced tachycardia in the pig: possible involvement of a new type of 5-hydroxytryptamine receptor

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1 The mechanism of 5-hydroxytryptamine (5-HT)-induced tachycardia is 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) receptors, or by an action similar to tyramine (guinea-pig). The present investigation is devoted to the analysis of the positive chronotropic effect of 5-HT in the pentobarbitone-anaesthetized pig.

2 Intravenous bolus injections of 5-HT (3, 10 and 30 $\mu g\,kg^{-1}$) in pigs resulted in dose-dependent increases in heart rate of 24 ± 2, 38 ± 3 and 51 ± 3 beats min^{-1}, respectively ($n = 39$). Topical application of a high concentration of 5-HT (150 $\mu g\,kg^{-1}$ in 5 ml) on the right atrium was also followed by tachycardia (38 ± 6 beats min^{-1}, $n = 4$).

3 A number of drugs which antagonize responses mediated by different 5-HT receptors – phenoxybenzamine, methiothepin, metergoline, methysergide and mesulergine ('5-HT_1-like' and 5-HT_2 receptors), ketanserin, cyproheptadine, pizotifen and mianserin (5-HT_2 receptors), and MDL 72222 and ICS 205-930 (5-HT_3 receptors) – did not attenuate the chronotropic responses to 5-HT.

4 The 5-HT-induced tachycardia was also not affected by antagonists at α- and β-adrenoceptors, muscarinic, nicotinic, histamine and dopamine receptors, and calcium channels.

5 Selective inhibitors of 5-HT-uptake, indalpine and fluvoxamine, themselves increased porcine heart rate and facilitated 5-HT-induced tachycardia both in magnitude and in duration.

6 A number of putative selective agonists at '5-HT_1-like' receptors or their possible subtypes (5-carboxamidotryptamine (5-CT), 8-hydroxy-2-(di-N,N-n-propylamino) tetralin (8-OH-DPAT), BEA 1654 and RU 24969), or at 5-HT_3 receptors (2-methyl-5-HT), elicited no or only a weak tachycardiac response in the pig. RU 24969, but not 8-OH-DPAT, seemed to potentiate the responses to 5-HT, whereas 5-CT slightly inhibited these responses.

7 It was concluded that the tachycardia induced by 5-HT in the pig does not involve the receptors for some common neurotransmitter substances but may be mediated by a new 5-HT receptor type that is clearly different from '5-HT_1-like', 5-HT_2 or 5-HT_3 receptors.

Introduction

Intravenous bolus injections of 5-hydroxytryptamine (5-HT) result in a transient decrease in heart rate (Page, 1958) due to a chemoreceptor reflex (von Bezold-Jarisch reflex) initiated by the stimulation of receptors on sensory vagal afferent fibres (Paintal, 1973). In the rat (Fozard, 1984; Richardson *et al.*, 1985) and the cat (Saxena *et al.*, 1985a) the bradycardiac effect has been shown to be mediated by 5-HT_3 receptors (for nomenclature of 5-HT receptors, see Bradley *et al.*, 1986; Saxena *et al.*, 1986b).

5-HT can also increase heart rate in a variety of non-mammalian and mammalian species, including man (Page, 1958; Hollander *et al.*, 1957; LeMessurier *et al.*, 1959). The tachycardiac action of 5-HT has been analysed in some species and it involves several different mechanisms (Trendelenburg, 1960; Saxena, 1986). In the cat 5-HT-induced tachycardia is mediated by myocardial '5-HT_1-like' receptors (Saxena *et al.*, 1985a; Connor *et al.*, 1986), and in the rat (Saxena & Lawang, 1985; Göthert *et al.*, 1986) and the dog (Feniuk *et al.*, 1981) 5-HT_2 receptors, present on the myocardium and adrenal medulla,

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respectively, seem to be involved. The third 5-HT receptor type characterized so far (5-HT_3 receptor), located on the postganglionic cardiac sympathetic fibres, mediates the effects of 5-HT in the rabbit (Fozard, 1984) while, in the guinea-pig, tachycardia has been attributed to β-adrenoceptors (Eglen *et al.*, 1985), probably activated by catecholamines released via a tyramine-like action (De Boer *et al.*, 1986; Dhasmana *et al.*, 1988). High concentrations of 5-HT can also cause tachycardia by such a mechanism in the rat (Göthert *et al.*, 1986) and in the hamster (González Alvarez & García Rodríguez, 1977).

In the present experiments the increase in heart rate caused by 5-HT in the pig has been investigated and the results reveal the involvement of a mechanism which is different from those proposed so far (see above). Preliminary results of this investigation were communicated to the British Pharmacology Society (Duncker *et al.*, 1985).

Methods

General

After an overnight fast, 42 young Yorkshire pigs (25–30 kg, 12–16 weeks old) were initially sedated with 120 mg (i.m.) azaperone and 120–150 mg (i.v.) metomidate. After the animals had been intubated, they were connected to a respirator for intermittent positive pressure ventilation with a mixture (1 : 2) of oxygen and nitrous oxide. The anaesthesia was maintained with a continuous infusion of pentobarbitone sodium (15–20 mg kg^{-1} h^{-1}, i.v.). Aortic blood pressure was recorded with a Statham pressure transducer via a cannula inserted into the left femoral artery. All drugs were injected into the left femoral or jugular vein cannulated for this purpose. The animal's temperature was maintained around 37°C by using an electric blanket and arterial blood gasses 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 30 min, bolus injections of 5-HT (3, 10 and 30 $\mu g\,kg^{-1}$, i.v.) were given at intervals of 10–15 min. Subsequently, one of the several antagonist drugs (for names, doses and number of experiments, see the Drug and Results sections), used to analyse the positive chronotropic effects of 5-HT, was slowly administered over two minutes. About 10 min later, the responses to the three doses of 5-HT were elicited again. In initial experiments it was noticed that several drugs, in doses sufficient for the purpose for which they were employed, did not modify the effects of 5-HT. Therefore, in order to restrict the number of animals to be used for this investigation, it was decided to use more than one such drug, rather than several doses of a particular drug, in any single experiment. The order of their use was varied and a total of 30 animals was used for the above purpose. Furthermore, in 3 animals the reproducibility of the 5-HT-induced tachycardia was checked after a period of 2 h.

In 13 animals (12 new and 1 from the above group) the effect of some drugs thought to be selective agonists at different 5-HT receptors was studied. Lastly, after the above experiments had been completed, on 4 occasions 5-HT was administered locally on the surface of the right atrium.

Data presentation and analysis

All data in the text and tables, unless otherwise stated, are presented as mean ± s.e. mean. The peak change in heart rate by the different doses of 5-HT (and other putative 5-HT receptor agonists) were recorded. The mean ± s.e. mean increases in heart rate by the three doses of 5-HT just before and after a particular antagonist drug were compared by use of Duncan's new multiple range test, once an analysis of variance (randomized block design) revealed that the samples represented different populations (Saxena, 1985). A P value of 0.05 or less (two-tailed) was considered statistically significant.

Drugs

The following drugs used in this study were kindly supplied by the sources mentioned: atropine sulphate (Pharmacy Department, Erasmus University Rotterdam, Rotterdam, The Netherlands), 5-carboxamidotryptamine maleate (5-CT; Dr W. Feniuk, Glaxo, Ware, U.K.), cimetidine hydrochloride (Smith Kline & French, Welwyn Garden City, U.K.), cyproheptadine hydrochloride (Merck, Sharpe and Dohme, Haarlem, The Netherlands), fluvoxamine maleate (Dr W. Wouters, Duphar, Weesp, The Netherlands); haloperidol base (Janssen Pharmaceutica, Beerse, Belgium), histamine phosphate (Pharmacy Department, Erasmus University Rotterdam, Rotterdam, The Netherlands), 8-hydroxy-2-(di-N,N-n-propylamino)tetralin (8-OH-DPAT; Dr J.R. Fozard, Merrell Dow Research Institute, Strasbourg, France), indalpine base (Dr A. Uzan, Pharmuka Laboratories, Gennevilliers, France), isoprenaline sulphate (Pharmacy Department, Erasmus University Rotterdam, Rotterdam, The Netherlands), ketanserin tartrate (Dr J.M. Van

Neuten, Janssen Pharmaceutica, Beerse, Belgium), mepyramine maleate (Rhone-Poulenc, Paris, France), noradrenaline bitartrate (Pharmacy Department, Erasmus University Rotterdam, Rotterdam, The Netherlands), mesulergine hydrochloride (Dr G. Engel, Sandoz, Basle, Switzerland), metergoline base (Farmatalia Carlo Erba, Torino, Italy), methiothepin methane-sulphonate (Hoffman La Roche, Mijdrecht, The Netherlands), 5-methoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl-1H-indole succinate (RU 24969; Roussel Laboratories, Hoevelaken, The Netherlands), 2-methyl-5-hydroxytryptamine maleate (2-methyl-5-HT; Dr W. Feniuk, Glaxo, Ware, U.K.), methysergide hydrogen maleate (Sandoz, Basle, Switzerland), mianserin hydrochloride (Organon, Oss, The Netherlands), pizotifen maleate (Sandoz, Basle, Switzerland), phentolamine hydrochloride (Ciba-Geigy, Basle, Switzerland), phenoxybenzamine hydrochloride (dibenzyline; Smith Kline & French, Philadelphia, U.S.A.), propranolol hydrochloride (Imperial Chemical Industries, Rotterdam, The Netherlands), 1αH,3α,5αH-tropan-3yl-3,5-dichlorobenzoate (MDL 72222; Dr J.R. Fozard, Merrell Dow Research Institute, Strasbourg, France), (3α-tropanyl)-1H-indole-3-carboxylic acid ester (ICS 205-930; Dr G. Engel, Ludwigshafen, F.R.G.). Other drugs were purchased: 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP; Sigma, St. Louis, U.S.A.), 5-hydroxytryptamine creatinine sulphate (5-HT; Merck, Darmstadt, F.R.G.) and hexamethonium bromide (Fluka, Buchs, Switzerland). The doses mentioned in the text refer to the salts of substances except in the case of 5-HT and 5-CT, where they refer to the base. All drugs were dissolved in saline before injection; fresh solutions were prepared for each experiment.

Results

Effects of 5-HT

Intravenous bolus injections of 5-HT (3, 10 and 30 $\mu g\,kg^{-1}$) were given in a total of 39 pigs where the baseline values of heart rate and mean arterial blood pressure were, respectively, 90 ± 3 beats min^{-1} and 78 ± 2 mmHg. None of the three doses of 5-HT produced consistent changes in arterial pressure and, therefore, they were not quantified further. Bradycardia due to the Bezold-Jarisch reflex, as is usual in several species, was also absent, probably due to the level of anaesthesia. These doses of 5-HT, however, induced dose-dependent increases in heart rate of 24 ± 2, 38 ± 2 and 51 ± 3 beats min^{-1}, respectively ($n = 39$). In 3 animals the reproducibility of the tachycardiac effects of 5-HT was tested; the increase in heart rate by the three doses of 5-HT were, respectively, 29 ± 2, 45 ± 2 and 65 ± 4 beats min^{-1} and after an interval of 2 h: 27 ± 3, 38 ± 2 and 59 ± 2 beats min^{-1}.

Topical application of a high concentration of 5-HT (150 $\mu g\,kg^{-1}$ in 5 ml) on the right atrium was followed by an increase in heart rate of 38 ± 6 beats min^{-1} from a basal value of 91 ± 5 beats min^{-1} in 4 pigs.

Tachycardiac effects of 5-HT after some antagonist drugs

To study the involvement of the three types of 5-HT receptors the effects of non-selective ('5-HT_1-like' and 5-HT_2: phenoxybenzamine, methiothepin, metergoline, methysergide and mesulergine) and selective (5-HT_2: ketanserin, cyproheptadine, pizotifen and mianserin; 5-HT_3: MDL 72222 and ICS 205-930) 5-HT receptor antagonists were studied. None of these drugs (except MDL 72222 against the lowest dose of 5-HT) reduced the magnitude of tachycardia induced by 5-HT. One such experiment involving MDL 72222, cyproheptadine and methysergide is shown in Figure 1, while the summary of all the data is presented in Table 1. It may be noted that, instead of an antagonism, phenoxybenzamine (highest dose of 5-HT) and cyproheptadine seemed to facilitate the chronotropic effects of 5-HT.

Since none of the above 5-HT antagonists was capable of reducing the 5-HT-induced tachycardia, a number of other commonly used antagonist drugs were investigated. Table 2 shows that the antagonists at adrenoceptors, cholinoceptors, histamine and dopamine receptors and at voltage-dependent calcium channels did not modify the responses to the three doses (3, 10 and 30 $\mu g\,kg^{-1}$) of 5-HT. The effectiveness of phentolamine, propanolol, hexamethonium and mepyramine was confirmed by blockade of, respectively, the noradrenaline (1 $\mu g\,kg^{-1}$)-induced pressor effect, and isoprenaline (0.1 $\mu g\,kg^{-1}$)-, DMPP (20 $\mu g\,kg^{-1}$)- and histamine (3 $\mu g\,kg^{-1}$)-induced tachycardia; the responses before and after the antagonists involved were respectively: noradrenaline, 36 ± 7 and 18 ± 4 mmHg ($n = 4$); isoprenaline: 48 ± 2 and 1 ± 1 beats min^{-1} ($n = 3$); DMPP, 54 ± 17 and 3 ± 2 beats min^{-1} ($n = 4$) and histamine, 15 ± 7 and 1 ± 1 beats min^{-1} ($n = 4$). The hypotensive response to histamine was also antagonized by mepyramine (-18 ± 2 mmHg before and -2 ± 0 mmHg after mepyramine). Cimetidine did not antagonize the tachycardia caused by histamine indicating that, as in the cat (Owen, 1977), the histamine-induced tachycardia in the pig is mediated by histamine H_1 receptors, probably on the adrenal medulla. The hypertensive response to noradrenaline remaining after phentolamine was apparently due to an

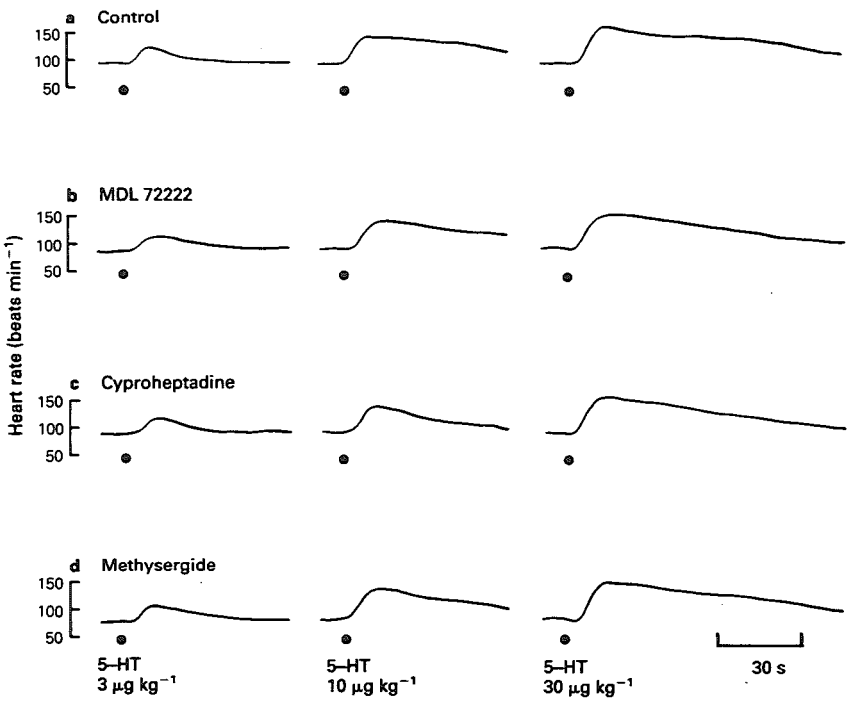

Figure 1 The effects of 5-hydroxytryptamine (5-HT; 3, 10 and 30 $\mu g\,kg^{-1}$) on heart rate in an anaesthetized pig before (a) and after successive administration of MDL 72222 (0.3 $mg\,kg^{-1}$) (b), cyproheptadine (0.5 $mg\,kg^{-1}$) (c) and methysergide (0.5 $mg\,kg^{-1}$) (d). Note that 5-HT caused a dose-dependent tachycardia which was not antagonized by these drugs.

Table 1 Effects of antagonists at different 5-hydroxytryptamine (5-HT) receptors on the 5-HT-induced increases in heart rate (beats min^{-1}) in the pig

			5-HT 3 $\mu g\,kg^{-1}$		*5-HT* 10 $\mu g\,kg^{-1}$		*5-HT* 30 $\mu g\,kg^{-1}$	
Antagonist	*Dose* ($mg\,kg^{-1}$)	n	*Before*	*After*	*Before*	*After*	*Before*	*After*
Phenoxybenzamine	1.0	3	38 ± 13	44 ± 10	46 ± 10	55 ± 12	44 ± 11	60 ± 10*
Methiothepin	0.5	6	22 ± 3	21 ± 4	40 ± 5	39 ± 4	53 ± 5	53 ± 3
Metergoline	0.5	5	29 ± 2	22 ± 3	49 ± 3	46 ± 6	63 ± 4	63 ± 6
Methysergide	0.5	6	26 ± 3	28 ± 3	50 ± 4	49 ± 3	63 ± 5	63 ± 3
Mesulergine	0.3	3	25 ± 1	26 ± 1	40 ± 2	45 ± 2	52 ± 4	56 ± 4
Ketanserin	0.5	4	20 ± 4	18 ± 4	42 ± 8	41 ± 7	58 ± 7	57 ± 7
Cyproheptadine	0.5	5	17 ± 3	23 ± 3*	40 ± 3	45 ± 4*	54 ± 3	60 ± 4*
Pizotifen	0.5	5	29 ± 2	26 ± 2	49 ± 3	45 ± 2	61 ± 5	56 ± 4
Mianserin	0.5	5	24 ± 2	23 ± 2	45 ± 1	44 ± 3	59 ± 3	54 ± 4
MDL 72222	0.3	4	28 ± 4	22 ± 5*	45 ± 4	45 ± 5	60 ± 5	58 ± 6
ICS 205-930	0.3	3	29 ± 2	21 ± 3	43 ± 4	41 ± 2	54 ± 6	56 ± 1

* $P < 0.05$, after vs before.

Table 2 Effects of some common antagonists on the 5-hydroxytryptamine (5-HT)-induced increases in heart rate (beats min^{-1}) in the pig

			5-HT 3 $\mu g kg^{-1}$		*5-HT* 10 $\mu g kg^{-1}$		*5-HT* 30 $\mu g kg^{-1}$	
Antagonist	*Dose* ($mg kg^{-1}$)	n	*Before*	*After*	*Before*	*After*	*Before*	*After*
Phentolamine	1.0	5	26 ± 4	24 ± 6	42 ± 4	38 ± 6	58 ± 5	51 ± 12
Propranolol	0.5	5	24 ± 4	27 ± 4	43 ± 3	43 ± 4	60 ± 4	60 ± 3
Atropine	0.5							
+ propranolol	0.5	3	37 ± 4	35 ± 4	50 ± 5	51 ± 1	55 ± 6	58 ± 2
Atropine	1.0							
+ hexamethonium	10.0	4	9 ± 2	10 ± 2	24 ± 11	37 ± 7	32 ± 7	53 ± 8
Cimetidine	1.0	4	38 ± 5	33 ± 3	48 ± 5	47 ± 4	58 ± 5	56 ± 6
Mepyramine	1.0	4	31 ± 5	35 ± 3	45 ± 7	47 ± 5	55 ± 7	53 ± 5
Haloperidol	1.0	3	31 ± 4	35 ± 2	45 ± 5	46 ± 3	51 ± 6	55 ± 4
Verapamil	0.1[a]	3	23 ± 4	23 ± 2	44 ± 2	34 ± 7	58 ± 2	45 ± 5

[a] This dose was followed by an infusion of 0.01 $mg kg^{-1} min^{-1}$.

increase in cardiac output, since it was eliminated after additional administration of propranolol.

Lastly, indalpine and fluvoxamine, which are known to interfere with the uptake of 5-HT by nerve terminals and blood platelets (Claasen *et al.*, 1977; Le Fur & Uzan, 1977; Ashkenazi *et al.*, 1983), were employed in an attempt to antagonize the tachycardiac responses to 5-HT. However, after-administration of these drugs the positive chronotropic responses to bolus injections of 5-HT were not reduced, but, on the contrary, these responses were facilitated both in magnitude and in duration (see Figure 2 for indalpine and Table 3 for both indalpine and fluvoxamine). Furthermore, the two 5-HT-

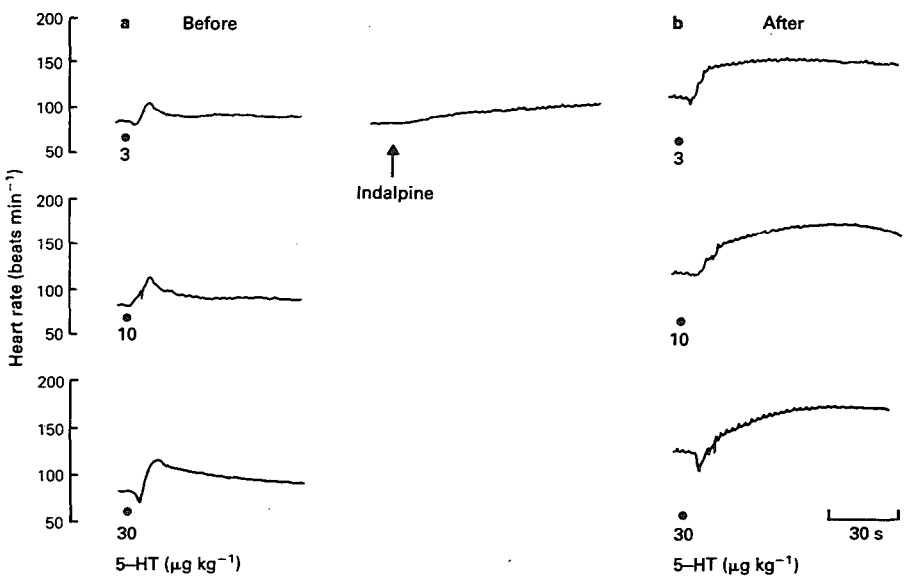

Figure 2 The effects of 5-hydroxytryptamine (5-HT; 3, 10 and 30 $\mu g kg^{-1}$) on heart rate in an anaesthetized pig before (a) and after (b) administration of indalpine (1 $mg kg^{-1}$). It can be seen that heart rate increased after indalpine and that this drug potentiated the tachycardiac responses to 5-HT.

Table 3 Effects of 5-hydroxytryptamine (5-HT)-uptake blockers on the 5-HT-induced increases in heart rate (beats min^{-1}) in the pig

			5-HT $3\,\mu g\,kg^{-1}$		*5-HT* $10\,\mu g\,kg^{-1}$		*5-HT* $30\,\mu g\,kg^{-1}$	
Antagonist	*Dose* ($mg\,kg^{-1}$)	n	*Before*	*After*	*Before*	*After*	*Before*	*After*
Indalpine	1.0	3	20 ± 3	48 ± 3*	30 ± 1	58 ± 3*	45 ± 3	54 ± 7*
Fluvoxamine	1.0	4	11 ± 2	26 ± 2*	21 ± 2	40 ± 8*	30 ± 3	48 ± 9*

* $P < 0.05$, after vs before.

Table 4 Effects of putative agonists of 5-hydroxytryptamine (5-HT) receptors on the 5-HT-induced increases in heart rate (beats min^{-1}) in the pig

			5-HT $3\,\mu g\,kg^{-1}$		*5-HT* $10\,\mu g\,kg^{-1}$		*5-HT* $30\,\mu g\,kg^{-1}$	
Agonist	*Dose* ($mg\,kg^{-1}$)	n	*Before*	*After*	*Before*	*After*	*Before*	*After*
RU-24969	1.0	3	14 ± 3	39 ± 5*	13 ± 3	41 ± 5*	24 ± 4	47 ± 8*
8-OH-DPAT	1.0	3	33 ± 13	33 ± 10	44 ± 4	47 ± 4	57 ± 7	61 ± 12
5-CT	0.1	3	27 ± 3	18 ± 2*	38 ± 2	28 ± 2*	59 ± 2	43 ± 1*

* $P < 0.05$, after vs before. 8-OH-DPAT = 8-hydroxy-2-(di-N,N-n-propylamino)tetralin and 5-CT = 5-carboxamidotryptamine.

uptake blockers caused a slowly developing increase in heart rate; the peak tachycardiac effects following indalpine and fluvoxamine were 49 ± 10 ($n = 3$) and 10 ± 5 ($n = 4$) beats min^{-1}, respectively.

Effects of some putative agonists of 5-HT

The effects of intravenous injections of 5-CT, 8-OH-DPAT, BEA 1654, RU 24969 and 2-methyl-5-HT on the heart rate were also studied. Compared to 5-HT, none of these drugs had any profound effect on porcine heart rate (Figure 3). In experiments with RU 24969, 8-OH-DPAT and 5-CT the effect of the highest dose of each compound was also studied on the heart rate responses to 5-HT. The tachycardia caused by 3, 10 and $30\,\mu g\,kg^{-1}$ of 5-HT was potentiated after administration of RU 24696 ($1\,mg\,kg^{-1}$), remained unchanged after 8-OH-DPAT ($1\,mg\,kg^{-1}$) and was slightly inhibited by 5-CT ($0.1\,mg\,kg^{-1}$) (see Table 4).

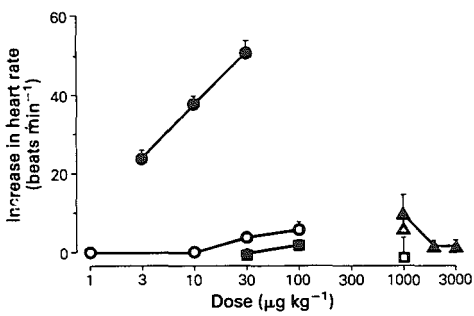

Figure 3. Heart rate responses to a number of agonists at 5-hydroxytryptamine (5-HT) receptors: 5-carboxamidotryptamine (○, $n = 4$), 2-methyl-5-HT (■, $n = 3$), 8-hydroxy-2-(di-N,N-n-propylamino)tetralin (△, $n = 3$), RU-24969 (□, $n = 4$) and BEA 1654 (▲, $n = 3$). Compared to 5-HT (●, $n = 39$), these agents elicited either no or only a slight increase in heart rate. The values of heart rates before administration of different doses of agonists varied between 74 ± 5 and 96 ± 13 beats min^{-1} and did not differ significantly.

Discussion

Consideration of known 5-HT receptors

Until recently 5-HT receptors had been classified on the basis of antagonism by dibenzyline (phenoxybenzamine) and morphine as 'D' and 'M' receptors, respectively (Gaddum & Picarelli, 1957), or on the basis of high binding affinity for [^{3}H]-5-HT and [^{3}H]-spiroperidol as 5-HT_1 and 5-HT_2, respectively (Peroutka & Snyder, 1979). However, neither of these classifications, though correct within themselves, adequately covered the pharmacological actions of 5-HT. Being aware of this drawback and prompted by the recent availability of more selective drug tools, Bradley *et al.* (1986) subdivided 5-HT receptors into three distinct groups named as '5-HT_1-like', 5-HT_2 and 5-HT_3. They advocated 5-CT ('5-HT_1-like') and 2-methyl-5-HT (5-HT_3) as selective agonists, and methiothepin and methysergide ('5-HT_1-like'), ketanserin and cyproheptadine (5-HT_2), and MDL 72222 and ICS 205-930 (5-HT_3)

as antagonists. It is to be noted that while the antagonists for 5-HT_2 and 5-HT_3 are selective, the drugs antagonizing '5-HT_1-like' receptors, being even more effective against the responses mediated via 5-HT_2 receptors, are not. The above agonist-antagonist criteria are clearly fulfilled with respect to 5-HT-induced tachycardia in the cat (5-CT in doses of 0.1 to 1 $\mu g\,kg^{-1}$ mimics, and methiothepin and methysergide, but not ketanserin and cyproheptadine, antagonize) (Saxena *et al.*, 1985a; Connor *et al.*, 1986), the rat (5-CT does not mimic and, ketanserin and cyproheptadine antagonize) (Saxena & Lawang, 1985) and the rabbit (2-methyl-5-HT mimics, and MDL 72222 and ICS 205-930 antagonize) (Fozard, 1984; Richardson *et al.*, 1985). However, in the present investigation in the anaesthetized pig, it was noticed that the above drugs neither mimicked nor antagonized the tachycardiac responses to 5-HT. Therefore, it can be concluded that the positive chronotropic effects of this amine in the pig are not mediated by either the '5-HT_1-like', 5-HT_2 or 5-HT_3 receptors.

Bradley *et al.* (1986) have pointed out that the receptor category denoted as '5-HT_1-like', being devoid of a selective antagonist, is not yet fully characterized but that it appears to be heterogeneous. However, neither the relationship of '5-HT_1-like' receptors with the 5-HT_1 binding sub-sites is clear nor the selectivity of drugs for the subclasses is as yet entirely satisfactory. Richardson & Engel (1986) have nevertheless attempted to subclassify '5-HT_1-like' receptors into three subclasses, namely 5-HT_{1A} (agonists; 8-OH-DPAT and 5-CT; antagonists: spiroperidol and methiothepin), 5-HT_{1B} (agonist: 5-CT; antagonists: 21-009 and methiothepin) and 5-HT_{1C} (agonist: (+)-S-α-methyl-5-HT; antagonists; mesulergine and methiothepin). Although not all of these drugs could be used in this investigation, the ineffectiveness of 8-OH-DPAT, 5-CT, mesulergine and methiothepin does not allow the effect of 5-HT on the porcine heart to be classified into any of the above putative, though not yet convincingly characterized, subclasses. The above conclusion is further supported by the finding that some more drugs which have affinities for 5-HT binding sites – phenoxybenzamine and metergoline (5-HT_1 and 5-HT_2; Peroutka & Snyder, 1979; Leysen *et al.*, 1981), pizotifen (5-HT_2; Leysen *et al.*, 1981; Hoyer *et al.*, 1985), BEA 1654 (5-HT_{1A}; Verdouw *et al.*, 1985; Middlemiss, unpublished), RU 24969 (5-HT_{1A} and 5-HT_{1B}; Hoyer *et al.*, 1985) and mianserin (5-HT_2 and 5-HT_{1C}; Hoyer *et al.*, 1985) – were all ineffective in our experiments.

Lastly, it has to be remarked that the doses of the compounds used were equal to or greater than those found effective in our earlier experiments concerning analyses of functional 5-HT receptors (see Saxena & Verdouw, 1982; 1984; 1985; Saxena *et al.*, 1985a,b; 1986a; Saxena & Lawang, 1985; Verdouw *et al.*, 1984; 1985). These doses also appear to be sufficient on the basis of their estimated affinity constants (see Hoyer *et al.*, 1985; Richardson & Engel, 1986).

Consideration of non-5-HT mechanisms

Since the chronotropic response to 5-HT could not be classified into any of the above categories, some common non-5-HT mechanisms were considered. The involvement of baroreceptors was ruled out by the fact that the changes in blood pressure were inconsistent and that instillation of 5-HT, though admittedly in a high concentration, on the right atrium also raised heart rate. Moreover, tachycardiac responses of similar magnitude were obtained in animals after complete ganglion (hexamethonium + atropine) or sympathetic and parasympathetic (propranolol + atropine) blockade. The use of atropine, hexamethonium, phentolamine and propranolol also precluded the involvement of the muscarinic and nicotinic receptors, α- and β-adrenoceptors and the release of catecholamines (by a tyramine-like action) in the mechanism of tachycardia produced by 5-HT in the pig.

Similarly, the increase in heart rate by 5-HT in the pig was not mediated by histamine H_1 and H_2 or dopamine receptors because the respective antagonists – mepyramine, cimetidine and haloperidol – were also incapable of modifying the responses to 5-HT.

Does a new type of 5-HT receptor mediate 5-HT-induced tachycardia in the pig?

The inability to find a specific mechanism responsible for the positive chronotropic action can suggest that 5-HT may be taken up into some neurone to displace a neurotransmitter agent (other than those mentioned above: noradrenaline, acetylcholine, histamine and dopamine). If an unidentified neurotransmitter is indeed displaced and released by 5-HT, this would involve an uptake process that is distinct from the one selectively inhibited (in the brain and blood platelets) by indalpine (Le Fur & Uzan, 1977; Ashkenazi *et al.*, 1983) and fluvoxamine (Claasen *et al.*, 1977) since these drugs increased (not reduced) the magnitude and duration of 5-HT-induced tachycardia. Though such a possibility cannot be entirely dismissed, it is perhaps more likely that the tachycardia elicited by 5-HT is mediated by a receptor type which is different from those characterized so far, i.e. '5-HT_1-like', 5-HT_2 or 5-HT_3 receptors (Bradley *et al.*, 1986; Saxena *et al.*, 1986b). This 5-HT receptor, apparently not much

dependent on extracellular calcium as indicated by the ineffectiveness of verapamil, may resemble the one that mediates either the positive inotropic effect of 5-HT in the kitten isolated papillary muscle (Kaumann, 1985; 1986) or the slow depolarization of myenteric type II/AH neurones in the guinea-pig small intestine (Mawe *et al.*, 1986). In the kitten isolated papillary muscle the responses to 5-HT, unlike that in the kitten isolated atria (Kaumann, 1985; 1986) or the cat heart *in vivo* (Saxena *et al.*, 1985a; Connor *et al.*, 1986), are neither mimicked by 5-CT nor antagonized by methysergide or phenoxybenzamine. Similarly, the above 5-HT receptor in the gut is insensitive to ICS 205-930 or lysergide, but is antagonized by dipeptides of 5-hydroxytryptophan and excited by hydroxylated indalpines (Mawe *et al.*, 1986). It is interesting to note that indalpine, which may be hydroxylated *in vivo* to stimulate the type of receptor being described by Mawe *et al.* (1986), caused tachycardia in our experiments. Alternatively, however, indalpine and fluvoxamine may be increasing the concentration of 5-HT at the receptor mediating the tachycardiac response to 5-HT, by blocking the uptake mechanism in the blood platelets or at some 5-HT neurones, possibly in the pig heart.

Lastly, it is still plausible that the stimulation of this new 5-HT receptor may lead to a release of a neurotransmitter substance. One such candidate can be calcitonin gene-related peptide which has recently been suggested as functioning as a neurotransmitter in the guinea-pig right atrium (Saito *et al.*, 1986).

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Chapter 6: ARTERIOVENOUS ANASTOMOTIC BLOOD FLOW IN THE PORCINE CAROTID CIRCULATION

1. Anatomical localization.

Arteriovenous anastomoses form direct connections between the large arterioles and venules, thereby bypassing the capillary bed. Using radioactive microspheres it has been shown that in the carotid circulation of the anaesthetized pig the entrapment of microspheres increases with microsphere size in the ears and the skin, whereas in the brain, muscles, tongue, bones, eyes, fat and salivary glands no enlarged entrapment is observed, suggestive for the presence of relatively small arteriovenous anastomoses in the ears and skin (Saxena & Verdouw, 1985a). However, using the largest microspheres (diameter: 35 µm), the arteriovenous shunt flow was only reduced by 16 % compared to the shunt flow measured with 15 µm microspheres, showing that the majority of arteriovenous shunt flow occurs through relatively large arteriovenous anastomoses. The localization of these blood vessels is still not clarified, but they are most likely located in the ears, the skin, the tongue, the lips and the nasal mucosa. From studies in other species (Hales, 1974; Marcus et al., 1976; Fan et al., 1979) it is known that only a small amount of 15 µm microspheres can be retrieved in cerebral venous blood, showing that the blood flow through arteriovenous anastomoses in the brain is probably very low. Since the dura mater is draining on the same venous system, blood flow through arteriovenous anastomoses in this tissue is probably very low.

2. Innervation

In conscious pigs arteriovenous blood flow is very low at an ambient temperature of 20°C (Verdouw et al., unpublished results), but during anaesthesia arteriovenous blood flow increases, resulting in a shunting of

70–80 % of the total carotid blood flow. This could be explained by a reduction in sympathetic outflow to the blood vessels, resulting in a decrease in vascular tone. However, electrical stimulation of the cervical sympathetic nervous system did not reduce the arteriovenous anastomotic blood flow (Verdouw et al., 1984a). Furthermore, intracarotid infusions of noradrenaline were also ineffective. Only intracarotid bolus injections of noradrenaline or clonidine caused a temporary decrease in shunt flow, which could be antagonized with phentolamine, whereas 5–HT caused a marked decrease in shunt flow (Saxena & Verdouw, 1982), indicating that the low potency of noradrenaline cannot be explained by an action of the anaesthesia on the vascular smooth muscle cells.

3. Physiological role

Arteriovenous anastomoses play an important role in thermoregulation. Thermoregulatory responses are under control of the hypothalamus, which receives information about temperature changes in the periphery from thermoreceptors in the skin and spinal cord, which is integrated with information from central thermoreceptors located in the hypothalamus itself. An increase in body temperature above a certain set–point will result in a scala of heat loss responses: blood is redistributed from the core of the body to the periphery; sweating (but not in the pig), followed by evaporation; panting; behavioural changes: reduction in muscular activity, resting in the shadow, saliva spreading on the fur.
An increase in arteriovenous anastomotic blood flow in the hind limb occurs during spinal heating in sheep (Hales et al., 1982), whereas spinal cooling results in a decrease in shunt flow. In this species adrenergic pathways play a role in this mechanism, which is mainly mediated by α–adrenergic receptors.

4. Pathophysiology.

In 1969 Heyck demonstrated in 7 patients that the arteriovenous oxygen saturation difference between arterial and jugular venous blood decreases during migraine attacks. In 6 patients he repeated these measurements after the relief of the attack (either spontaneously or after dihydroergotamine treatment) and found that the arteriovenous oxygen saturation difference returned to normal (Heyck, 1969). It is not clear from this study during which phase of the migraine attack the blood samples were collected, nor from which type of migraine (e.g. classic or common migraine) the patients were suffering. Initially, Heyck explained his results by an opening of arteriovenous anastomoses, but later he emphasized that other factors can also influence the arteriovenous oxygen saturation difference (Heyck, 1981). Several factors (alone or in combination) can explain Heyck's results:

a) opening of arteriovenous anastomoses, allowing arterial blood to go directly into the venous system.

b) an increase in capillary blood flow, exceeding the metabolic requirements.

c) a reduction in oxygen consumption by the cranial tissues without a reduction in perfusion.

d) disturbance in the process of CO_2 and O_2 exchange.

4.1. Opening of arteriovenous anastomoses

Opening of arteriovenous anastomoses can explain the decrease in arteriovenous oxygen saturation difference, since the arterial blood is allowed to enter the venous system, without the exchange of oxygen and carbondioxide in the capillary bed. An identical theory has been postulated by Fontaine (1957), who reported higher oxygen saturation values in venous samples from the limbs of patients with varicose veins than in blood samples from healthy control subjects. Injections of radioactive iodine albumine aggregrates (Lindemayer et al., 1972) or

radioactive technetium labelled, 25 µm albumin microspheres (Hehne et al., 1974) into the femoral artery, failed to reveal evidence of arteriovenous shunting. This clearly shows that a decrease in arteriovenous oxygen saturation difference does not necessarily need to be caused by an increase in arteriovenous shunt flow, but can be caused by other factors like disturbance of the oxygen–carbondioxide exchange, which might occur during 'sterile inflammation' of blood vessels. In other clinical situations opening of arteriovenous anastomoses also occurs. Cervical sympathectomy causes ipsilateral miosis, ptosis, conjunctival hyperaemia and reddening of the skin, caused by a massive dilatation of arteriovenous anastomoses and to a lesser extent by dilatation of arterioles, but does not induce migraine. In contrast, cervical sympathectomy has been used successfully in the past for the treatment of patients with severe migraine attacks (Dandy, 1931). Furthermore, opening of arteriovenous anastomoses during heat stress does not necesarily cause migraine or headache.

As long as perfusion pressure remains unchanged, opening of arteriovenous anastomoses without simultaneous dilatation of the arterioles will lead to a reduction of capillary blood flow. The local rise in venous blood pressure could even lead to stoppage of capillary blood flow and ischemia. However, simultaneous relaxation of the arterioles could prevent these effects. Both relaxation of arterioles and opening of arteriovenous anastomoses could therefore explain the pulsatile character of the migraine headache. In the past it has been suggested that ischemia might be the cause of the painful sensations. However, from skin transplantation experience it is known that the metabolic needs of the skin are extremely low. When ischemia would be the cause of the pain, one would expect that the pain should not occur only in the temporal area, but also in the lips, ears, tongue and nose, where both arteriovenous anastomoses and afferent pain fibers are abundantly present.

4.2 Increase in capillary blood flow

Since many patients have a pale face during the migraine attack, Heyck assumed that no increase in capillary blood flow occured. However, changes in skin flow do not necessarily reflect changes in blood flow to other tissues like muscles, tongue, fat, eyes, bones, salivary glands and the brain as has been demonstrated in the anaesthetized pig using radioactive microspheres (e.g. Duncker et al., 1986). An increase in capillary blood flow can occur by vasodilatation by changes in parasympathetic and/or sympathetic nerve activity, by circulating vasoactive substances, by the release of vasoactive substances from the endothelium, or by changes in autoregulatory mechanisms. Therefore, the increased oxygen saturation of jugular venous blood during the migraine attack, as described by Heyck, can also be explained by an increase in capillary blood flow in certain tissues, exceeding the metabolic needs of the tissue.

4.3 Reduction of oxygen consumption

No data on oxygen consumption and carbondioxide production in cranial tissues of migraine sufferers are available yet. In the future, these biochemical processes might be studied using positron emission tomography.

4.4 Disturbances in carbondioxide-oxygen exchange.

Increased permeability of the vascular wall in certain cranial vascular beds will result in oedema and infiltration of plasma proteins and other constituants (sterile inflammation ?), which might reduce the carbondioxide-oxygen exchange between the tissue and the blood, resulting in an increased oxygen saturation of jugular venous blood.

However, despite the fact that the role of arteriovenous anastomoses in migraine is still unclear, it is evident that some drugs that are active in the treatment of migraine (like methysergide, dihydroergotamine, ergotamine, isomepthene, GR43175) reduce cranial arteriovenous anastomotic blood flow in anaesthetized animals like the cat, dog and pig (see Saxena, 1987; Perren et al., 1989).
Due to the technique used, it is difficult to define, whether these drugs act directly on the arteriovenous anastomoses or that they act on certain large arterioles which are located between the small arteries and the arteriovenous anastomoses. Therefore, it is better to speak about reduction in arteriovenous blood flow instead of constriction of arteriovenous anastomoses.
The fact that drugs that reduce arteriovenous anastomotic blood flow are also effective in migraine does not necessarily imply that there is a causal relationship. The receptors, which are mediating the reduction in shunt flow, might be also involved in the therapeutic effect of these antimigraine drugs, but could be located on other anatomical structures.

5. 5-HT receptors in the cranial circulation of the pig.

Intracarotid administration of 5-HT decreases arteriovenous anastomotic blood flow, whereas arteriolar blood flow increases, especially to the skin, which becomes bright red (Saxena & Verdouw, 1982). These effects are mimicked by 5-carboxamidotryptamine, which has a high affinity for 5-HT_1-like receptors. The 5-HT_2 antagonists ketanserin and WAL 1307 did not cause important changes in these responses to 5-HT (Verdouw et al., 1984b; Saxena et al., 1986). Furthermore cyproheptadine did not modify the effects of 5-carboxamidotryptamine (Saxena & Verdouw, 1985b). Methysergide and BEA 1654, compounds with a high affinity for 5-HT_1 binding sites, did not cause a large increase in arteriolar blood flow, as seen during 5-HT or 5-carboxamidotryptamine infusions, suggestive for a heterogeneity of the 5-HT1-like receptors in the carotid vascular bed.

In earlier studies of our group the circulating blood volume was enlarged by infusion of plasma-expanders and saline, resulting in carotid blood flow values exceeding 200 ml min^{-1} (Saxena & Verdouw, 1982;1984;1985a;1985b, Verdouw et al., 1984a; 1984b; 1985). Since cardiac output in anaesthetized pig is in the range of 2 - 2.5 l min^{-1} and vertebral blood flow is also supplying the head, about 20-25 % of the cardiac output would perfuse the cranial structures, which must be considered as very high. Therefore, in the following studies it was decided not to follow this procedure, which resulted in much lower carotid blood flows (80 - 120 ml min^{-1}), which are more in the range described by Buckley et al. (1979). Furthermore, the risk of binding of drugs to the plasma expander could be avoided.

From the earlier experiments it was clear that 5-HT might act on 5-HT_1-like receptors. Therefore, the effects of the 5-HT_3 receptor antagonist MDL 72222 and the 5-HT_1-like and 5-HT_2 receptor antagonist methiothepin on the 5-HT-induced changes in carotid blood flow and its distribution were determined (Chapter 8).

In order to study the involvement of the putative 5-HT_{1A} and 5-HT_{1B} receptors, 8-OH-DPAT, ipsapirone (TVX Q7821) and RU 24969 were tested in the carotid circulation of the pig (Chapter 9 & 10).

Since ergotamine also possesses affinity for the 5-HT_1 binding sites, this compound was also tested in the carotid vascular bed of the cat and the pig (Chapter 11).

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Chapter 7: 5-HT RECEPTORS IN THE CAROTID CIRCULATION

1. Introduction.

A possible role of 5-HT in migraine has been suggested after the observation that the urinary concentration of 5-hydroxy-indolacetic acid (5-HIAA), the main metabolite of 5-HT, was increased in some patients during migraine attacks (Sicuteri et al., 1961; Curran et al., 1965), indicating that the turnover of 5-HT was enhanced. Since 5-HT is mainly present in the gastrointestinal tract (GIT), the thrombocytes and the brain, changes in the 5-HT metabolism in these regions might explain this phenomenon. Changes in GIT motility frequently occur during migraine attacks (vomiting, diarrhea), which might be the reflection of changes in parasympathetic and/or sympathetic nerve activity. These motility changes could result in an enhanced release of 5-HT from enterochromaffin cells and 5-HT-containing neurons in the myenteric plexus. 5-HT will be released and transported by the portal circulation to the liver, where 5-HT will be metabolized by monoamine oxidase (MAO). Free serotonin that escapes this metabolic process will either be taken up by thrombocytes or will be transported to the lungs, which also rapidly inactivates the amine. Therefore it is very unlikely that 5-HT will have any effect on the arterial circulation after GIT release, but it might explain the high urinary 5-HIAA levels. However, thrombocytes transport 5-HT to every part of the circulation. Therefore, the release of 5-HT in the arterial system might have systemic effects. A decrease in thrombocyte 5-HT concentration has been described by many authors (see Anthony, 1987), which cannot be explained by a reduced 5-HT uptake by these cells (Anthony & Lance, 1975). However, plasma β-thromboglobuline levels increase during the attacks, indicating an increased release of substances from the thrombocytes (Gawel et al., 1979). This release might be induced by "serotonin releasing factors" (Dvilansky et al., 1976; Mueck-Seler et al., 1979), which are still not characterized. The amount of 5-HT that is released from thrombocytes

can be estimated using the data of Anthony and Lance (1975). In a subject of 70 kg with a blood volume of 5000 ml and a thrombocyte concentration of 400,000 thrombocytes per ml, the total number of thrombocytes will be 2.10^9. According to Anthony and Lance the 5-HT concentration of thrombocytes decreased from 514 to 384 ng per 10^9 thrombocytes. Therefore 2 x (514-384) = 260 ng 5-HT will be released in a volume of 5000 ml: 5-HT concentration increase: 52 ng per liter = 0.3 nM. Since it is very unlikely that all thrombocytes will release 5-HT at the same moment, and considering that 5-HT is also removed from the circulation by lungs, liver and vascular endothelium, this increase in 5-HT concentration will probably not occur. Only a local, massive release of 5-HT from thrombocytes may have important vascular effects.

2. Effect of 5-HT and related vasoactive agents on carotid blood flow in different species.

2.1. Cat.

Intravenous infusion of 5-HT reduces total carotid blood flow by a reduction in arteriovenous anastomotic blood flow, whereas arteriolar blood flow is almost unchanged. Intracarotid infusion of 5-HT also reduces arteriovenous shunt flow both in intact and spinal cats (Saxena & Verdouw, 1982). Furthermore, many studies have reported about the effects of ergotamine on the carotid circulation. Ergotamine reduces the number of microspheres detected in the jugular vein (Johnston & Saxena, 1978), and reduces carotid blood flow and arteriovenous shunt flow (Spierings & Saxena, 1980). Dihydroergotamine mimicks the effect of ergotamine, whereas methysergide is ineffective (Spierings & Saxena, 1980). Indalpine, a selective inhibitor of 5-hydroxytryptamine, reduces the number of microspheres in jugular venous blood, indicating a decrease in arteriovenous shunting, probably induced by the increased 5-HT levels

in the blood (Saxena & Tangri, 1983). GR43175, a selective 5-HT1-like receptor agonist, also reduces arteriovenous shunting in the circulation (Perren et al., 1989).

2.2. Rabbit

In anaesthetized rabbits, intra-atrial infusion of 5-HT (10 μg kg^{-1} min^{-1}) induces an increase on both extracerebral and cerebral blood flow despite a fall in blood pressure. In conscious animals, intravenous infusion of 5-HT also results in an increase in extracerebral and cerebral blood flow, whereas blood pressure remains unchanged (Saxena et al., 1977). Intravenous injection of methysergide in anaesthetized rabbits clearly reduces arteriovenous shunting in the circulation. Therefore, the subsequent response to 5-HT is markedly inhibited (Forsyth & Saxena, 1978).

2.3. Dog

Intravenous infusion of 5-HT in anaesthetized dogs causes an initial increase in carotid blood flow, followed by a decrease, which is related to the simultaneous blood pressure changes. However, carotid resistance is mildly decreased (Vyden et al., 1974). In cervical vagosympathectomized dogs intracarotid bolus injection of 5-HT causes a decrease in carotid blood flow, without any effect on blood pressure. Since the internal carotid artery and the occipital artery were ligated, the decrease in carotid blood flow can be related to a decrease in external carotid blood flow. In the same animal model ergotamine also reduces external carotid blood flow. After increasing doses of ergotamine the original 5-HT-induced decrease in blood flow is converted into an increase in blood flow (Saxena & de Vlaam-Schluter, 1974). Using radioactive microspheres it has been demonstrated that the ergotamine-induced decrease in external carotid blood flow is mainly caused by an reduction in arteriovenous anastomotic blood flow, which cannot be prevented by

pretreatment with phentolamine and/or pizotifen, excluding the involvement of α-adrenoceptors, 5-HT_2 and $histamine_1$ receptors. Methysergide also reduces total and internal carotid blood flow by an increase in arterial resistance (Saxena, 1974). In isolated, internal and external carotid arteries it has been demonstrated that 5-HT causes a potent vasoconstriction in the internal carotid artery, whereas the external carotid artery only shows a minimal response (Chiba et al., 1978). However, intracarotid infusion of 5-HT causes an increase in external carotid blood flow in dogs with an ligated internal carotid artery, whereas this infusion causes a decrease in carotid blood flow in dogs with a ligated external carotid artery. Methysergide is able to block both effects. However, the α-adrenoceptor antagonist zolertine, the ganglion blocking agent chlorisondamine and the vasodilator diazoxide only block the external carotid artery response (Vidrio & Hong, 1975).

2.4. Monkey

Intracarotid infusions of 5-HT induce a dose-dependent reduction in internal and external carotid blood flow in the Macaca nemestrinas (Spira et al., 1978). In the same species the effects of methysergide, pizotifen and ergotamine have been tested (Mylecharane et al., 1978). Methysergide causes a transient increase in resistance in both external and internal carotid resistance, but complete recovery is obtained after 10 minutes.

The 5-HT-induced increase in external and internal carotid resistance is reduced after methysergide. Pizotifen has no effect by itself, but during intracarotid infusion of 5-HT the increase in external carotid resistance is enhanced, whereas the increase in internal carotid blood flow is attenuated. Ergotamine produces a long-lasting, marked increase in external carotid resistance, but a rather mild increase in internal carotid resistance. The effects of 5-HT on both vascular beds are therefore reduced.

In the baboon (African papiopapiogera) the effect of intracarotid 5-HT infusion has also been studied (Grimson et al., 1969). Total carotid blood flow was measured during clamping of the external carotid artery (measurement of internal carotid blood flow) or during clamping of the internal carotid artery (measurement of the internal carotid blood flow). 5-HT causes a decrease in internal carotid blood flow, whereas the amine induces an increase in external carotid blood flow. In both type of experiments no significant changes in blood pressure were observed.

It has been generally accepted that vascular alterations occur during migraine attacks. Therefore, we decided to characterize the actions of 5-HT on the carotid circulation of the anaesthetized pig.

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Chapter 8

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.

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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

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Summary. The present study concerns the effects of MDL 72222 (0.5 mg · kg^{-1}, i.v.), a 5-hydroxytryptamine$_3$ (5-HT$_3$) receptor antagonist, and methiothepin (1.0 mg · kg^{-1}, i.v.), an antagonist of both 5-HT$_2$ and "5-HT$_1$-like" receptors, on the responses to local infusions of 5-HT (2.0 μg · kg^{-1} · min^{-1}) on the total common carotid artery blood flow and its complete distribution in anaesthetized pigs. As reported earlier, more than 80% of the carotid blood bypassed the capillary circulation via cranial arteriovenous anastomoses, while approximately 15% and 2% was distributed to the extracerebral structures and brain, respectively. The total carotid blood flow did not change or was moderately reduced by 5-HT, but the amine consistently caused a 85% reduction in arteriovenous anastomotic blood flow and a 5-fold increase in blood flow to the extracerebral tissues, mainly the skin and ears. The colour of the skin and ears changed to bright pink. Complete recovery from the effects of 5-HT was observed once the infusion was stopped. MDL 72222 and methiothepin did not themselves affect carotid haemodynamics. The responses to 5-HT were not modified by MDL 72222 except that the reduction of the total carotid blood flow by 5-HT was augmented. In contrast, methiothepin almost completely abolished both the reduction of arteriovenous anastomotic blood flow and the increase in tissue blood flow following 5-HT-infusion. The colour of the skin and ears also did not become pink. In conjunction with our earlier findings that the constriction of arteriovenous anastomoses and the dilatation of arterioles caused by 5-HT within the carotid territory of the pig are not attenuated by 5-HT$_2$ receptor antagonists (cyproheptadine, methysergide, ketanserin and WAL 1307), and are mimicked by agonists at "5-HT$_1$-like" receptors (5-carboxamidotryptamine and BEA 1654), our results clearly establish that these responses are mediated by "5-HT$_1$-like" receptors.

Key words: Arteriovenous anastomoses – Blood flow – Carotid artery – 5-Hydroxytryptamine – 5-Hydroxytryptamine$_1$ receptors – MDL 72222 – Methiothepin – Regional blood flow – Serotonin – Skin blood flow

Introduction

Early studies using electromagnetic blood flow probes showed that drugs like mianserin, methysergide, cyproheptadine or pizotifen, which effectively block the 5-hydroxytryptamine$_2$ (5-HT$_2$) receptor-mediated constriction of the isolated segments of the carotid and other "large" conducting arteries (see Müller-Schweinitzer and Weidmann 1978; Cohen et al. 1983; Müller-Schweinitzer and Engel 1983), do not effectively antagonize the vasoconstrictor effect of 5-HT in the total carotid vascular bed in vivo (Saxena et al. 1971; Saxena 1972; Saxena and de Vlaam-Schluter 1974; Spira et al. 1976; Mylecharane et al. 1978). Subsequently, studies where the entire distribution of common carotid artery blood flow was followed with radioactive microspheres revealed that 5-HT, as a result of a constriction of arteriovenous anastomoses and a dilatation of arterioles (particularly in the skin and ears), causes a redistribution of carotid blood flow in favour of the nutrient (capillary) compartment at the expense of the non-nutrient (arteriovenous anastomotic) compartment, irrespective of whether the total carotid artery blood flow decreases, remains unchanged or increases (Saxena 1978; Saxena and Verdouw 1982). The constriction of arteriovenous anastomoses and dilatation of arterioles within the carotid territory seem to involve mainly "atypical", possibly "5-HT$_1$-like", receptors since the effects of 5-HT are (i) only poorly antagonized (arteriovenous anastomoses) or even enhanced (arterioles) by selective 5-HT$_2$ receptor antagonists, cyproheptadine (Saxena and Verdouw 1982), low dose (0.3 mg · kg^{-1}) methysergide (Saxena and Verdouw 1984), and ketanserin and WAL 1307 (Verdouw et al. 1984b), and (ii) mimicked by 5-carboxamidotryptamine (5-CT) (Saxena and Verdouw 1985a) and BEA 1654 (Verdouw et al. 1985), both of which have a selective affinity for 5-HT$_1$ recognition sites and are considered as putative 5-HT$_1$ receptor agonists (Feniuk et al. 1981, 1984; Engel et al. 1983; Saxena and Lawang 1985; Verdouw et al. 1985; Charlton et al. 1986). For a more definitive characterization of "5-HT$_1$-like" receptors, Bradley et al. (1986) have suggested that a response should, in addition to the above two criteria, be unaffected by antagonists of 5-HT$_3$ ("M") receptors, like MDL 72222 (Fozard 1984) or ICS 205–930 (Richardson et al. 1985) and be amenable to blockade by high doses of, for example, methiothepin (metitepine) or methysergide which, though having a higher affinity for 5-HT$_2$ recognition sites, also show appreciable affinity for 5-HT$_1$ recognition sites (Peroutka and Snyder 1979; Leysen et al. 1981). In this investigation, therefore, we have now studied the effects of MDL 72222 and methiothepin on the responses to local infusions of 5-HT on the carotid arteriovenous anastomoses and arterioles in the anaesthetized pig.

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Materials and methods

Experimental set-up. After an overnight fast, young Yorkshire pigs (body weight: 16–29 kg, age: 12–16 weeks) 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 (15–20 mg · kg^{-1} · h^{-1}, i.v.) completed the anaesthesia. Aortic blood pressure was recorded with a Statham pressure transducer via a cannula inserted into the left femoral artery. Respiratory rate and tidal volume were adjusted or sodium bicarbonate (8.4%) was infused to keep arterial blood gases within the normal ranges: $90 < P_{O_2}$ (mm Hg) < 150; $35 < P_{CO_2}$ (mm Hg) < 45. The animal's temperature was maintained at around 37°C using an electric blanket.

Distribution of common carotid blood flow. The common carotid arteries were dissected free and bilateral cervical vagosympathectomy was performed to avoid reflex influences on the carotid circulation. Blood flow in one of the common carotid arteries was measured with a suitable precalibrated blood flow probe connected to a sine wave electromagnetic blood flow meter (Skalar, Delft, The Netherlands). Two 0.5 mm (external diameter) needles, connected to suitable polyethylene tubing, were inserted directly into the main ärtery for intracarotid infusions of 5-HT and microsphere injections. The distribution of carotid blood flow into nutrient (tissue; capillary) and non-nutrient (arteriovenous anastomotic) fractions was determined with the radioactive microsphere method using 15 ± 1 (S.D.) µm diameter spheres, labelled with ^{141}Ce, ^{113}Sn, ^{103}Ru, ^{46}Sc or ^{95}Nb (NEN Company, Dreieich, FRG). For each measurement a suspension of microspheres, labelled with one of the nuclides, was ultrasonicated and injected in 0.5 ml saline over a 15–20 s period. To facilitate uniform mixing, the spheres were injected against the direction of the blood flow.

At the end of each experiment the animal was killed with an overdose of pentobarbitone sodium. The various tissues of the right and left half of the head, neck muscles, heart, kidneys and lungs were dissected out, weighed and placed in vials. The radioactivity in the vials was counted for 5–10 min in a γ-scintillation counter (Packard, model 5986) equipped with a multichannel pulse height analyser (Conrac) using suitable windows for discriminating the different isotopes. The microsphere and other data were processed by a PDP-11/70 computer using a set of specially developed programmes (Saxena et al. 1980). The amount of carotid blood distributed to the individual tissues ($\dot{Q}_{tis[car]}$) of the head was calculated by: $\dot{Q}_{tis[car]}$ (ml · min^{-1}) = (I_{tis}/I_{tot}) × $\dot{Q}_{car}$, where I_{tis} and I_{tot} are, respectively, the radioactivity (c · min^{-1}) in a particular tissue and that detected in all tissues collectively, and $\dot{Q}_{car}$ is carotid blood flow (ml · min^{-1}). Since we ensured complete entrapment of microspheres in the lungs by the absence of significant amounts of radioactivity in the heart and kidneys, the values determined for the lungs represent the arteriovenous anastomotic part of the carotid circulation (for details, see Saxena and Verdouw 1982).

Experimental procedure. In all experiments the baseline values were determined after the preparation had been stable for at least 45 min after completion of the surgical procedure. The measurements consisted of the recording of heart rate, mean arterial blood pressure and common carotid artery blood flow, while a batch of microspheres (100,000–150,000) was injected for the determination of tissue and anastomotic blood flow. All measurements were repeated following a 10 min intracarotid infusion of 2.0 µg · kg^{-1} · min^{-1} 5-HT and after a recovery period of 20 min. Subsequently, 1.0 mg · kg^{-1} methiothepin ($n = 6$) or 0.5 mg · kg^{-1} MDL 72222 ($n = 4$) was slowly injected via an i.v. catheter. After 10–20 min, when arterial pressure and heart rate were stable, another 10 min infusion of 2.0 µg · kg^{-1} · min^{-1} 5-HT was given and all measurements were repeated before and after the infusion.

Statistical analysis. The significance of the differences between the 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 (Steel and Torrie 1980). For comparing the effects of 5-HT before and after the antagonists, the changes induced by 5-HT were first calculated before subjecting data to statistical analysis. Statistical significance was accepted at $P < 0.05$ (two-tailed). All data are expressed as means ± SEM.

Drugs. Apart from anaesthetics, the drugs used in this study were: 5-hydroxytryptamine creatinine sulphate (Janssen Chimica, Beerse, Belgium), methiothepin maleate (courtesy: Dr. P. J. Pijper, Hoffman La Roche B.V., Mijdrecht, The Netherlands) and 1αH, 3α,5αH-tropan-3-yl-3,5-dichlorobenzoate (MDL 72222; courtesy: Dr. J. R. Fozard, Merrell Dow Research Institute, Strasbourg, France). The doses of 5-HT and MDL 72222 are in terms of the base and substance, respectively, while that of methiothepin refers to the salt.

Results

Systemic haemodynamics

In Table 1 are summarized the values of mean arterial blood pressure and heart rate recorded during the experiments. The administration of methiothepin (1.0 mg · kg^{-1}) resulted

Table 1. Effects of intracarotid infusions of 5-HT (2.0 µg · kg^{-1} · min^{-1}) on mean arterial blood pressure (MAP) and heart rate (HR) in anaesthetized pigs before and after MDL 72222, 0.5 mg · kg^{-1} ($n = 4$) or methiothepin, 1 mg · kg^{-1} ($n = 6$)

	Baseline	5-HT	Recovery	Antagonist	5-HT after antagonist
MAP (mm Hg)					
Methiothepin	87± 5	91± 8	83± 5	77±4	86±4
MDL 72222	104± 6	100± 6	95± 4	88±2	86±4
HR (beats · min^{-1})					
Methiothepin	114± 7	115± 7	95± 5	88±4*	96±4**,***
MDL 72222	113±11	115±11	99±12	93±9	92±7

* $P < 0.05$ vs recovery; ** $P < 0.05$ vs antagonist (MDL 72222 or methiothepin); *** the 5-HT-induced change after antagonist was significantly different ($P < 0.05$) from the 5-HT-induced change before the antagonist

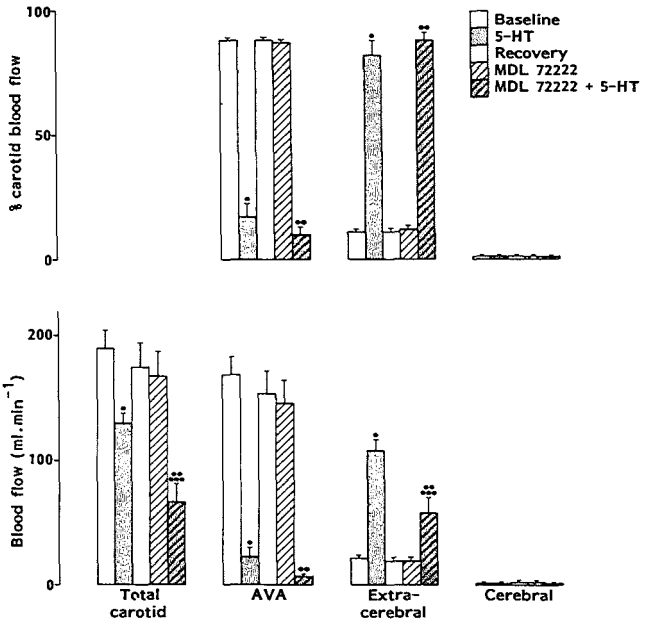

Fig. 1. Changes induced by intracarotid infusions of 5-HT (2.0 $\mu g \cdot kg^{-1} \cdot min^{-1}$) in the total carotid blood flow and its distribution into non-nutrient (arteriovenous anastomotic; AVA) and nutrient (extracerebral and cerebral) parts in 4 pigs. MDL 72222 (0.5 $mg \cdot kg^{-1}$, i.v.) did not affect carotid haemodynamics or modify the responses to 5-HT other than potentiating the reduction of the total carotid blood flow and, therefore, reducing extracerebral vasodilatation. * $P < 0.05$ vs baseline; ** $P < 0.05$ vs MDL 72222; *** 5-HT-induced change after MDL 72222 was significantly different ($P < 0.05$) from the 5-HT-induced change before MDL 72222

in a small decrease in heart rate, but arterial blood pressure remained unchanged. MDL 72222 (0.5 $mg \cdot kg^{-1}$) caused no effect. Infusions of 5-HT did not have major effects on these variables; only a moderate tachycardia was noticed with 5-HT after methiothepin.

Carotid haemodynamics

Effect of MDL 72222 on the responses to 5-HT. A large part (> 80%) of carotid blood flow was distributed via arteriovenous anastomoses. Intracarotid infusions of 5-HT (2.0 $\mu g \cdot kg^{-1} \cdot min^{-1}$) decreased the arteriovenous anastomotic fraction, but increased the fraction distributed to the extracerebral structures; the cerebral fraction, which was less than 2% of carotid blood flow, did not change (Fig. 1). The decrease in the arteriovenous anastomotic blood flow was more than the increase in blood flow to the extracerebral structures. Complete recovery from the effects of 5-HT was noticed within 20 min of stopping the infusion. MDL 72222 (0.5 $mg \cdot kg^{-1}$), which by itself did not affect the total carotid blood flow or its fractionation, failed to modify the effects of 5-HT on the distribution (%) of carotid blood flow. However, as there was an exaggerated decrease in the total carotid blood flow by 5-HT after MDL 72222, the enhancement of extracerebral blood flow by 5-HT was somewhat less (Fig. 1).

The increase in blood flow to the extracerebral tissues after 5-HT was mainly in the skin and ears (Fig. 2) which became bright pink in colour. After MDL 72222 the 5-HT-induced increase in the blood flow to the tissues, in terms of percent distribution, was similar but the absolute ($ml \cdot min^{-1}$) increase in the blood flow to the skin and ears was less, probably due to a more marked diminution of the total carotid blood flow (Fig. 1). However, the remaining increase in the skin and ear blood flow was of such a magnitude that the colour change was still indistinguishable from that after the control 5-HT infusions.

Effect of methiothepin on the responses to 5-HT. The effects of methiothepin on the carotid vascular responses to 5-HT are shown in Figs. 3 and 4. In this series, the infusion of 5-HT did not affect the total carotid blood flow. Nevertheless, the arteriovenous anastomotic blood flow was decreased and the extracerebral blood flow was increased by 5-HT. Administration of methiothepin did not itself have any influence on the total carotid blood flow or its distribution. The drug, however, strongly reduced the effects of 5-HT on both the arteriovenous anastomotic and extracerebral tissue blood flows. The change in the colour of the head skin and ears, normally observed during infusions of 5-HT, was conspicuously absent when the amine was infused after methiothepin administration.

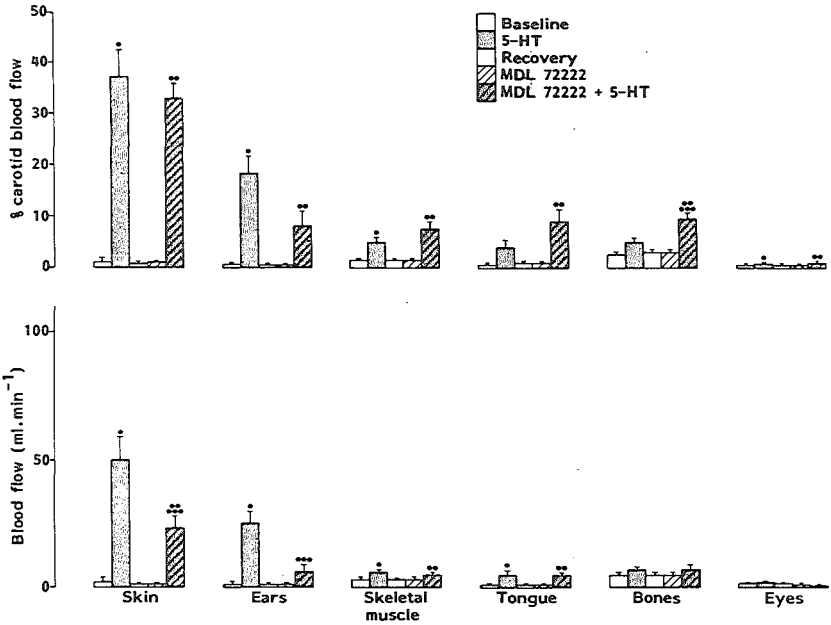

Fig. 2. Changes induced by intracarotid infusions of 5-HT (2.0 $\mu g \cdot kg^{-1} \cdot min^{-1}$) in the distribution of carotid blood to extracerebral tissues in 4 pigs. MDL 72222 (0.5 $mg \cdot kg^{-1}$, i.v.) itself did not cause any change in tissue blood flows or their responses to 5-HT except that skin and ear blood flows increased less and the bone fraction increased more when compared to control 5-HT-infusion. * $P < 0.05$ vs baseline; ** $P < 0.05$ vs MDL 72222; *** 5-HT-induced change after MDL 72222 was significantly different ($P < 0.05$) from the 5-HT-induced change before MDL 72222

Discussion

This study confirmed our earlier observations that in anaesthetized pigs with bilateral cervical vagosympathectomy 80–85% of the common carotid artery blood flow is shunted via arteriovenous anastomoses and that 5-HT is able to reduce the anastomotic blood flow to very low levels (Saxena and Verdouw 1982, 1984; Verdouw et al. 1984b). The carotid arteriovenous anastomoses have been located in the head skin and ears (Saxena and Verdouw 1985b) and it is in these tissues that 5-HT prominently increases blood flow, reduces the difference in blood flow measured with 10 and 35 µm microspheres and causes colour change from light pink to characteristic bright pink (Saxena and Verdouw 1982, 1984, 1985b; Verdouw et al. 1984b; present results). The dilatation of porcine skin vessels is in agreement with the vasodilatation reported in the human skin (Maricq 1972). In the dog, however, Dobbins et al. (1983) have reported that 5-HT increases arterial perfusion pressure in the forelimb skin vasculature. The results of our study suggest that the increase in the perfusion pressure observed by these authors may be due to the constriction of arteriovenous anastomoses (and not arterioles) by 5-HT. This suggestion is reinforced by the fact that the effects of 5-HT were selectively blocked by methysergide but not by cyproheptadine (Dobbins et al. 1983), thus indicating the involvement of "5-HT_1-like" receptors.

Barring some exceptions, for example the dog auricular and basilar arteries where a part of the contractile response to 5-HT may be mediated via "5-HT_1-like" receptors (Apperley et al. 1980; Peroutka et al. 1983), isolated segments of "large" conducting arteries contract mainly in response to the activation of 5-HT_2 receptors since the effects of 5-HT are susceptible to ketanserin, pizotifen, methysergide or cyproheptadine (Apperley et al. 1976; Black et al. 1981; Van Nueten et al. 1981; Cohen et al. 1983; Müller-Schweinitzer and Engel 1983; Blackshear et al. 1985; Clancy and Maayani 1985). In addition, 5-HT_2 receptors apparently also mediate the constriction of arterioles (resistance vessels) in the pig carotid (Saxena and Verdouw 1982, 1984; Verdouw et al. 1984b), and the rat mesenteric (McLennan and Taylor 1984) circulations as indicated by unmasking and/or facilitation of 5-HT-induced vasodilatatory responses by 5-HT_2 receptor antagonists.

The constriction of arteriovenous anastomoses and the dilatation of "small" resistance vessels (arterioles) elicited by 5-HT in the carotid vascular bed are resistant to antagonism by a number of 5-HT_2 receptor antagonists (Saxena and Verdouw 1982, 1984; Verdouw et al. 1984b). Furthermore, it has also been shown that two agonists of "5-HT_1-like" receptors, 5-CT (Feniuk et al. 1981, 1984, 1985; Engel et al. 1983; Saxena et al. 1985b; Saxena and Lawang 1985) and BEA 1654 (Verdouw et al. 1985), mimick the effects of 5-HT, and their effects are not modified by ketanserin and/

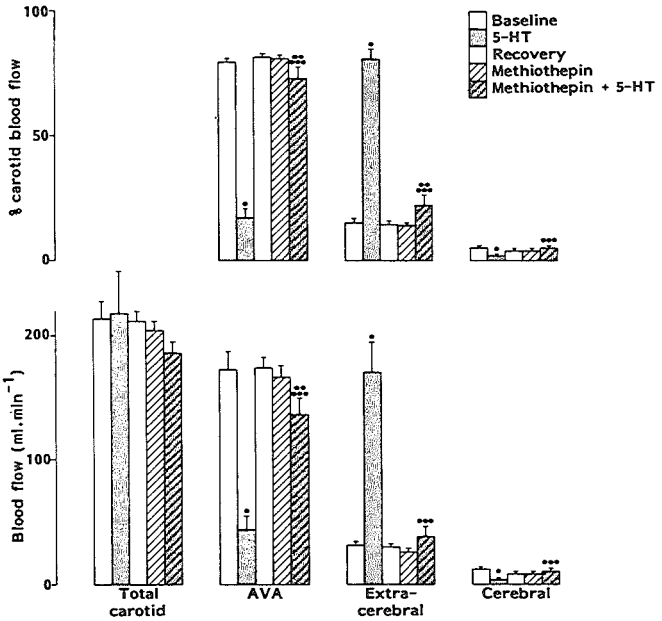

Fig. 3
Changes induced by intracarotid infusions of 5-HT (2.0 μg · kg^{-1} · min^{-1}) in the total carotid blood flow and its distribution into non-nutrient (arteriovenous anastomotic; AVA) and nutrient (extracerebral and cerebral) parts in 6 pigs. Methiothepin (1.0 mg · kg^{-1}, i.v.) did not affect carotid haemodynamics, but it almost completely antagonized the reduction of AVA and cerebral blood flow and the increase in the extracerebral tissue blood flow. * $P < 0.05$ vs baseline; ** $P < 0.05$ vs methiothepin; *** 5-HT-induced change after methiothepin was significantly different ($P < 0.05$) from the 5-HT-induced change before methiothepin

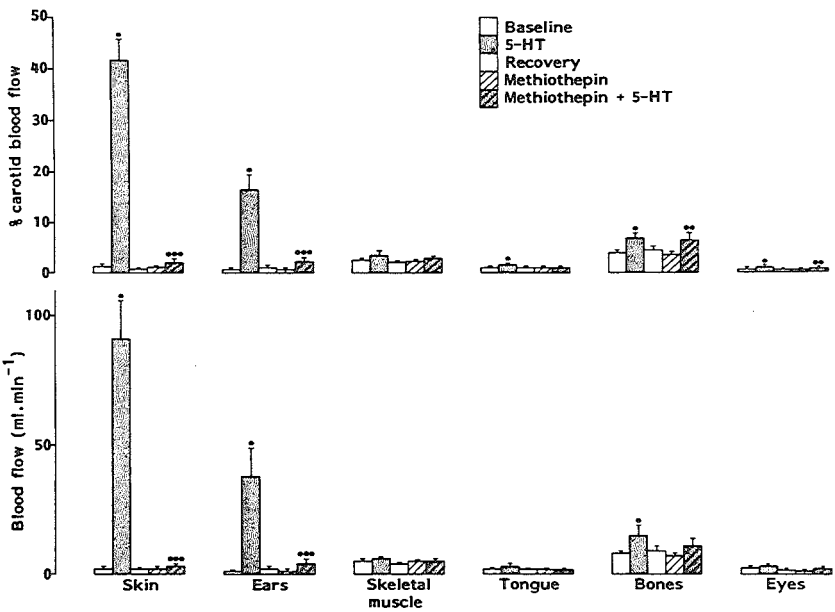

Fig. 4. Changes induced by intracarotid infusions of 5-HT (2.0 μg · kg^{-1} · min^{-1}) in the distribution of carotid blood to extracerebral tissues in 6 pigs. Methiothepin (1.0 mg · kg^{-1}, i.v.) itself did not cause any change in tissue blood flows, but it effectively antagonized the increase in the skin and ear blood flows by 5-HT. * $P < 0.05$ vs baseline; ** $P < 0.05$ vs methiothepin; *** 5-HT-induced change after methiothepin was significantly different ($P < 0.05$) from the 5-HT-induced change before methiothepin

or phentolamine (Saxena and Verdouw 1985a; Verdouw et al. 1985). In the present investigation, we provide further evidence for the involvement of "5-HT_1-like" receptors in mediating arteriovenous anastomotic constriction and arteriolar dilatation. MDL 72222, a 5-HT_3 receptor antagonist (Fozard 1984), was ineffective whereas methiothepin was quite effective in antagonizing the above effects, and the accompanying colour changes, caused by 5-HT. In contrast to ketanserin, cyproheptadine and WAL 1307, all of which have a high affinity for 5-HT_2 recognition sites but not for 5-HT_1 recognition sites, methiothepin also has a noticeable affinity for the latter (see Peroutka and Snyder 1979; Leysen et al. 1981; Verdouw et al. 1985). Admittedly, methiothepin exhibits α-adrenoceptor blocking properties, but such a mechanism can be discounted in the antagonism of the carotid vascular responses to 5-HT. Thus, we have earlier observed that the effects of 5-HT are not amenable to blockade to drugs (ketanserin, WAL 1307 and phentolamine) which can block α-adrenoceptors (Verdouw et al. 1984b; Saxena, Verdouw and Heiligers, unpublished). Moreover, in the carotid circulation of the pig, noradrenaline is not an effective constrictor agent (Verdouw et al. 1984a).

Although we have now conclusively demonstrated that "5-HT_1-like" receptors mediate carotid arteriovenous anastomotic constriction and arteriolar dilatation, we cannot categorically state whether or not these two responses to 5-HT are coupled with each other, i.e. the reduction in arteriovenous anastomotic blood flow results form "steal" as a consequence of dilatation in the nutrient vascular channels or *vice versa*. However, a comparison of data obtained with two calcium channel antagonists, nimodipine and nifedipine, in the pig carotid circulation suggests that 5-HT does actively constrict arteriovenous anastomoses. In the face of a 5.5-fold increase in nutrient vascular conductance, the calcium channel antagonists reduced arteriovenous anastomotic conductance by about 40% (Duncker et al. 1986), whereas in association with a smaller increase (3- to 4.5-fold) in nutrient conductance, 5-HT decreases arteriovenous anastomotic conductance much more prominently (by 70–80%); see Saxena and Verdouw 1982 and present results. We also do not yet know if the "5-HT_1-like" receptors demonstrated in the pig carotid circulation are associated with any one of the three sub-types of 5-HT_1 recognition sites (Pedigo et al. 1981; Pazos et al. 1984). The agonist activity of methysergide in the carotid circulation (Saxena 1974; Saxena and Verdouw 1984) suggests that the carotid vascular "5-HT_1-like" receptors may resemble those present in the dog saphenous vein (Apperley et al. 1980; Feniuk et al. 1981, 1985), but may de different from those, for example, demonstrated in the cat heart (Saxena et al. 1985b).

Apart from the now well-established presence of the 5-HT_1 and 5-HT_2 receptors, the porcine carotid vascular bed may have yet another type of 5-HT receptor. In the present experiments, before the antagonist treatment, 5-HT either decreased (MDL 72222 series) or had no effect (methiothepin series) on the total carotid blood flow. However, as described earlier with the 5-HT-induced contraction of the cat urinary bladder (Saxena et al. 1985a), MDL 72222 conspicuously potentiated the 5-HT_2 receptor-mediated carotid vasoconstrictor response. This indicates that 5-HT_3 receptors may be mediating vascular smooth muscle relaxation by a local neuronal action.

Lastly, the characterization of the 5-HT receptors in the different segments of the carotid vascular bed explains the weak antagonist action of 5-HT_2 receptor antagonists against the increase in the total carotid vascular resistance elicited by bolus injections of 5-HT (Saxena et al. 1971; Saxena 1972; Spira et al. 1976; Mylecharane et al. 1978). It is now obvious that the antagonism will depend upon the relative recruitment of "5-HT_1-like" and 5-HT_2 receptors in the overall response to 5-HT in the carotid vascular bed. This fact also helps explain why divergent results can be obtained in *in vitro* and *in vivo* studies dealing with 5-HT and its antagonists.

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Chapter 9

CAROTID HAEMODYNAMICS IN PIGS DURING INFUSION OF 8-OH-DPAT: REDUCTION IN ARTERIOVENOUS SHUNTING IS MEDIATED BY 5-HT_1-LIKE RECEPTORS.

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Carotid haemodynamics in pigs during infusions of 8-OH-DPAT: reduction in arteriovenous shunting is mediated by 5-HT_1-like receptors

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1 The effects of intracarotid infusions of 8-hydroxy-2-[di-n-propyl-amino]-tetralin (8-OH-DPAT) on heart rate, blood pressure and carotid blood flow and its distribution were studied in anaesthetized pigs by use of radioactive microspheres of 15 μm diameter.

2 Control experiments with physiological saline showed that systemic and carotid haemodynamics remain essentially unchanged during the experimental period. In contrast to results obtained in rat, cat and dog experiments, 8-OH-DPAT did not decrease arterial blood pressure.

3 8-OH-DPAT, which has a high affinity and is selective for the 5-HT_{1A} recognition site, caused a dose-related decrease in arteriovenous anastomotic (non-nutrient) blood flow, resulting in a decrease in carotid blood flow. At the highest dose used, a small increase in arteriolar (nutrient) blood flow was observed.

4 The decrease in arteriovenous anastomotic and carotid blood flow induced by 8-OH-DPAT was not significantly modified by pretreatment with the 5-HT_2 receptor antagonist ketanserin (0.5 mg kg^{-1}), but was markedly reduced by pretreatment with methiothepin (1 mg kg^{-1}), which blocks both the 5-HT_1-like and 5-HT_2 receptors.

5 It is concluded that the effects of 8-OH-DPAT on arteriovenous anastomotic blood flow are mediated by 5-HT_1-like receptors. These receptors, however, cannot yet be classified as belonging to 5-HT_{1A} receptor subtype. Since a number of antimigraine drugs reduce arteriovenous shunting, it is tempting to suggest that 8-OH-DPAT may have similar clinical efficacy.

Introduction

Intracarotid administration of 5-hydroxytryptamine (5-HT) in the anaesthetized pig decreases arteriovenous anastomotic (non-nutrient) blood flow but increases arteriolar (nutrient) blood flow. The increase in arteriolar blood flow is especially marked in the ears and skin which change to bright red colour (Saxena & Verdouw, 1982). These effects of 5-HT are antagonized by methiothepin, which blocks both 5-HT_1-like and 5-HT_2 receptors, but not by the 5-HT_2 receptor antagonist ketanserin or the 5-HT_3 receptor antagonist MDL 72222 (Verdouw *et al.*, 1984). Furthermore, the effects of 5-HT are mimicked by 5-carboxamidotryptamine (Saxena & Verdouw, 1985). Therefore, according to the recent criteria for the classification of functional 5-HT receptors (Bradley *et al.*, 1986), 5-HT_1-like receptors mediate the constriction of arteriovenous anastomoses as well as the dilatation of arterioles (Saxena *et al.*, 1986).

The 5-HT_1-like receptors in the carotid circulation appear to be heterogeneous. Methysergide (Saxena & Verdouw, 1984) and BEA 1654 (Verdouw *et al.*, 1985), which have affinity for 5-HT_1-like binding sites, reduce arteriovenous shunting but, unlike 5-HT (Saxena & Verdouw, 1982) or 5-carboxamidotryptamine (Saxena & Verdouw, 1985), only slightly increase arteriolar blood flow. More recently, another 5-HT_1-like receptor agonist, GR 43175, has been described. This compound lacks 5-HT_1-like receptor-mediated vascular and intestinal smooth muscle relaxant actions, but contracts dog saphenous vein and inhibits noradrenaline release

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from sympathetic nerve terminals (Humphrey *et al.*, 1988). GR 43175 also reduces arteriovenous anastomotic blood flow without affecting arteriolar blood flow in the carotid circulation of the cat (Feniuk *et al.*, 1987).

The subdivision of functional 5-HT receptors into 5-HT_1-like, 5-HT_2 and 5-HT_3 receptors has been partially based on results from radioligand binding studies. Peroutka &. Snyder (1979) demonstrated that two distinct 5-HT recognition sites exist in the central nervous system. The sites labelled by [^{3}H]-5-HT were designated as 5-HT_1-'receptors' and those labelled by [^{3}H]-spiperone were called 5-HT_2-'receptors'; the former have been further subdivided into 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} and 5-HT_{1D} recognition sites (Pedigo *et al.*, 1981; Pazos & Palacios, 1985; Heuring & Peroutka, 1987). In contrast to the good agreement between 5-HT_2 recognition sites and functional 5-HT_2 receptors, it is still unclear whether the subtypes of the 5-HT_1 recognition site correspond to 5-HT_1-like receptors, which undoubtedly, are heterogeneous (Bradley *et al.*, 1986; Humphrey & Richardson, 1988). The barrier here is the lack of sufficiently selective compounds to discriminate unequivocally between the different 5-HT_1 recognition site subtypes. One possible exception appears to be the 5-HT_{1A} recognition site where 8-OH-DPAT displays a high affinity and about 1000 fold selectivity compared to the other subtypes of 5-HT_1 recognition sites (Middlemiss & Fozard, 1983; Hoyer *et al.*, 1985; Heuring & Peroutka, 1987). Therefore, in an attempt to characterize further the 5-HT_1-like receptors in the porcine carotid circulation we studied the effects of 8-OH-DPAT on carotid blood flow and its distribution in the anaesthetized pig.

Methods

General

After an overnight fast young Yorkshire pigs (body weight: 19–29 kg) 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 (20–26 mg kg^{-1} h^{-1}, 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). Aortic blood pressure was recorded with a Statham pressure transducer (P23 ID) connected to a cannula inserted into the right femoral artery. 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, i.d.) 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 suitable polyethylene tubing, was inserted into this common carotid artery for the administration of microspheres and drugs. The animals were insulated with a blanket to maintain body temperature at 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, Federal Republic of Germany). 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 γ-scintillation counter (Packard, Minaxi Autogamma 5000) equipped with a multichannel analyzer 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 (Saxena *et al.*, 1980; Saxena & Verdouw, 1982) by: $Q_{tis[car]}$ (ml min^{-1}) = (I_{tis}/I_{tot}) × $Q_{[car]}$, 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^{-1}). Because there was complete trapping of 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 arterial blood flow (see Johnston & Saxena, 1978; Saxena & Verdouw, 1982).

Experimental protocol

Four series of experiments ($n = 6$ 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 intracarotid infusions of 8-OH-DPAT on carotid blood

flow distribution were assessed in untreated animals (Series 2) and in animals that were pretreated with either 0.5 mg kg^{-1} ketanserin (Series 3) or 1 mg kg^{-1} methiothepin (Series 4) administered at a rate of 1 ml min^{-1} during a period of 10 min, followed by a 10 min period of intracarotid infusion of physiological saline.

In all experiments the baseline values were determined after the preparation had been stable for at least 45 min after completion of the surgical procedure. 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, in Series 2–4 the measurements were repeated after consecutive 10 min intracarotid infusions of 8-OH-DPAT, 0.3, 1.0, 3.0 and 10.0 μg kg^{-1} min^{-1} at the rate of 1 ml min^{-1}, always given in this order. In the first series, in which only physiological saline was infused, an identical protocol was followed.

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 (Steel & Torrie, 1980). 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: 8-OH-DPAT (8-hydroxy-2-[di-n-propylamino]-tetralin (courtesy of Dr. J. Traber, Troponwerke, Cologne, Federal Republic of Germany), ketanserin tartrate (courtesy of Dr J.M. Van Nueten, Janssen Pharmaceutica, Beerse, Belgium) and methiothepin maleate (courtesy of Dr P.J. Pijper, Hoffman La Roche B.V., Mijdrecht, The Netherlands). The doses of 8-OH-DPAT are in terms of the base, whereas those of ketanserin and methiothepin refer to the salt.

Results

Physiological saline experiments

The effects of four consecutive infusions of physiological saline on heart rate, mean arterial blood pressure, carotid blood flow and its distribution are summarized in Table 1. Heart rate and blood pressure showed a tendency to decrease, but a statistically significant change was only observed in heart rate after the last saline infusion. Total carotid blood flow and arteriovenous anastomotic blood flow tended to increase, but the changes were not statistically significant. Nutrient (arteriolar) blood flow remained unaffected.

8-OH-DPAT experiments

Systemic haemodynamics The values for heart rate, mean arterial blood pressure and carotid blood flow before and after the infusion of either ketanserin or methiothepin are shown in Table 2. Both mean arterial blood pressure and heart rate decreased after intracarotid infusion of ketanserin (0.5 mg kg^{-1}), whereas after methiothepin infusion (1 mg kg^{-1}) only mean arterial blood pressure was lowered. Carotid blood flow was not changed significantly by infusion of either antagonist. In Table 3 are summarized the values of mean arterial blood pressure and heart rate recorded during the intracarotid infusions of 8-OH-DPAT. 8-OH-DPAT did not affect mean arterial blood pressure, but decreased heart rate in both

Table 1 Mean arterial blood pressure, heart rate and carotid blood flow and its distribution during four consecutive infusions of physiological saline

		Saline infusions			
	Baseline	*First*	*Second*	*Third*	*Fourth*
MAP	97 ± 12	92 ± 11	91 ± 11	88 ± 11	87 ± 11
HR	109 ± 13	109 ± 12	105 ± 12	102 ± 11	99 ± 10*
CBF	108 ± 17	108 ± 20	115 ± 20	123 ± 22	124 ± 23
AVA	87 ± 18	89 ± 20	95 ± 21	100 ± 23	102 ± 24
Nutrient	20 ± 3	19 ± 2	20 ± 3	22 ± 3	22 ± 3

Saline was infused for 10 min periods at a rate of 1 ml min^{-1} ($n = 6$). Abbreviations and units: MAP, mean arterial blood pressure (mmHg); HR, heart rate (beats min^{-1}); CBF, total carotid blood flow (ml min^{-1}); AVA, arteriovenous anastomotic blood flow (ml min^{-1}); Nutrient, arteriolar blood flow (ml min^{-1}).
* Significantly different ($P < 0.05$) from the baseline value.

Table 2 Mean arterial blood pressure, heart rate and carotid blood flow before and after ketanserin and methiothepin

	Antagonist pretreatment	*Baseline*	*Immediately after antagonist infusion*	*10 min after antagonist infusion*
MAP	Ketanserin	94 ± 7	85 ± 7*	84 ± 7*
	Methiothepin	98 ± 10	86 ± 10*	80 ± 7*
HR	Ketanserin	114 ± 8	107 ± 6	100 ± 5*
	Methiothepin	100 ± 8	97 ± 8	96 ± 8
CBF	Ketanserin	125 ± 14	111 ± 14	112 ± 13
	Methiothepin	102 ± 8	105 ± 11	97 ± 9

Ketanserin (0.5 mg kg^{-1}; $n = 6$) or methiothepin (1 mg/kg^{-1}; $n = 6$) was infused into the carotid artery at a rate of 1 ml min^{-1} during a period of 10 min. Thereafter an infusion of physiological saline (1 ml min^{-1}) was given. Abbreviations and units: MAP, mean arterial blood pressure (mmHg); HR, heart rate (beats min^{-1}); CBF, carotid blood flow (ml min^{-1}).
* Significantly different ($P < 0.05$) from the baseline value.

untreated and ketanserin- or methiothepin-pretreated animals during intracarotid infusions of the three highest doses (1, 3 and 10 μg kg^{-1}).

Carotid blood flow and its distribution The effects of intracarotid infusions of 8-OH-DPAT on carotid blood flow and its fractionation over non-nutrient, arteriovenous anastomotic blood flow and nutrient, arteriolar blood flow are shown in Figure 1. As was the case in the experiments with physiological saline, a large fraction (70%) of the total carotid blood flow was diverted through the arteriovenous anastomoses in untreated as well as in ketanserin- or methiothepin-pretreated animals before the infusions of 8-OH-DPAT were started. In the untreated and ketanserin-pretreated animal, 8-OH-DPAT infusions induced a dose-dependent decrease in total carotid blood flow, which was directly related to a decrease in arteriovenous anastomotic blood flow. Only at the highest dose tested did arteriolar blood flow show an increase in the untreated animals, whereas in the ketanserin-pretreated animals arteriolar blood flow tended to decrease. In the methiothepin-pretreated animals the effects of 8-OH-DPAT infusions on total carotid and arteriovenous anastomotic blood flow were markedly reduced. Furthermore, a small but dose-related decrease in arteriolar blood flow was observed.

Analysis of regional carotid blood flow distribution showed that 8-OH-DPAT infusion at the highest dose caused an increase in blood flow to the skin, ears, tongue, bones and brain in the untreated animals (Figure 2). No changes in colour of the skin and ears, as seen with 5-HT and 5-carboxamidotryptamine (Saxena & Verdouw, 1982; 1985), were observed during the experiments. In the group pretreated with ketanserin, blood flow to the skin and ears increased at the highest dose of 8-OH-

Table 3 Effect of intracarotid infusions of 8-hydroxy-2[di-n-propyl-amino]tetralin (8-OH-DPAT) on mean arterial blood pressure and heart rate

	Antagonist pretreatment	8-OH-DPAT (μg kg^{-1} min^{-1}) 0	0.3	1.0	3.0	10.0
MAP	None	79 ± 7	78 ± 7	76 ± 6	76 ± 7	75 ± 6
	Ketanserin	84 ± 7	84 ± 8	84 ± 8	84 ± 8	85 ± 8
	Methiothepin	80 ± 7	80 ± 7	78 ± 7	74 ± 5	74 ± 6
HR	None	109 ± 7	104 ± 6	99 ± 5*	93 ± 4*	88 ± 3*
	Ketanserin	100 ± 5	96 ± 6	92 ± 5*	90 ± 4*	85 ± 2*
	Methiothepin	96 ± 8	94 ± 8	90 ± 8*	86 ± 6*	82 ± 5*

8-OH-DPAT was infused in 10 min periods at a rate of 1 ml min^{-1} in untreated ($n = 6$), ketanserin (0.5 mg kg^{-1}; $n = 6$) or methiothepin (1 mg kg^{-1}; $n = 6$) pretreated animals. Abbreviations and units: MAP, mean arterial blood pressure (mmHg); HR, heart rate (beats min^{-1}).
* Significantly different ($P < 0.05$) from the value before the administration of 8-OH-DPAT.

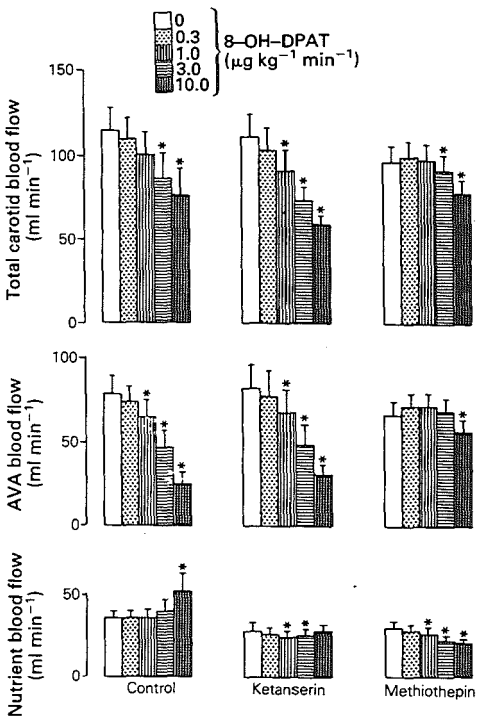

Figure 1 Total carotid blood flow, arteriovenous anastomotic (AVA) blood flow and arteriolar (nutrient) blood flow in untreated (control) and ketanserin (0.5 mg kg^{-1}) or methiothepin (1 mg kg^{-1}) pretreated pigs ($n = 6$ each) before and after intracarotid infusions of increasing doses of 8-hydroxy-2-[di-n-propylamino]tetralin (8-OH-DPAT). * $P < 0.05$ vs baseline.

DPAT, but blood flow to the tongue, bones and brain remained unchanged, while that to the skeletal muscles and eyes was reduced. Pretreatment with methiothepin prevented the 8-OH-DPAT-induced increase in blood flow to the skin, ears, tongue and brain, whereas blood flow to the muscles, bones and eyes decreased.

Discussion

Validity of the experimental model

Our experiments, in which four consecutive intracarotid infusions of physiological saline were given to the anaesthetized pig, clearly show that no appreciable changes occur in either systemic or carotid haemodynamics over a period of about 40–60 min. Similarly, we have previously shown that within minutes of stopping the infusions of 5-HT, which is quickly metabolised, the distribution of carotid blood flow is restored to that observed during the baseline period (Saxena & Verdouw, 1982; 1984; Verdouw *et al.*, 1984). Therefore, we can conclude that no time-dependent changes or changes due to the injection of microspheres themselves are seen in carotid haemodynamics during the experimental period in the animal model used here.

Effects of 8-OH-DPAT

In contrast to the rat (Gradin *et al.*, 1985; Martin & Lis, 1985; Fozard *et al.*, 1987), cat (Ramage & Fozard, 1987; McCall *et al.*, 1987; Doods *et al.*, 1988) and dog (Di Francesco *et al.*, 1988), 8-OH-DPAT did not lower blood pressure in the anaesthetized pig. Whether this is caused by differences in species, anaesthesia or the level of baseline arterial blood pressure, remains unclear. The decrease in heart rate observed in our experiments cannot be unequivocally attributed to an action of 8-OH-DPAT, since this was also observed in experiments in which only physiological saline was infused. The use of nitrous oxide as an anaesthetic might be an explanation for this phenomenon. Nitrous oxide is reported to reduce heart rate and cardiac output with systemic arterial blood pressure being maintained by increased total peripheral resistance (Eisele & Smith, 1972).

Intracarotid infusion of 8-OH-DPAT, which is a selective agonist at 5-HT$_{1A}$ receptors (Middlemiss & Fozard, 1983; Hoyer *et al.*, 1985), caused a dose-related decrease in carotid blood flow in the anaesthetized pig. This effect on carotid blood flow, unrelated to changes in mean arterial blood pressure, was associated with a selective decrease in the arteriovenous anastomotic fraction. Pretreatment with methiothepin, but not with ketanserin, considerably reduced the effects of 8-OH-DPAT, indicating that 8-OH-DPAT was acting on 5-HT$_1$-like receptors.

The increase in tissue blood flow to the skin and ears observed during the infusion of the highest dose of 8-OH-DPAT is probably also mediated by 5-HT$_1$-like receptors since this effect was antagonized by methiothepin but not be ketanserin. However, it should be noted that like methysergide and BEA 1654 (Saxena & Verdouw, 1984; Verdouw *et al.*, 1985) and GR 43175 (Feniuk *et al.*, 1987), but in contrast to 5-HT and 5-carboxamidotryptamine (Saxena & Verdouw, 1982, 1985), the effect of 8-OH-DPAT on total nutrient (arteriolar) blood flow was not marked. Compatible with this difference, intracarotid infusions of 5-HT and 5-carboxamidotryptamine changed the colour of the

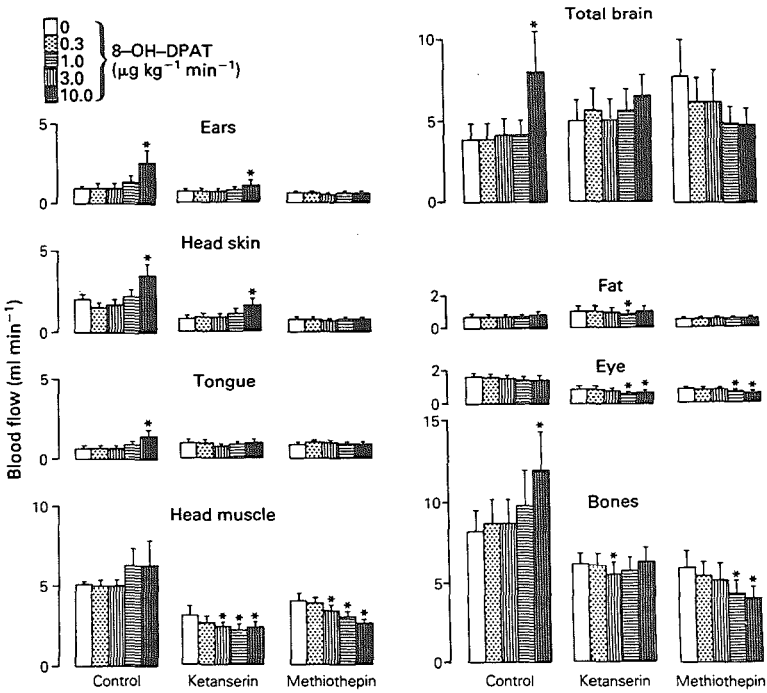

Figure 2 The distribution of carotid blood flow to various cranial tissues in untreated (control), ketanserin (0.5 mg kg^{-1}) or methiothepin (1 mg kg^{-1}) pretreated pigs ($n = 6$ each) before and after intracarotid infusions of increasing doses of 8-hydroxy-2-[di-n-propyl-amino]tetralin (8-OH-DPAT). * $P < 0.05$ vs baseline.

skin and ears to bright red (Saxena & Verdouw, 1982; 1985), but infusions of 8-OH-DPAT did not. The above findings are suggestive of heterogeneity of the 5-HT$_1$-like receptors mediating the constriction of arteriovenous anastomoses and the dilatation of arterioles in the carotid vascular bed.

The selectivity of 8-OH-DPAT for the 5-HT$_{1A}$ recognition sites (Middlemiss & Fozard, 1983; Hoyer *et al.*, 1985) obviously tempts one to suggest that the 5-HT$_1$-like receptors mediating the reduction of arteriovenous blood flow belong to the 5-HT$_{1A}$ subtype. However, it is still unclear whether this is so, since no selective antagonists for the putative 5-HT$_{1A}$ receptor are as yet available. Moreover, in initial experiments, ipsapirone, which also has a high affinity for the 5-HT$_{1A}$ recognition site (Peroutka, 1986; Traber & Glaser, 1987) and reduces blood pressure in anaesthetized cats by an agonist action on putative 5-HT$_{1A}$ receptors in the brain (Ramage & Fozard, 1987), was found to be inactive in our experimental model (Bom *et al.*, 1988). It remains possible that the inactivity of ipsapirone is due to a low efficacy of the drug at the putative 5-HT$_{1A}$ receptors or to some differences in 5-HT$_1$ recognition sites in the brain and in the carotid circulation.

Lastly we would like to draw some attention to the possible clinical relevance of the main finding of this study, i.e. the constriction of arteriovenous anastomoses by 8-OH-DPAT. It should be recalled that dilatation of arteriovenous anastomoses has been suggested to form part of the pathophysiology of migraine headache (Heyck, 1969; Saxena, 1978; 1987). Indeed, a number of proven and effective antimigraine drugs, including ergotamine, methysergide and isometheptene (Johnston & Saxena, 1978; Spierings & Saxena, 1980a,b; Saxena & Verdouw, 1984; Saxena, 1987), constrict arteriovenous anastomoses. Also, GR 43175, the newly developed agonist at 5-HT$_1$-like receptors (Humphrey *et al.*, 1988), reduces arteriovenous anastomotic blood flow (Feniuk *et al.*, 1987) and has shown promise in the treatment of acute attacks of migraine (Doenicke *et al.*, 1988). It is therefore possible that 8-OH-DPAT

may also have an antimigraine action. However, we do not know how the behavioural effects of 8-OH-DPAT described in animals (Tricklebank, 1985) would manifest in man.

In conclusion the present study demonstrates that 8-OH-DPAT, which has a high affinity and is selective for 5-HT_{1A} recognition sites, decreases common carotid arterial blood flow in the pig by selectively reducing arteriovenous anastomotic blood flow. Since methiothepin, but not ketanserin, antagonized the 8-OH-DPAT-induced constriction of arteriovenous anastomoses, this response is mediated by 5-HT_1-like receptors. It is, however, not yet certain whether these 5-HT_1-like receptors correlate with the 5-HT_{1A} recognition sites.

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Chapter 10

THE 5-HT_1-LIKE RECEPTOR MEDIATING REDUCTION IN PORCINE CAROTID ARTERIOVENOUS SHUNTING BY RU 24969 IS NOT RELATED TO EITHER 5-HT_{1A} OR THE 5-HT_{1B} SUBTYPE.

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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

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Using the radioactive microsphere technique in anaesthetized pigs, we studied the systemic and carotid haemodynamic effects of intracarotid infusions (0.3, 1, 3 and 10 $\mu g/kg \cdot min$) of 5-methoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1H-indole succinate (RU 24969), a drug with high affinity for 5-HT_{1A} and 5-HT_{1B} recognition sites. Unlike in the rat, RU 24969 did not elicit hypotension in the pig. Instead, the two highest doses of the drug caused a slight increase in blood pressure. RU 24969 reduced common carotid artery blood flow by decreasing the non-nutrient, arteriovenous anastomotic blood flow; the nutrient, arteriolar blood flow was mildly increased. The decrease in common carotid and arteriovenous anastomotic blood flow was only slightly attenuated in animals pretreated with the 5-HT_2 receptor antagonist, ketanserin (0.5 mg/kg i.a.), but was markedly reduced in animals pretreated with the 5-HT_1-like and 5-HT_2 receptor antagonist, methiothepin (1.0 mg/kg i.a.). However, these responses were not modified after pretreatment with the putative 5-HT_{1A} and 5-HT_{1B} receptor antagonist, (±)-pindolol (4.0 mg/kg i.v.). The slight increase in arteriolar blood flow was attenuated in the animals pretreated with either ketanserin, methiothepin or (±)-pindolol. It is concluded that the RU 24969-induced reduction in common carotid and arteriovenous anastomotic blood flow is mediated mainly by 5-HT_1-like receptors, which do not seem to correspond to either the 5-HT_{1A} or 5-HT_{1B} receptor subtypes.

Arteriovenous anastomoses; Carotid blood flow; 5-Hydroxytryptamine (5-HT, serotonin); 5-HT receptors; 5-HT_1-like receptors; Migraine; RU 24969

1. Introduction

Intracarotid infusion of 5-hydroxytryptamine (5-HT) into anaesthetized pigs causes a reduction in cephalic arteriovenous anastomotic blood flow and an increase in arteriolar blood flow (Saxena and Verdouw, 1982). According to the classification of Bradley et al. (1986), these effects are mediated mainly by 5-HT_1-like receptors, since the effects are strongly antagonizied by methiothepin, which blocks both 5-HT_1-like and 5-HT_2 receptors, but only slightly by the 5-HT_2 receptor antagonist, ketanserin, or the 5-HT_3 receptor antagonist, MDL 72222 (Verdouw et al., 1984; Saxena et al., 1986). Furthermore, 5-carboxamidotryptamine, which has a high affinity for the 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} recognition sites (Hoyer, 1988), mimics the effects of 5-HT (Saxena and Verdouw, 1985).

In subsequent studies, we reported that 8-OH-DPAT, which has a high affinity for the 5-HT_{1A}

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recognition site (Middlemiss and Fozard, 1983; Hoyer et al., 1985; Hoyer, 1988), reduced arteriovenous anastomotic blood flow without increasing arteriolar blood flow (Bom et al., 1989b). However, it is still unclear whether 5-HT_{1A} recognition sites correspond to the 5-HT_1-like receptor mediating the decrease in arteriovenous anastomotic shunting, because ipsapirone, another compound with high affinity for the 5-HT_{1A} recognition site (Traber and Glaser, 1987), was found to be ineffective (Bom et al., 1988). Both 5-HT and 5-carboxamidotryptamine increase arteriolar blood flow, especially to the skin which becomes bright red; this effect is not observed during infusions of either 8-OH-DPAT or ipsapirone, thus it is unlikely that the 5-HT_{1A} receptor is involved. Nevertheless, in order to further study the possible involvement of 5-HT_{1A} and/or 5-HT_{1B} receptors in the distribution of nutrient and non-nutrient cephalic blood flow, we have investigated the carotid vascular effects of RU 24969 in the anaesthetized pig. RU 24969 has a high affinity for both 5-HT_{1A} and 5-HT_{1B} recognition sites in rat and bovine brain tissue, and to a lesser extent for the 5-HT_2 recognition site (Hoyer et al., 1985; Peroutka, 1986; Hoyer, 1988).

2. Material and methods

2.1. Experimental set-up

Fasted young Yorkshire pigs (n = 24; body weight: 18-37 kg) were sedated with azaperone (120 mg i.m.) and metomidate (120-150 mg i.v.), intubated and artificially ventilated with a mixture (1 : 2) of oxygen and nitrous oxide. A continuous infusion of pentobarbitone sodium (15-30 mg/kg · h i.v.) completed the anaesthesia. The respiratory rate and tidal volume were adjusted to keep arterial blood gases and pH within normal limits (pH: 7.35-7.45; PaO_2: 90-150 mmHg; $PaCO_2$: 35-45 mm Hg). The right femoral artery and vein were cannulated for the measurement of aortic blood pressure (Statham transducer, model P23 ID) and infusions of (±)-pindolol, respectively. The heart rate was derived from the pressure signals. Both common carotid arteries were dissected free and a bilateral cervical vagosympathectomy was performed. The blood flow in one of the common carotid arteries was measured with a precalibrated electromagnetic flow probe (2.25-2.50 mm internal diameter; Transflow 601-system, Skalar, Delft, The Netherlands). A hub-less 0.5 mm (external diameter) needle, connected to suitable polyethylene tubing, was inserted into this common carotid artery for the administration of microspheres, RU 24969, ketanserin and methiothepin. The animals were packed in a thermo-insuluating blanket to maintain the body temperature between 37-38 °C.

2.2. Distribution of common carotid artery blood flow

The distribution of common carotid artery blood flow was determined with 15 ± 1 (S.D.) μm diameter microspheres labelled with either ^{141}Ce, ^{113}Sn, ^{103}Ru, ^{95}Nb or ^{46}Sc (NEN Company, Dreieich, West Germany). For each measurement a suspension of microspheres (with $1\text{-}1.5 \times 10^5$ spheres), labelled with one of the nuclide, was mechanically agitated and injected into the carotid artery against the direction of blood flow to facilitate uniform mixing. At the end of the experiments the animals were killed and the heart, kidneys, lungs and the various cranial tissues were dissected out, weighed and placed in vials. The radioactivity in these vials was counted for 5-10 min in a γ-scintillation counter (Packard, Minaxi Autogamma 5000) using suitable windows for discriminating the different isotopes. The ratio between the radioactivity in a particular tissue and the radioactivity in the sum of all tissues was calculated with a specially developed computer program (Saxena et al., 1980). By multiplying this ratio with the value for the total carotid blood flow, determined just before the microsphere injection, the carotid blood flow to a particular tissue could be determined. Little or no radioactivity was detected in the heart or kidneys, signifying a nearly complete entrapment of the microspheres in the lungs. Therefore, the amount of radioactivity in the lungs was used as an *index* for the arteriovenous anastomotic part of the common carotid artery blood flow.

2.3. Experimental protocol

After a post-surgical stabilization period of 45 min, the animals were divided into four groups and received either physiological saline, ketanserin (0.05 mg/kg · min) and methiothepin (0.1 mg/kg · min) at a rate of 1.0 ml/min for a period of 10 min (total dose 0.5 and 1.0 mg/kg i.a.; respectively) or (±)-pindolol (0.2 mg/kg · min) at a rate of 1.0 ml/min for a period of 20 min (total dose 4.0 mg/kg i.v.). This was followed by an infusion of physiological saline at the same flow rate for a period of 10 min. Then, baseline measurements of heart rate, mean arterial blood pressure, common carotid artery blood flow and its distribution were made. These measurements were repeated after the intracarotid administration of RU 24969 (0.3, 1.0, 3.0 and 10.0 μg/kg · min) at 10 min intervals.

2.4. Statistical analysis

The significance of the differences between the variables was evaluated with 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). Statistical significance was accepted at $P < 0.05$ (two-tailed). All data are expressed as means ± S.E.M.

2.5. Drugs

Apart from the anaesthetics, the drugs used in this study were: ketanserin tartrate (gift: Dr. J.M. Van Nueten, Janssen Pharmaceutica, Beerse, Belgium), methiothepin maleate (gift: Dr. P.J. Pijper, Hoffman La Roche B.V., Mijdrecht, The Netherlands), (±)-pindolol (gift: Sandoz B.V., Uden, The Netherlands) and 5-methoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl)1H-indole succinate (RU 24969; gift: Roussel Laboratories, Hoevelaken, The Netherlands). With the exception of (±)-pindolol (which was dissolved in ethanol 30% with physiological saline), all drugs were dissolved in physiological saline. The doses of ketanserin, methiothepin and RU 24969 refer to the salts.

3. Results

3.1. Systemic haemodynamics

The effects of intracarotid infusions of physiological saline, ketanserin (total dose: 0.5 mg/kg), methiothepin (total dose: 1.0 mg/kg), (±)-pindolol (total dose: 4.0 mg/kg) and increasing doses of RU 24969 (0.3, 1.0, 3.0 and 10.0 μg/kg · min) on the mean arterial blood pressure and heart rate are summarized in table 1. The heart rate was lowered by ketanserin, whereas the mean arterial blood

TABLE 1

Effect of intracarotid infusion of physiological saline (n = 6), ketanserin (0.5 mg/kg; n = 6), methiothepin (1.0 mg/kg; n = 6) or (±)-pindolol (4.0 mg/kg; n = 6) and subsequent doses of RU 24969 on heart rate and mean arterial blood pressure in pigs.

Pretreatment group	Baseline	Pretreatment	RU 24969 (μg/kg·min)			
			0.3	1.0	3.0	10.0
Heart rate (beats/min)						
Saline	96 ± 5	94 ± 4	92 ± 4	87 ± 3 [b]	82 ± 3 [b]	79 ± 3 [b]
Ketanserin	98 ± 5	87 ± 4 [a]	84 ± 3	81 ± 3 [b]	78 ± 2 [b]	76 ± 2 [b]
Methiothepin	106 ± 4	102 ± 7	102 ± 8	99 ± 8	95 ± 7 [b]	91 ± 6 [b]
(±)-Pindolol	122 ± 10	125 ± 6	130 ± 7	131 ± 7	128 ± 8	129 ± 8
Mean arterial blood pressure (mm Hg)						
Saline	66 ± 7	65 ± 6	71 ± 8	74 ± 8	75 ± 9	75 ± 10
Ketanserin	80 ± 7	72 ± 7	71 ± 8	76 ± 5	80 ± 7 [b]	82 ± 8 [b]
Methiothepin	83 ± 3	75 ± 3	72 ± 5	74 ± 4	76 ± 5	75 ± 7
(±)-Pindolol	92 ± 9	104 ± 10 [a]	100 ± 10	104 ± 9	103 ± 10	101 ± 9

[a] $P < 0.05$ vs. baseline; [b] $P < 0.05$ vs. pretreatment.

pressure was increased after pretreatment with (±)-pindolol. Furthermore, the heart rate decreased during the intracarotid infusion of increasing doses of RU 24969 in saline-, ketanserin- and methiothepin-pretreated animals, an effect which has also been observed during repeated infusions of physiological saline (Bom et al., 1989b). The heart rate remained essentially unchanged during the experimental period in the animals pretreated with (±)-pindolol. The mean arterial blood pressure was increased by the two highest doses of RU 24969 only in the animals pretreated with ketanserin.

3.2. Carotid haemodynamics

The effects of intracarotid infusions of RU 24969 on the total carotid blood flow and its distribution over non-nutrient, arteriovenous anastomotic blood flow and nutrient, arteriolar blood flow are shown in fig. 1. Irrespective of the pretreatment a large fraction (70%) of the total carotid blood flow was diverted through the cephalic arteriovenous anastomoses. In the saline-pretreated animals increasing doses of RU 24969 caused a marked decrease in arteriovenous blood flow and a less pronounced increase in arteriolar

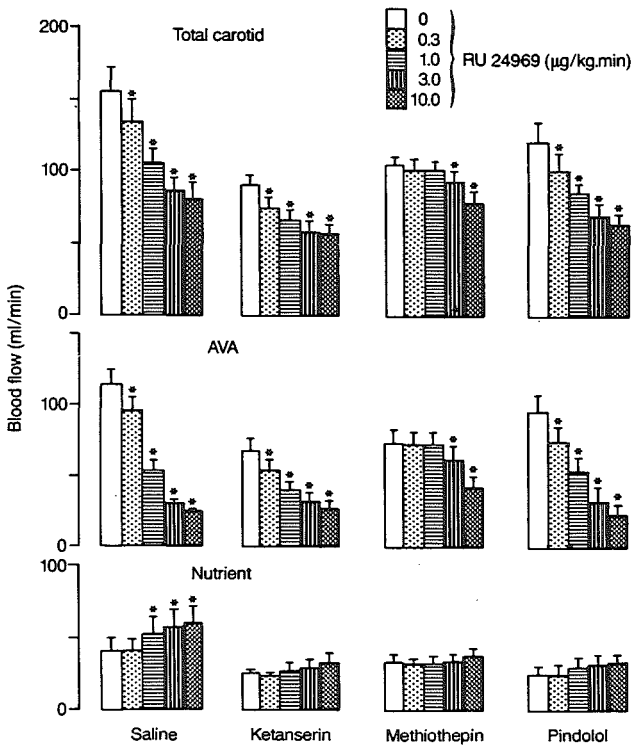

Fig. 1. Effect of intracarotid infusion of RU 24969 (0.3, 1.0, 3.0 and 10 µg/kg·min) on the total carotid artery blood flow and in its arteriovenous anastomotic (AVA) and nutrient (tissue) fractions in groups of six pigs pretreated with either physiological saline, ketanserin (0.5 mg/kg i.a.), methiothepin (1.0 mg/kg i.a.) or (±)-pindolol (4.0 mg/kg i.v.). * $P < 0.05$ vs. baseline.

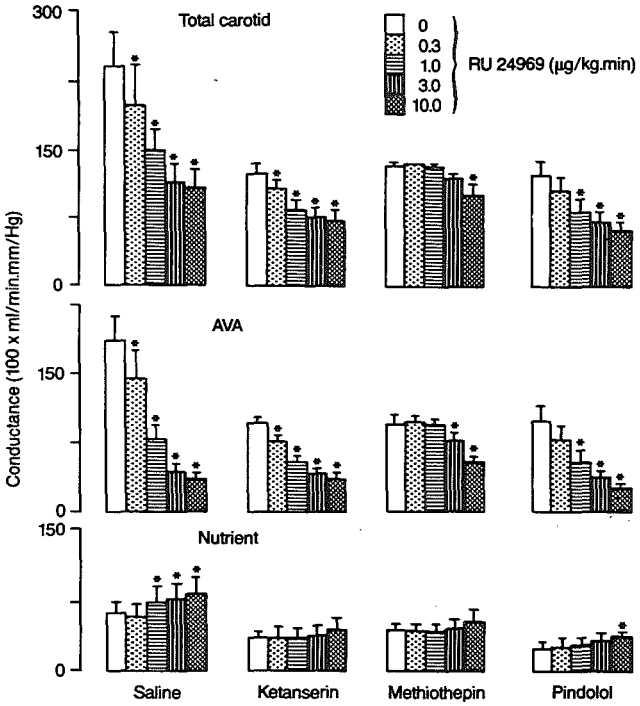

Fig. 2. Effect of intracarotid infusion of RU 24969 (0.3, 1.0, 3.0 and 10 µg/kg·min) on the total carotid conductance and in its arteriovenous anastomotic (AVA) and nutrient (tissue) fractions in groups of six pigs pretreated with either physiological saline, ketanserin (0.5 mg/kg i.a.), methiothepin (1.0 mg/kg i.a.) or (±)-pindolol (4.0 mg/kg i.v.). * $P < 0.05$ vs. baseline.

blood flow, resulting in a decrease in the total carotid blood flow. In animals pretreated with ketanserin (total dose: 0.5 mg/kg) the effects of RU 24969 were attenuated (more pronounced on the arteriolar blood flow than on the total carotid blood flow and its arteriovenous anastomotic part), whereas in the methiothepin-pretreated (total dose: 1.0 mg/kg) animals these effects were markedly suppressed. In contrast, pretreatment with (±)-pindolol (total dose: 4.0 mg/kg) did not modify the effects of RU 24969 on either the total carotid blood flow or its arteriovenous anastomotic fraction, although the effects of RU 24969 in the arteriolar (nutrient) fraction were attenuated. The effects of the intracarotid infusion of RU 24969 on vascular conductance, approximated by the ratio of blood flow and mean arterial blood pressure, showed a similar pattern (fig. 2).

The effects of RU 24969 on the fractionation of nutrient, arteriolar flow are shown in figs. 3 and 4. RU 24969 increased the arteriolar blood flow to the ears, skin, tongue, salivary glands and fat, whereas the arteriolar blood flow to the eyes, muscles, brain and bones remained unchanged. No change in colour of the skin or ears was observed. The RU 24969-induced increase in

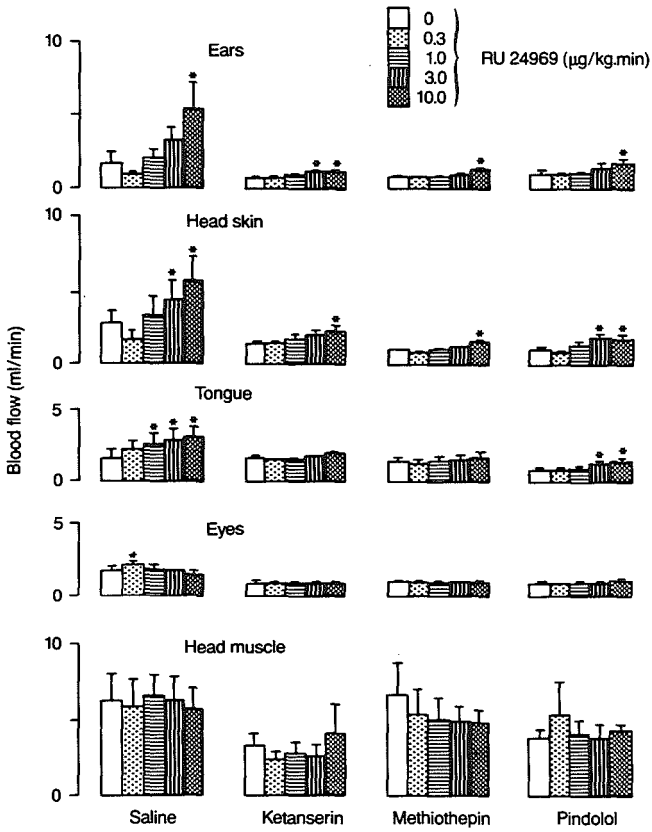

Fig. 3. Effect of intracarotid infusion of RU 24969 (0.3, 1.0, 3.0 and 10 μg/kg·min) on carotid blood flow to the ears, head skin, tongue, eyes and head muscles in groups of six pigs pretreated with either physiological saline, ketanserin (0.5 mg/kg i.a.), methiothepin (1.0 mg/kg i.a.) or (±)-pindolol (4.0 mg/kg i.v.). * $P < 0.05$ vs. baseline.

arteriolar blood flow to the above mentioned tissues was reduced in the ketanserin-, methiothepin- and (±)-pindolol-pretreated animals.

4. Discussion

4.1. Systemic haemodynamics

In contrast to the hypotensive effect observed in ketanserin-treated rats (Doods et al., 1985), RU 24969 did not decrease the mean arterial blood pressure in anaesthetized pigs. However, there was a tendency for the blood pressure to increase in the ketanserin-pretreated animals. The cause of this difference between rats and pigs remains unclear, but it must be emphasized that 8-OH-DPAT did not have the hypotensive action in the anaesthetized pig (Bom et al., 1989b) that is observed in the rat (Doods et al., 1985; Fozard et al., 1987), cat (Ramage and Fozard, 1987) and dog (Di Francesco et al., 1988). The effects of pretreat-

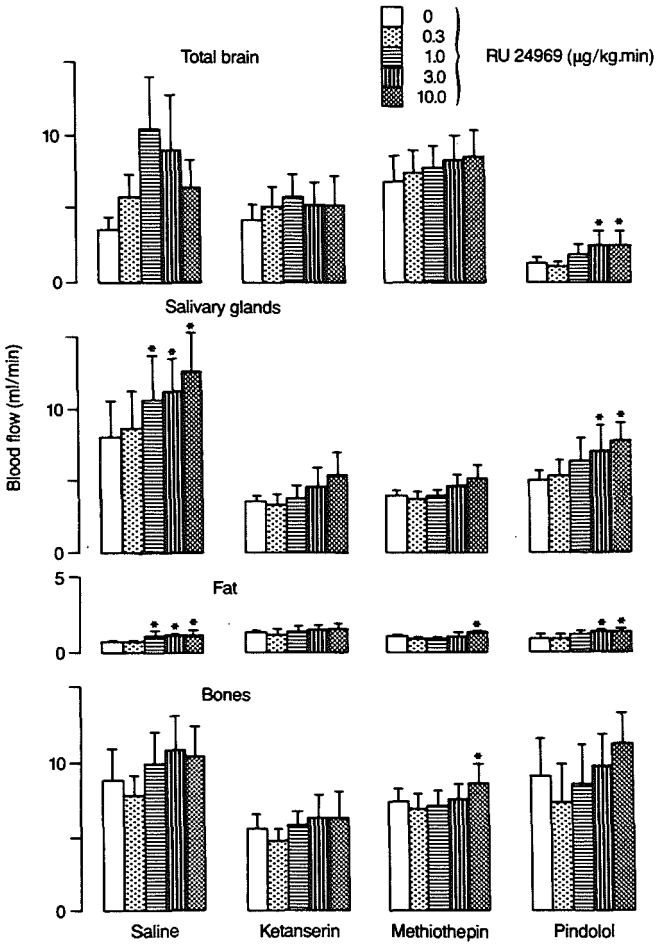

Fig. 4. Effect of intracarotid infusion of RU 24969 (0.3, 1.0, 3.0 and 10 μg/kg·min) on carotid blood flow to the brain, salivary glands, fat and bones in groups of six pigs pretreated with either physiological saline, ketanserin (0.5 mg/kg i.a.), methiothepin (1.0 mg/kg i.a.) or (±)-pindolol (4.0 mg/kg i.v.). * $P < 0.05$ vs. baseline.

ment with (±)-pindolol on the mean arterial blood pressure and heart rate during the experimental period might be explained in terms of the intrinsic sympathomimetic activity of this β-adrenoceptor antagonist (Scriabine and Taylor, 1984). Firstly, (±)-pindolol is able to increase renin release (Weber et al., 1977), thereby suggesting that the increase in mean arterial blood pressure we observed could have been a consequence of (±)-pindolol pretreatment. Secondly, nitrous oxide (which was used as an anaesthetic) is reported to reduce the heart rate and cardiac output while the systemic arterial blood pressure is maintained by an increased total peripheral resistance (Eisele and

Smith, 1972), which could explain the bradycardia observed in the saline-, ketanserin- or methiothepin-pretreated animals; however, this effect was not observed after pretreatment with (±)-pindolol, possibly because of its intrinsic activity on cardiac β-adrenoceptors, which has been described elsewhere (Louis et al., 1977).

4.2. Carotid haemodynamics

Despite the tendency of the blood pressure to increase, intracarotid infusion of RU 24969 caused a dose-related reduction in the total common carotid blood flow, which was directly related to a decrease in the non-nutrient, arteriovenous anastomotic blood flow. Pretreatment with ketanserin slightly attenuated these effects, whereas the reduction in the total carotid blood flow and arteriovenous anastomotic blood flow was markedly reduced after pretreatment with methiothepin. Therefore, RU 24969 seems to reduce arteriovenous anastomotic shunting mainly via 5-HT_1-like receptors (Saxena et al., 1986). However, as described previously for 5-HT (Saxena and Verdouw, 1982; Verdouw et al., 1984), 5-HT_2 receptors could also be involved to a lesser extent in the reduction of arteriovenous anastomotic blood flow induced by RU 24969. Indeed, it is known from radioligand binding studies that RU 24969 has affinity for 5-HT_2 recognition sites (Peroutka, 1986).

The exact reason for the increase in arteriolar blood flow (to the ears, skin, tongue and salivary glands) induced by RU 24969, which was susceptible to ketanserin, methiothepin and (±)-pindolol, is difficult to explain. It could be secondary to the reduction of the arteriovenous anastomotic part of the carotid blood flow; however, another drug, indorenate, did not increase the arteriolar blood flow despite similarly decreasing the arteriovenous anastomotic blood flow (unpublished observations). Another possibility is that vasodilator substances are released from thrombocytes and/or blood vessels by a 5-HT receptor mechanism; Kokkas and Boeynaems (1988) have recently shown that 5-HT can release prostacyclin from the dog saphenous vein by a 5-HT_2 receptor-mediated action. Finally, both (+)- and (−)-pindolol have been reported to possess a low affinity for 5-HT_2 receptors (Hoyer, 1988); nonetheless, because of the fact that methiothepin as well as (±)-pindolol apparently blocked the above mentioned response, we cannot rule out the possibility that 5-HT_{1A} and/or 5-HT_{1B} receptors could be partly involved in the effects of RU 24969 on the arteriolar fraction.

4.3. Nature of 5-HT_1-like receptors involved in cephalic arteriovenous anastomotic flow changes

5-HT_1-like receptors are heterogeneous (Bradley et al., 1986; Humphrey and Feniuk, 1988; Saxena, 1989) and it is, therefore, important to establish the exact nature of the 5-HT_1-like receptor mediating the constriction of arteriovenous anastomoses in the carotid vascular bed. Besides 5-HT (Saxena and Verdouw, 1982) and 5-carboxyamidotryptamine (Saxena and Verdouw, 1985), a number of other drugs having affinity for the 5-HT_{1A} recognition sites (see Middlemiss and Fozard, 1983; Hoyer et al., 1985; Hoyer, 1988) – 8-OH-DPAT (Bom et al., 1989b), BEA 1654 (Verdouw et al., 1985), methysergide (Saxena and Verdouw, 1984) and RU 24969 (present results) – diminish arteriovenous anastomotic blood flow in the pig. This could suggest that the 5-HT_1-like receptors involved correspond to the 5-HT_{1A} recognition site. However, ipsapirone (TVX Q 7821), another compound with high affinity for the 5-HT_{1A} recognition site (Peroutka, 1986; Traber and Glaser, 1987), proved to be ineffective in this model (Bom et al., 1988). Taken together, these findings imply that either the 5-HT receptors on carotid arteriovenous anastomoses in the pig do not correspond to the 5-HT_{1A} recognition sites in the brain or that ipsapirone has a low efficacy at the 5-HT_{1A} receptors on the carotid vessels (Bom et al., 1989b). Our observation that the decrease in both total carotid and arteriovenous anastomotic blood flow induced by RU 24969 was not affected by (±)-pindolol further reveals that these carotid 5-HT_1-like receptors do not belong to either the 5-HT_{1A} or 5-HT_{1B} subtype. Both RU 24969 and (−)-pindolol have high affinity for 5-HT_{1A} and 5-HT_{1B} recognition sites (Hoyer, 1988), and some behavioral effects of 8-OH-DPAT and RU 24969

in the rat can be dose dependently (0.5 to 4.0 mg/kg) blocked by (−)-pindolol (Tricklebank et al., 1984; 1987).

Furthermore, it should be noted that the 5-HT_1-like receptors mediating the constriction of arteriovenous anastomoses and the dilatation of arterioles in the carotid vascular bed seem to be heterogeneous because 5-HT and 5-carboxamidotryptamine induce these effects and change skin colour to bright pink (Saxena and Verdouw, 1982; 1985), whereas methysergide, BEA 1654, 8-OH-DPAT and RU 24969 (Saxena and Verdouw, 1984; Verdouw et al., 1985; Bom et al., 1989b; present results) *primarily* reduce arteriovenous anastomotic shunting. Since a change in the skin colour was not observed with RU 24969, a mediation by either 5-HT_{1A} or 5-HT_{1B} receptors in this phenomenon is not very likely.

4.4. 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 isometheptene (see Saxena, 1978; 1987), powerfully reduce cephalic arteriovenous anastomotic shunting, although, as demonstrated for ergotamine (Bom et al., 1989a), a reduction in cephalic arteriovenous anastomotic blood flow does not necessarily have to be mediated by 5-HT_1-like receptors. Two new, highly selective agonists at 5-HT_1-like receptors, AH25086 and sumatriptan (GR43175), have recently been shown to constrict cephalic arteries in several species and to diminish feline carotid arteriovenous anastomotic blood flow (Feniuk et al., 1987; Humphrey et al., 1989; in press; Saxena and Ferrari, 1989). Furthermore, these new drugs do not seem to cross the blood brain barrier or have an antinociceptive effect even after intrathecal administration (Humphrey et al., 1989; in press), yet they are remarkably effective against acute migraine attacks (Brandt et al., 1987; Doenicke et al., 1988; Perrin et al., 1989). Since RU 24969 powerfully reduces cephalic arteriovenous anastomotic blood flow, the drug may also possess an antimigraine action.

In conclusion, this study demonstrates that RU 24969, which has a high affinity for 5-HT_{1A} and 5-HT_{1B} recognition sites, reduces total carotid blood flow in the anaesthetized pig by selectively affecting arteriovenous anastomotic blood flow. This effect of RU 24969, which was highly susceptible to methiothepin but not much to ketanserin and which was resistant to blockade by (±)-pindolol, is mediated by 5-HT_1-like receptors that do not seem to belong to either the 5-HT_{1A} or 5-HT_{1B} subtype.

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Chapter 11

REDUCTION OF CEPHALIC ARTERIOVENOUS SHUNTING BY ERGOTAMINE IS NOT MEDIATED BY 5-HT_1-LIKE OR 5-HT_2 RECEPTORS.

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Reduction of cephalic arteriovenous shunting by ergotamine is not mediated by 5-HT_1-like or 5-HT_2 receptors

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1 The potent, antimigraine drug ergotamine has affinity for both 5-HT_1 and 5-HT_2 binding sites and constricts arteriovenous anastomoses. Since 5-HT also constricts arteriovenous anastomoses (mainly via 5-HT_1-like receptors), this study investigates the involvement of 5-HT receptors in the ergotamine-induced reduction of arteriovenous shunting in the carotid circulation of the cat and pig.

2 In the cat, ergotamine (3, 10 and 30 $\mu g\ kg^{-1}$, i.v.) reduced carotid blood flow, predominantly by a reduction in arteriovenous anastomotic blood flow. Pretreatment with ketanserin (0.5 $mg\ kg^{-1}$, i.v.) or methiothepin (1 $mg\ kg^{-1}$, i.v.) did not antagonize the effects of ergotamine.

3 In the pig, ergotamine (2.5, 5, 10 and 20 $\mu g\ kg^{-1}$, i.v.) also reduced carotid blood flow and arteriovenous shunting, which was not affected by pretreatment with methiothepin (1 $mg\ kg^{-1}$, i.v.).

4 These results suggest that the reduction by ergotamine in the shunting of carotid arterial blood via cephalic arteriovenous anastomoses is not mediated by 5-HT_1-like or 5-HT_2 receptors.

Introduction

Ergotamine is a potent and effective drug for the treatment of acute attacks of migraine headaches where, among other pathophysiological factors (for references, see Blau, 1987), an opening of arteriovenous anastomoses has been suggested (Heyck, 1969; Saxena, 1978; 1987). Indeed, ergotamine decreases blood flow in the carotid circulation in a variety of species, including man (Saxena & De Vlaam-Schluter, 1974; Spira *et al.*, 1976; Puzich *et al.*, 1983). In anaesthetized cats (Johnston & Saxena, 1978), pigs (Schamhardt *et al.*, 1979) and dogs (Saxena *et al.*, 1983), the decrease in carotid blood flow by ergotamine is almost entirely due to a reduction in the cephalic arteriovenous anastomotic ('shunt') fraction. The constrictor effect of ergotamine on arteriovenous anastomoses in the dog is not antagonized by phentolamine and/or pizotifen, thereby excluding the involvement of α-adrenoceptors and histamine H_1-receptors, as well as the 'D' 5-hydroxytryptamine (5-HT) receptors (Saxena *et al.*, 1983) which are claimed to be involved in ergotamine-induced constrictions of isolated extracranial arteries (Müller-Schweinitzer & Weidmann, 1978; Müller-Schweinitzer, 1986).

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The receptors for 5-HT have recently been subdivided into three distinct types, called 5-HT_1-like, 5-HT_2 (generally equivalent to 'D' 5-HT) and 5-HT_3 receptors (Bradley *et al.*, 1986; Humphrey & Richardson, 1988). The selective agonists at the three receptor types are, respectively, 5-carboxamidotryptamine, α-methyl-5-HT and 2-methyl-5-HT. Ketanserin and MDL 72222 selectively antagonize 5-HT_2 and 5-HT_3 receptors respectively, whilst methiothepin and methysergide are non-selective antagonists at both 5-HT_2 and 5-HT_1-like receptors. The 5-HT_1-like receptors mediate the constriction of arteriovenous anastomoses by 5-HT in the carotid circulation of the pig, since this response is mimicked by 5-carboxamidotryptamine (Saxena & Verdouw 1985a) and other selective 5-HT_1-like agonists such as BEA 1654 (Verdouw *et al.*, 1985), AH25086 (Humphrey *et al.*, 1987) and GR43175 (Feniuk *et al.*, 1987; Humphrey *et al.*, 1988), and is blocked by methiothepin but not by ketanserin or MDL 72222 (Verdouw *et al.*, 1984; Saxena *et al.*, 1986). Recent radioligand experiments show that ergotamine possesses a high affinity for 5-HT_1 binding sites (Leysen & Gommeren, 1984; Richardson *et al.*, 1986). In this investigation, therefore, we address the question whether 5-HT_1-like

receptors mediate the ergotamine-induced constriction of arteriovenous anastomoses. For this purpose the effects of ergotamine on the carotid circulation has been compared in untreated cats and pigs with those in animals pretreated with ketanserin or methiothepin.

Methods

Experimental set-up

Cats The experimental set-up used in the cats has been described in detail (Johnston & Saxena, 1978; Saxena & Verdouw, 1982). Briefly, the animals weighing between 2.1 and 4.4 kg, were anaesthetized with a mixture of α-chloralose (60 mg kg^{-1}, i.p.) and urethane (700 mg kg^{-1}, i.p.). Bilateral cervical vagosympathectomy was performed (to reduce reflex influences on the carotid circulation) and the trachea was cannulated and connected to a respirator for intermittent positive pressure ventilation. Arterial blood gasses were monitored and kept within normal limits (pH, 7.35–7.45; Pao_2, 90–150 mmHg; $Paco_2$, 35–45 mmHg). Aortic blood pressure was measured with a Statham transducer (model P23 Ac) connected to a polyethylene cannula inserted in a femoral artery. Heart rate was derived from the pressure signals. The femoral vein was cannulated for drug administration. Blood flow in the left common carotid artery was measured with electromagnetic flow probes (1.5–2 mm i.d.; Transflow 600-system, Skalar, Delft, The Netherlands). A catheter was placed in the left lingual artery to allow retrograde injection of microspheres into the carotid artery. The body temperature was maintained around 37°C by use of an electric heating pad.

Pigs As described earlier (Saxena & Verdouw, 1982), fasted young Yorkshire pigs (body weight: 19–29 kg) were sedated with azaperone (120 mg, i.m.) and metomidate (120–150 mg, i.v.), intubated and given intermittent positive pressure ventilation with a mixture (1:2) of oxygen and nitrous oxide. A continuous infusion of pentobarbitone sodium (20–26 mg kg^{-1} h^{-1}, i.v.) completed the anaesthesia. Respiratory rate and tidal volume were adjusted to keep arterial blood gases and pH within normal limits (see above). The right femoral artery and vein were cannulated for the measurement of aortic blood pressure (Statham transducer, model P23 ID) and administration of ergotamine, respectively. Heart rate was derived from the pressure signals. The common carotid arteries were dissected free and both cervical vagosympathetic trunks were severed. Blood flow in one of the common carotid arteries was measured with a precalibrated flow probe (2.5–3 mm, i.d.; Transflow 601-system, Skalar, Delft). A hub-less 0.5 mm (external diameter) needle, connected to suitable polyethylene tubing, was inserted in this common carotid artery for the administration of microspheres and methiothepin (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, West Germany). 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 τ-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 (Saxena *et al.*, 1980; Saxena & Verdouw, 1982) by: $Q_{tis[car]}$ (ml min^{-1}) = (I_{tis}/I_{tot}) × $Q_{[car]}$, 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^{-1}). Because there was complete entrapment of 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 (see Johnston & Saxena, 1978; Saxena & Verdouw, 1982).

Experimental protocol

Cats About 45 min after completion of the surgical procedures baseline measurements of heart rate, blood pressure and common carotid blood flow and its distribution (using 20,000–50,000 radioactive microspheres) were made. The cats were then divided into three groups receiving pretreatment with either physiological saline (0.9% NaCl, w/v) (2 ml, i.v.), ketanserin (0.5 mg kg^{-1}, i.v.) or methiothepin (1 mg kg^{-1}, i.v.) This pretreatment was followed by the administration of ergotamine in cumulative doses (3, 10, 30 μg kg^{-1}, i.v.) at intervals of 15 min. The systemic and carotid haemodynamic measure-

Table 1 Effects of saline ($n = 7$), ketanserin (0.5 mg kg^{-1}, i.v.; $n = 7$) or methiothepin (1 mg kg^{-1}, i.v.; $n = 7$) and subsequent cumulative doses of ergotamine on heart rate and mean arterial blood pressure in cats

Pretreatment	*Baseline*	*Pretreatment*	*Ergotamine* (μg kg^{-1}, i.v.) 3	10	30
Heart rate (beats min^{-1})					
Saline	181 ± 20	182 ± 18	154 ± 14**	135 ± 8**	129 ± 7**
Ketanserin	212 ± 8	177 ± 8*	155 ± 8**	141 ± 9**	133 ± 9**
Methiothepin	189 ± 12	162 ± 9*	139 ± 7**	131 ± 7**	127 ± 8**
Mean arterial blood pressure (mmHg)					
Saline	90 ± 7	80 ± 3	80 ± 5	82 ± 7	104 ± 7**
Ketanserin	108 ± 8	71 ± 7*	80 ± 8	85 ± 7**	91 ± 6**
Methiothepin	95 ± 7	72 ± 7*	76 ± 8	85 ± 9**	92 ± 10**

* Significantly ($P < 0.05$) different vs baseline;
** Significantly ($P < 0.05$) different vs pretreatment.

ments were repeated at the end of each intervention (pretreatment and the three ergotamine doses).

Pigs After a post-surgical stabilization period of about 45 min, the animals were divided into two groups receiving intracarotid infusions (rate: 1 ml min^{-1} for a period of 10 min) of either physiological saline or methiothepin (total dose: 1 mg kg^{-1}). Ten minutes later, in all experiments baseline measurements of heart rate, arterial blood pressure, common carotid arterial blood flow and its distribution (with 100,000–150,000 spheres) were made. These measurements were repeated after the administration of ergotamine (2.5, 5, 10 and 20 μg kg^{-1} in cumulative doses) at intervals of 15 min.

Statistical analysis

The significance of the differences between the 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 (Steel & Torrie, 1980). 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: ergotamine tartrate (Gynergeen ampoules Sandoz B.V., Uden, The Netherlands), ketanserin tartrate (courtesy: Dr J.M. van Nueten, Janssen Pharmaceutica, Beerse, Belgium) and methiothepin maleate (courtesy: Dr P.J. Pijper, Hoffmann-La Roche B.V., Mijdrecht, The Netherlands). The drugs were diluted with (ergotamine) or dissolved in physiological saline. The doses of ergotamine, ketanserin and methiothepin refer to the salts.

Results

Systemic haemodynamics

In the cat experiments, pretreatment with saline did not change arterial blood pressure or heart rate. Pretreatment with ketanserin (0.5 mg kg^{-1}) or methiothepin (1 mg kg^{-1}) was followed by a decrease

Table 2 Effect of cumulative doses of ergotamine on heart rate and mean arterial blood pressure in untreated ($n = 6$) and methiothepin (1 mg kg^{-1})-treated pigs ($n = 6$)

Pretreatment	*Baseline*	*Ergotamine* (μg kg^{-1}, i.v.) 2.5	5.0	10.0	20.0
Heart rate (beats min^{-1})					
Saline	104 ± 10	91 ± 7*	84 ± 5*	79 ± 5*	76 ± 4*
Methiothepin	101 ± 12	96 ± 10	87 ± 9*	81 ± 8*	77 ± 7*
Mean arterial blood pressure (mmHg)					
Saline	76 ± 4	86 ± 5	86 ± 5	86 ± 5	80 ± 7
Methiothepin	79 ± 5	83 ± 5	89 ± 8	85 ± 10	84 ± 11

* Significantly different ($P < 0.05$) vs baseline.

in both variables. Irrespective of the type of pretreatment, cumulative doses of ergotamine (3, 10 and 30 $\mu g\,kg^{-1}$, i.v.) caused a decrease in heart rate, whereas arterial blood pressure showed a marked increase (Table 1). In the pig experiments ergotamine had no effect on mean arterial blood pressure in either the saline- or methiothepin-treated animals. Heart rate decreased dose-relatedly after ergotamine in both groups (Table 2).

Carotid haemodynamics

Cats In the saline group of animals, where saline-treatment itself had no effect on carotid blood flow or its distribution, cumulative doses of ergotamine (3, 10 and 30 $\mu g\,kg^{-1}$, i.v.) caused a decrease in total carotid blood flow. This decrease was predominantly in the arteriovenous anastomotic fraction (non-nutrient) but the nutrient (arteriolar; capillary) fraction was also reduced (Figure 1). Pretreatment with ketanserin (0.5 $mg\,kg^{-1}$) or methiothepin (1 $mg\,kg^{-1}$) caused a decrease in total carotid blood flow which was similar in the arteriovenous anastomotic and nutrient fractions. Neither pretreatment modified the effects of ergotamine on total carotid and arteriovenous anastomotic blood flows though the decrease in nutrient flow seemed attenuated (Figure 1). The decrease in the nutrient blood flow by ergotamine in the animals pretreated with saline was distributed over a number of cephalic tissues, particularly the ears, skin, muscles and salivary glands, but not the brain (data not shown). Pretreatment with ketanserin reduced blood flow, especially to the muscles, ears and skin. Methiothepin also reduced blood flow to the muscles and ears. Both drugs seemed to prevent ergotamine-induced decreases in nutrient blood flow, but the pre-ergotamine blood flow values were less after ketanserin and methiothepin when compared to saline (Figure 1).

Since both potential antagonist drugs, ketanserin and methiothepin, decreased mean arterial blood pressure, the effects of these drugs on blood flow may be related to reduced perfusion pressure; hence vascular conductances within the carotid bed were calculated as ratios of blood flow and mean arterial blood pressure values. The data reveal that the decrease in blood flow by ketanserin and methiothepin was clearly related to the drop in blood pressure, probably caused by vasodilatation in other vascular beds. Furthermore, it is observed that ergotamine-induced decreases in vascular conductances in total carotid, arteriovenous anastomotic and nutrient beds were not much altered by either ketanserin or methiothepin (Figure 2). The examination of individual tissues shows that vascular conductance decreased with ergotamine in the saline pretreated animals in the ears, skin, eyes, muscles, bones and

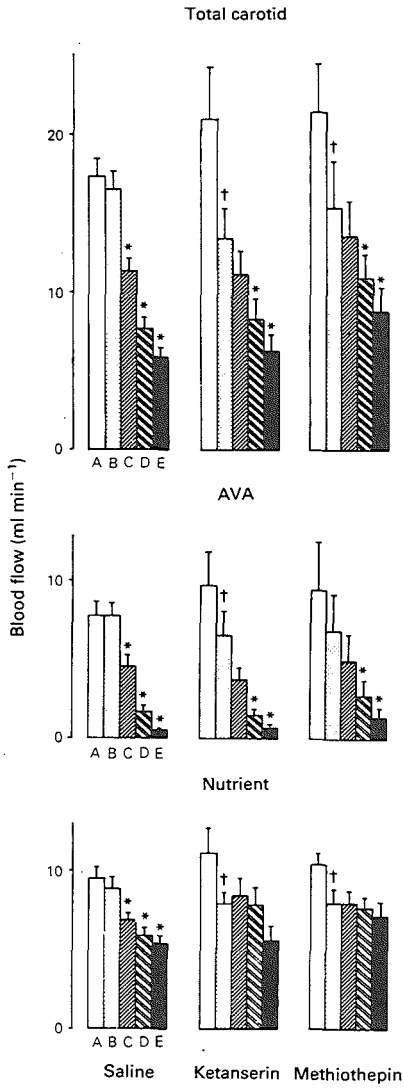

Figure 1 Effect of ergotamine (3, 10 and 30 $\mu g\,kg^{-1}$, i.v.) on the total carotid blood flow and its arteriovenous anastomotic (AVA) and nutrient (tissue) fractions in 6 cats each pretreated with either physiological saline, ketanserin (0.5 $mg\,kg^{-1}$, i.v.) or methiothepin (1.0 $mg\,kg^{-1}$, i.v.). Baseline, A; pretreatment, B; ergotamine 3 $\mu g\,kg^{-1}$, C; 10 $\mu g\,kg^{-1}$, D and 30 $\mu g\,kg^{-1}$, E. † Significantly ($P < 0.05$) different vs baseline; * significantly ($P < 0.05$) different vs pretreatment.

salivary glands, but not in the brain. In antagonist pretreated animals the effects of ergotamine were apparently either less or absent in several tissues; however, as with blood flow values, the pre-ergotamine conductance values were lower than in the saline group (data not shown).

Pigs As in the cats, ergotamine dose-dependently reduced common carotid blood flow in the saline-pretreated pigs by decreasing arteriovenous anastomotic blood flow; the nutrient (arteriolar; capillary) fraction was unaffected. Pretreatment with methiothepin (1 mg kg^{-1}) did not much modify the effects of ergotamine (Figure 3). After administration of ergotamine, blood flow to some tissues (brain, eyes, fat, bones or salivary glands) did not change, but in others there was a tendency to increase (ears, skin and tongue) or decrease (muscles). The increases in tissue blood flow seemed to be attenuated in animals pretreated with methiothepin (data not shown).

Discussion

Shunting of microspheres

The present results confirm the observations that a large fraction (cat: 35–45%; dog: 40–45%; pig: 75–80%) 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 (Johnston & Saxena; 1978; Saxena & Verdouw, 1982; Saxena *et al.*, 1983). 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 (Saxena & Verdouw, 1985b), but such 'shunt' vessels have also been located in the nasal mucosa (Änggård, 1974), tongue (Krönert *et al.*, 1980), dura mater (Rowbotham & Little, 1965) and rete mirabile (Gillilan & Markesbery, 1963). The opening of arteriovenous anastomoses, possibly secondary to a decrease in blood 5-HT concentrations (Lance, 1982), may be involved in the pathophysiology of migraine (see Heyck, 1969; Saxena, 1978; 1987).

Effects of ergotamine

Ergotamine decreased heart rate in both cats and pigs. In the cat the ergotamine-induced bradycardia is due to an agonist action on presynaptic dopamine receptors located on the cardiac sympathetic nerve terminals (Saxena & Cairo-Rawlins, 1983), but in the rat presynaptic α_2-adrenoceptors have been implicated (Roquebert & Grenié, 1986).

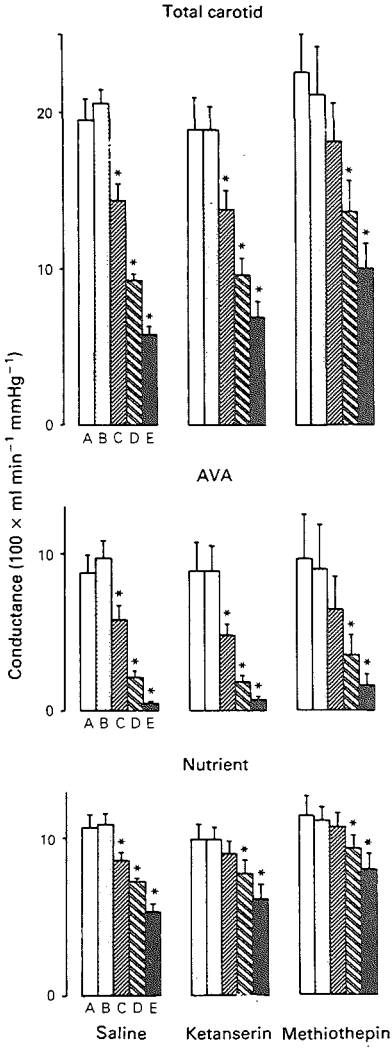

Figure 2 Effect of ergotamine (3, 10 and 30 μg kg^{-1}, i.v.) on vascular conductances in the total carotid bed and its arteriovenous anastomotic (AVA) and nutrient (tissue) fractions in 6 cats each pretreated with either physiological saline, ketanserin (0.5 mg kg^{-1}, i.v.) or methiothepin (1.0 mg kg^{-1}, i.v.). Baseline, A; pretreatment, B; ergotamine 3 μg kg^{-1}, C; 10 μg kg^{-1}, D and 30 μg kg^{-1}, E. * Significantly ($P < 0.05$) different vs pretreatment.

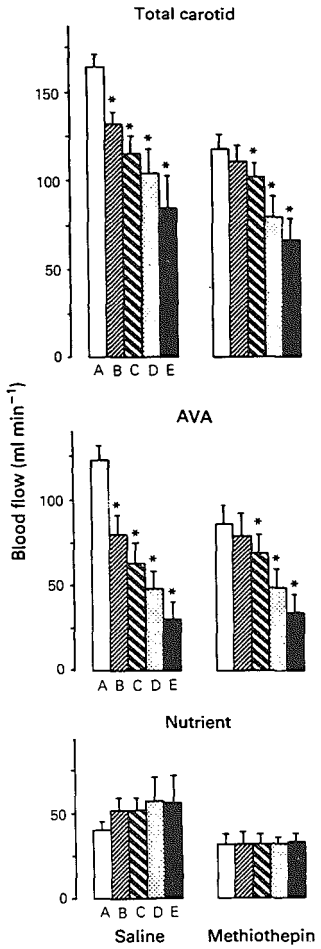

Figure 3 Effect of ergotamine (2.5, 5, 10 and 20 $\mu g\,kg^{-1}$, i.v.) on the total carotid blood flow and its arteriovenous anastomotic (AVA) and nutrient (tissue) fractions in 6 pigs each pretreated with either physiological saline or methiothepin (1.0 $mg\,kg^{-1}$, i.a.). No ergotamine, A; ergotamine 2.5 $\mu g\,kg^{-1}$, B; 5 $\mu g\,kg^{-1}$, C; 10 $\mu g\,kg^{-1}$, D and 20 $\mu g\,kg^{-1}$, E. * Significantly ($P < 0.05$) different vs baseline (pre-ergotamine) values.

In both species studied, ergotamine caused a dose-related decrease in carotid blood flow, predominantly by affecting the arteriovenous anastomotic fraction. Pretreatment with the 5-HT_2 receptor antagonist ketanserin or the mixed 5-HT_1-like and 5-HT_2 receptor antagonist methiothepin did not modify these effects. The doses of ketanserin (0.5 $mg\,kg^{-1}$) and methiothepin chosen (1 $mg\,kg^{-1}$) were more than adequate, since they antagonize several cardiovascular responses mediated by 5-HT_1-like receptors (e.g.: 5-HT-induced hypotension in the rat and cat, tachycardia in the cat and arteriovenous anastomotic constriction and arteriolar dilatation in the porcine carotid bed; Saxena & Lawang, 1985; Saxena *et al.* 1985; 1986; Connor *et al.*, 1986) or by 5-HT_2 receptors (e.g.: 5-HT-induced hypertension in the rat; Saxena & Lawang, 1985). Therefore, despite demonstration of a high affinity for both 5-HT_1 and 5-HT_2 binding sites (Leysen & Gommeren, 1984) and for 5-HT_2 receptors in 'large' conducting arteries (Müller-Schweinitzer & Weidmann, 1978; Müller-Schweinitzer, 1986), the reduction in the carotid blood flow following constriction of cephalic arteriovenous anastomoses by ergotamine does not appear to be mediated by either 5-HT_1-like or 5-HT_2 receptors. As shown earlier in the dog, phentolamine and pizotifen were also unable to modify the responses to ergotamine, excluding the involvement of α-adrenoceptors as well as histamine H_1-receptors (Saxena *et al.*, 1983). A possible mediation by 5-HT_3 receptors can not be ruled out from the present experiments, but neither 5-HT_3 receptors mediate constriction of arteriovenous anastomoses (Saxena *et al.*, 1986) nor does ergotamine have an agonist action on these receptors. Thus receptors involved in the ergotamine-induced reduction of arteriovenous anastomotic blood flow in the carotid vascular bed are yet to be characterized. In this context it may be recalled that 5-HT can act on receptors unrelated to 5-HT_1-like, 5-HT_2 or 5-HT_3 types in isolated papillary muscle of kitten (Kaumann, 1985) and in the pig heart (Bom *et al.*, 1988) and that vasodilator dopamine receptors have been described on arteriovenous anastomoses in the canine paw pad (Bell *et al.*, 1978; Bell & Lang, 1979). Whether ergotamine can act on these receptors is not yet known. Another mechanism explaining the action of ergotamine could be related to prostaglandin formation. In canine vein strips the constrictor response to ergotamine is in part mediated by production of a prostaglandin-like substance (Müller-Schweinitzer & Weidmann, 1978) and prostaglandin $F_{2\alpha}$ can increase vascular resistance in the external carotid bed of the monkey (Spira *et al.*, 1978).

The vasoconstrictor action of ergotamine in some tissues was attenuated in both ketanserin- and methiothepin-pretreated animals. It may suggest that the vasoconstriction by ergotamine in the nutrient circulation may be partly mediated by α-adrenoceptors and/or 5-HT_2 receptors (see Saxena & De Vlaam-Schluter, 1974; Müller-Schweinitzer & Weidmann, 1978).

In conclusion this study shows that the effects of ergotamine on arteriovenous anastomotic blood flow in cat and pig are not mediated by 5-HT_1-like or 5-HT_2 receptors. The involvement of α-adrenoceptors and histamine H_1-receptors has been excluded in earlier studies.

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Chapter 12: DISCUSSION

1. Characterization of 5-HT receptors.

Functional 5-HT_2 and 5-HT_3 receptors in the cardiovascular system can be adequately characterized using antagonists like ritanserin, ketanserin and cyproheptadine (5-HT_2 receptors) and MDL 72222 and ICS 205-930 (5-HT_3 receptors). Other 5-HT receptors, which are neither 5-HT_2 nor 5-HT_3 receptors are more difficult to characterize. From functional studies, showing effects of 5-HT which could not be blocked by 5-HT_2 or 5-HT_3 receptor antagonists, the existence of 'atypical' 5-HT receptors has been suggested (Saxena et al., 1971; Apperley et al., 1980; Saxena & Verdouw, 1984; Feniuk et al.,1985). Based on the knowledge obtained from radioligand binding studies on cerebral membrane preparations, these receptors have been classified as 5-HT_1-like receptors (Bradley et al.,1986). 5-Carboxamidotryptamine, which has a high affinity for 5-HT_1 recognition sites, proved to be a potent and selective agonist, mimicking the effects of 5-HT in several functional tests, like the 5-HT-induced tachycardia in the cat (Saxena et al., 1985; Connor et al., 1986), contraction of the dog saphenous vein (Feniuk et al., 1985) and reduction of arteriovenous anastomotic blood flow in the pig (Saxena & Verdouw, 1985). Unfortunately, no selective antagonist for the 5-HT_1-like receptor is available yet. However, methiothepin, an antagonist with a high affinity for both 5-HT_1 and 5-HT_2 recognition sites, can be used in functional tests, but other actions of methiothepin like α-adrenoceptor antagonism or noradrenaline uptake inhibition have to be considered.

Since radioligand binding studies revealed the presence of subtypes of 5-HT_1 recognition sites, many investigators studied the effects of these ligands in functional studies to get an impression of the efficacy of these compounds. However, due to the lack of selective antagonists for these putative subtypes of 5-HT_1-like receptors, it is still not possible to demonstrate that the subtypes of 5-HT_1 binding sites in cerebral

membranes actually correspond to subtypes of functional 5-HT_1-like receptors in the cardiovascular system. Pindolol, which has a high affinity for 5-HT_{1A} and 5-HT_{1B} recognition sites, did not antagonize the reduction in porcine cranial arteriovenous blood flow induced by RU 24969, which has affinity for the same recognition sites (Bom et al., 1989a). This shows that compounds with affinity for certain 5-HT recognition sites in cerebral membranes and without agonistic action on peripheral 5-HT receptors do not necessarily behave as a 5-HT antagonist. Lack of antagonism by cyanopindolol has also been described for GR43175-induced contraction of dog saphenous vein and basilar artery (Humphrey et al., 1988; Connor et al., 1989). Furthermore, spiperone does not block the effects of 8-OH-DPAT and GR 43175 on dog saphenous vein and carotid vascular resistance (Perren et al., 1989). Another disadvantage of binding studies is the dependency on highly selective ligands with high affinity for a certain recognition site. For many years it was claimed that no 5-HT_3 recognition sites were present in the brain, until they were discovered by using better ligands. The fact, that until now 5-HT_{1B} recognition sites have only been found in rodents, does not necessarily mean that there are no 5-HT_{1B} recognition sites in the brains of other species !

Therefore, it is questionable whether binding data are useful for the prediction of drug effects in functional models. Binding data might be useful for screening of new compounds to decide which functional tests are the first to be done, but the 'classical' pharmacological approach still has to be preferred. This is clearly demonstrated in our experiments using ergotamine in the cranial circulation of the cat and the pig. Since it has been shown that the effects of ergotamine on shunt flow are not antagonized by pizotifen and/or phentolamine (Saxena et al., 1983) it was assumed that ergotamine was acting on 5-HT_1-like receptors, because this compound also has a high affinity for 5-HT_1 recognition sites (Leysen & Gommeren, 1984; Richardson et al., 1986). However, methiothepin does not block this effect of ergotamine (Bom et al., 1989b), which leaves us with four unanswered questions: 1) is the

therapeutic effect of drugs in the acute migraine attack related to their action on 5-HT receptors ? 2) is ergotamine acting on a receptor that is not a 5-HT receptor ? 3) is ergotamine acting on a subtype of 5-HT_1-like receptors that cannot be blocked by methiothepin ? 4) is ergotamine acting on a 5-HT receptor in the cardiovascular system, that is not a 5-HT_1-like, 5-HT_2, or 5-HT_3 receptor ? The first three questions are still difficult to answer, but there exist experimental evidence for the possibility suggested in the fourth question. In the kitten isolated papillary muscle 5-HT, but not 5-carboxamidotryptamine, has a positive inotropic effect that cannot be blocked by methysergide or phenoxybenzamine (Kaumann, 1985). In pig coronary arteries 5-HT inhibits the release of noradrenaline from sympathetic nerves, which is also resistant to 5-HT_1-like, 5-HT_2 and 5-HT_3 receptor antagonists and which is not mimicked by 5-carboxamidotryptamine (Molderings et al., 1989). However, noradrenaline release is also inhibited by N,N-dimethyl-5-HT, 5-aminotryptamine, tryptamine and RU 24969, a compound with affinity for 5-HT_{1A} and 5-HT_{1B} recognition sites. In the pig 5-HT also causes tachycardia, which is resistant to 5-HT receptor antagonists and antagonists at α- and β-adrenoceptors, muscarinic, nicotinic, histamine and dopamine receptors, and calcium channnels (Bom et al., 1988). 8-OH-DPAT, BEA 1654, 5-carboxamidotryptamine and 2-methyl-5-HT are ineffective, as RU 24969, which is in contrast to the inhibitory effect of this compound on noradrenaline release in the pig coronary artery. It is still uncertain whether the above-mentioned effects of 5-HT are mediated by an identical 5-HT receptor or by different 5-HT receptors or other mechanisms. Hopefully, the development of new 5-HT antagonists will give an answer to this problem, which might lead to a reproposal for the criteria of 5-HT receptor classification.

2. Cranial arteriovenous anastomoses.

In contrast to conscious pigs, the arteriovenous anastomotic blood flow in anaesthetized pigs is high (70–80 % of total carotid blood flow), irrespective of sympathectomy. Electrical stimulation of the cervical sympathetic nerves does not modify arteriovenous anastomotic shunt flow, indicating that the large shunt flow is not related to a central depressant effect of the anaesthesia on sympathetic outflow (Verdouw et al. 1984). An α–adrenoceptor blocking effect of the anaesthetic agent used might be involved, since intracarotid infusions of noradrenaline did not have a marked effect on shunt flow, whereas bolus injections caused a short–lasting decrease in shunt flow. Furthermore, an effect of the anaesthesia on circulating vasoconstricting hormones (5–HT ?) cannot be excluded.

3. Role of arteriovenous anastomoses in migraine.

An increase in oxygen saturation of external jugular and frontal venous blood during migraine attacks has been described by Heyck (Heyck, 1969). Treatment with dihydroergotamine returned the venous oxygen saturation to normal, which led to the hypothesis that opening of arteriovenous anastomoses might contribute to this phenomenon (Heyck, 1981). Unfortunately, this study only includes 7 patients and for unknown reasons no further studies have been done on the time course of frontal venous oxygen saturation changes during migraine attacks and its relief by drugs like ergotamine, dihydroergotamine and GR43175 (Doenicke et al., 1988). These rather harmless experiments might give an answer on the question whether opening of arteriovenous anastomoses precedes the migraine attack or whether opening of arteriovenous anastomoses is just an epiphenomenon of the migraine attack. Opening of arteriovenous anastomoses might be a reflection of autonomic disturbances that occur during migraine attacks like vomiting, diarrhea, nasal stuffiness, Horner's syndrome and unilateral pupillary dilatation.

In conclusion, the drug-induced reduction in cranial arteriovenous anastomotic blood flow in experimental animals is a good model to study putative anti-migraine drugs, since 5-HT_1-like receptor-mediated effects can be studied and ergotamine-like actions can be studied simultaneously.

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SUMMARY

The autacoid 5-hydroxytryptamine (5-HT; serotonin) plays a role in many (patho-) physiological processes. This thesis describes the effects of 5-HT on heart rate and carotid blood flow distribution in anaesthetized cats and pigs. Using selective antagonists and agonists the 5-HT receptor subtypes were characterized according to, now generally accepted, criteria (Chapter 2). After a review of the literature concerning heart rate changes in mammals (Chapter 3), the 5-HT-induced epicardial chemoreflex is described (Chapter 4). Topical application of 5-HT or nicotine on the epicardium of anaesthetized cats leads to stimulation of afferent vagal nerve fibers, mediating the epicardial chemoreflex: bradycardia, inhibition of sympathetic nerve activity and hypotension. Topical or intravenous administration of the selective 5-HT_3 receptor antagonist MDL 72222 abolishes the reflex effects of 5-HT but not of nicotine, showing that MDL 72222 does not possess a local anaesthetic action. Furthermore, pretreatment with MDL 72222 revealed the presence of a tachycardic response to 5-HT, which is resistant to pretreatment with propranolol or phentolamine, but can be blocked by phenoxybenzamine.

The 5-HT-induced tachycardia was also studied in anaesthetized vagotomized pigs (Chapter 5). However, the dose-dependent increase in heart rate induced by 5-HT cannot be blocked by any of the known antagonists for 5-HT receptors. This response is also resistant to antagonists at α- and β-adrenoceptors, muscarinic, nicotinic, histamine and dopamine receptors, and calcium channels. 5-HT uptake inhibitors induce an increase in heart rate by themselves and potentiate the 5-HT response. Putative selective agonists at 5-HT_1-like or 5-HT_3 receptors do not induce tachycardia in the pig. Therefore one can conclude that the 5-HT-nduced tachycardia in the pig is not mediated by 5-HT_1-like, 5-HT_2 or 5-HT_3 receptors, but may be mediated by a new type of 5-HT receptor.

In Chapter 6 the role of arteriovenous anastomoses in the cardiovascular system is discussed, particularly their possible role in the pathophysiology

of migraine. Chapter 7 summarizes the effects of 5-HT in the carotid bed of several species. Using 15 µm radioactive microspheres the amount of blood flowing through arteriovenous anastomoses and arterioles can be estimated in experimental animals. Intracarotid infusion of 5-HT in the common carotid artery of anaesthetized, vagosympathectomized pigs results in a decrease in arteriovenous anastomotic blood flow and an increase in arteriolar blood flow, especially to the skin and ears, which become bright pink. These responses cannot be antagonized by the 5-HT_2 receptor antagonist ketanserin or the 5-HT_3 receptor antagonist MDL 72222, but can be blocked by methiothepin, which blocks both 5-HT_1-like and 5-HT_2 receptors. Furthermore, the selective 5-HT_1-like agonist 5-carboxamidotryptamine mimicks the effects of 5-HT in the carotid bed. From this it can be concluded that the effects of 5-HT on both arteriovenous anastomotic blood flow and arteriolar blood flow are mediated by 5-HT_1-like receptors (Chapter 8). Therefore, the effects of other putative 5-HT_1-like receptor agonists were studied: 8-OH-DPAT & ipsapirone (Chapter 9) and RU 24969 (Chapter 10). Intracarotid infusion of 8-OH-DPAT and RU 24969 decrease carotid blood flow by a reduction in arteriovenous blood flow, whereas in contrast to 5-HT and 5-carboxamido-tryptamine no important changes in arteriolar blood flow are observed. Ipsapirone does not have any effect on carotid blood flow and its distribution. The effects of 8-OH-DPAT and RU 24969 can be blocked by methiothepin but not by ketanserin, indicating that these effects are mediated by 5-HT_1-like receptors. However, the effects of RU 24969 cannot be antagonized by the putative 5-HT_{1A} and 5-HT_{1B} receptor antagonist pindolol. Lack of selective antagonists for the other putative 5-HT_1 receptor subtypes makes it difficult to characterize the actions of 8-OH-DPAT and RU 24969 more precisely.

The antimigraine drug ergotamine is also known to reduce arteriovenous shunting in the cranial circulation of the dog and this drug has affinity for 5-HT_1 recognition sites in cerebral membranes. In order to study the possible involvement of 5-HT receptors, the effects of intravenous injection of ergotamine were studied in both cat and pig (Chapter 11).

Ergotamine induces a reduction in arteriovenous shunting in both species, but this effect cannot be antagonized by either the 5-HT_2 receptor antagonist ketanserin or the 5-HT_1-like and 5-HT_2 receptor antagonist methiothepin, suggesting that this effect is not mediated by 5-HT_1-like or 5-HT_2 receptors. Since phentolamine and pizotifen were ineffective antagonists in the dog carotid bed, a possible involvement of α-adrenoceptors or histamine H_1 receptors is also unlikely.

In conclusion this thesis shows that 1) the 5-HT-induced epicardial chemoreflex in cats is mediated by 5-HT_3 receptors; 2) the 5-HT-induced tachycardia in pigs is not mediated by 5-HT_1-like, 5-HT_2 or 5-HT_3 receptors; 3) the effects of 5-HT on carotid blood flow and its distribution in pigs are mediated by 5-HT_1-like receptors; 4) 8-OH-DPAT and RU 24969 are also acting on this type of 5-HT receptor; 5) Ergotamine reduces arteriovenous anastomotic blood flow, which is not mediated by 5-HT_1-like or 5-HT2 receptors.

SAMENVATTING

Het locale hormoon 5-hydroxytryptamine (5-HT; serotonine) speelt een rol in diverse (patho-) physiologische processen. Dit proefschrift beschrijft het effect van 5-HT op hartfrequentie en distributie van de Carotis-doorbloeding in genarkotiseerde katten en varkens. Door gebruik te maken van selectieve antagonisten en agonisten werden de subtypes van 5-HT receptoren gekarakteriseerd volgens, nu algemeen, geaccepteerde criteria (Hoofdstuk 2). Na een uitgebreid literatuur-overzicht betreffende hartfrequentie-veranderingen in zoogdieren (Hoofdstuk 3), wordt de door 5-HT opgewekte epicardiale chemoreflex beschreven (Hoofdstuk 4). Locale toediening van zowel 5-HT als nicotine op het epicard van genarkotiseerde katten wordt gevolgd door stimulatie van afferente zenuwvezels van de N.vagus, hetgeen leidt tot de epicardiale chemoreflex: bradycardie, afname van de sympathicus activiteit en hypotensie. Locale of intraveneuse toediening van de selectieve 5-HT_3 receptor antagonist MDL 72222 heft de door 5-HT opgewekte reflex op, terwijl de door nicotine opgewekte reflex onveranderd blijft, hetgeen een locaal anesthetische werking van MDL 72222 uitsluit. Bovendien wordt door voorbehandeling met MDL 72222 de aanwezigheid van een positief chronotroop effect van 5-HT duidelijk, hetgeen niet door propranolol of phentolamine, maar wel door phenoxybenzamine geantagoneerd kan worden.

De door 5-HT opgewekte tachycardie werd tevens onderzocht in genarkotiseerde varkens, waarbij een bilaterale cervicale vagotomie was uitgevoerd (Hoofdstuk 5). 5-HT veroorzaakt een dosis-afhankelijke toename in de hartfrequentie, hetgeen echter niet geblokkeerd kan worden door welke van de tot nu toe bekende 5-HT receptor antagonisten dan ook. Deze tachycardie wordt niet geantagoneerd door antagonisten voor α- of β-adrenerge, muscarine, nicotine, histamine of dopamine receptoren en ook niet door de calcium antagonist verapamil. Remmers van de opname van 5-HT veroorzaken een toename in de hartfrequentie en versterken de door 5-HT opgewekte tachycardie. Mogelijk selectieve agonisten voor

5-HT_1-achtige en 5-HT_2 receptoren veroorzaken bovendien geen belangrijke veranderingen in hartfrequentie. Uit bovengenoemde resultaten kan men concluderen dat noch 5-HT_1-achtige, noch 5-HT_2 of 5-HT_3 receptoren betrokken zijn bij door 5-HT opgewekte tachycardie in het varken. De aanwezigheid van een nieuw type 5-HT receptor in het varkenshart moet daarom overwogen worden.
In hoofstuk 6 wordt de rol van arterioveneuse anastomosen in de bloedsomloop besproken, in het bijzonder hun rol in de pathophysiologie van migraine. Het effect van 5-HT op het Carotis vaatbed in diverse species is samengevat in hoofdstuk 7. Met behulp van radioactieve microsferen met een diameter van 15 µm kan de doorbloeding van zowel arterioveneuse anastomosen als arteriolen bepaald worden. Infusie van 5-HT direct in de A.carotis communis van genarkotiseerde varkens, waarbij een vagosympathectomie is uitgevoerd, leidt tot een afname in arterioveneuse doorbloeding en een toename in de arteriolaire doorbloeding, vooral in de huid en de oren, die helder rood worden. Deze effecten kunnen niet geantagoneerd worden door de 5-HT_2 receptor antagonist ketanserin of door de 5-HT_3 receptor antagonist MDL 72222, maar wel door methiothepine, dat zowel 5-HT_1-achtige als 5-HT_2 receptoren blokkeert. Bovendien leidt infusie van de selectieve 5-HT_1-achtige receptor agonist 5-carboxamidotryptamine tot dezelfde effecten als 5-HT infusie. Uit het bovenstaande kan geconcludeerd worden dat zowel de afname van arterioveneuse anastomose doorbloeding als de toename in arteriolaire doorbloeding geinduceerd worden door stimulatie van 5-HT_1-achtige receptoren (Hoofdstuk 8). Met deze kennis werd besloten de effecten van andere agonisten voor mogelijk 5-HT_1-achtige receptoren te onderzoeken: 8-OH-DPAT & ipsapirone (Hoofdstuk 9) en RU 24969 (Hoofdstuk 10). Infusie van 8-OH-DPAT en RU 24969 vermindert de Carotis doorbloeding door een daling in de doorbloeding van de arterioveneuse anastomosen, terwijl, in tegenstelling tot 5-HT en 5-carboxamidotryptamine, de arteriolaire doorbloeding nauwelijks verandert. Ipsapirone infusie heeft geen enkel effect op de Carotis doorbloeding en de distributie over arterioveneuse anastomosen en arteriolen. De effecten

van 8-OH-DPAT en RU 24969 kunnen geblokkeerd worden door methiothepine, maar niet door ketanserine, hetgeen erop duidt dat deze effecten geinduceerd worden door stimulatie van 5-HT_1-achtige receptoren. Echter, de effecten van RU 24969 kunnen ook niet geblokkeerd worden door pindolol, een mogelijke antagonist van 5-HT_{1A} en 5-HT_{1B} receptoren. Het ontbreken van selectieve antagonisten voor andere mogelijke subtypes van 5-HT_1-achtige receptoren maakt het tot op heden moeilijk de effecten van 8-OH-DPAT en RU 24969 nader te karakteriseren.

Het antimigraine geneesmiddel ergotamine heeft ook de eigenschap de arterioveneuse doorbloeding in de craniele circulatie te verminderen, zoals aangetoond is bij de hond, en bezit tevens affiniteit voor 5-HT_1 bindingsplaatsen in cerebrale membranen. Daarom werd de mogelijke betrokkenheid van 5-HT receptoren onderzocht in zowel de kat als het varken (Hoofdstuk 11). In beide species vermindert ergotamine de doorbloeding van de arterioveneuse anastomosen, maar dit effect kan niet geantagoneerd worden door de 5-HT_2 receptor antagonist ketanserine of de 5-HT_1-achtige en 5-HT_2 receptor antagonist methiothepine, hetgeen er op duidt dat noch 5-HT_1-achtige, noch 5-HT_2 receptoren bij dit effect betrokken zijn. Aangezien phentolamine en pizotifen onwerkzaam waren in de hond, is een mogelijke betrokkenheid van α-adrenoceptors of histamine H_1 receptoren ook onwaarschijnlijk.

Samengevat, dit proefschrift toont aan dat 1) de door 5-HT opgewekte epicardiale chemoreflex in de kat geinduceerd wordt door stimulatie van 5-HT_3 receptoren; 2) de door 5-HT geinduceerde tachycardie in het varken niet via stimulatie van 5-HT_1-achtige, 5-HT_2 of 5-HT_3 receptoren verloopt; 3) 5-HT_1-achtige receptoren betrokken zijn bij de door 5-HT geinduceerde veranderingen in Carotis doorbloeding en de distributie over arterioveneuse anastomosen en arteriolen; 4) 8-OH-DPAT en RU 24969 eveneens hun effect uitoefenen via dit 5-HT receptor subtype; 6) ergotamine de doorbloeding van de arterioveneuse anastomoses vermindert, een effect waarbij echter noch 5-HT_1-achtige, noch 5-HT_2 receptoren betrokken zijn.

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The author of this thesis was born in The Hague, The Netherlands, in 1954. After secondary school, he studied medicine from 1973 to 1981 at the Erasmus University of Rotterdam. During this study he was involved in research projects at the Departments of Experimental Echocardiography, Clinical Echocardiography, Experimental Cardiology and the Laboratory for Biomechanics of the Thorax Centre. After obtaining the medical degree he served as an officer in the Royal Dutch Army Medical Corps. From 1983 he worked as research scientist at the Abteilung für Herz- und Kreislaufphysiologie der Medizinischen Einrichtungen der Universität Düsseldorf, Federal Republic of Germany. In 1985 the preparation of this thesis was started at the Institute for Pharmacology at the Erasmus University Rotterdam in cooperation with the Department of Experimental Cardiology.

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