5-Hydroxytryptamine Receptors Mediating Carotid and Systemic Haemodynamic Effects: The Relation to Acute Antimigraine Therapy

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5-Hydroxytryptamine Receptors Mediating Carotid and Systemic Haemodynamic Effects: The Relation to Acute Antimigraine Therapy

5-Hydroxytryptamine receptoren betrokken bij carotid en systemische haemodynamische effecten: de relatie tot acute antimigraine therapie

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Voor mijn ouders en grootouders
A la memoria de Diana Díaz Díaz

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Chapter 1

5-HT receptor classification

1.1 Events leading to the Bradley *et al.* (1986) 5-HT receptor classification

The presence of a vasoconstrictor substance in blood was suspected for 130 years (Ludwig & Schmidt, 1868) and, 50 years ago, Page and associates at the Cleveland Clinic (Cleveland, Ohio, USA) succeeded in isolating 'serotonin' from the blood (Rapport *et al*., 1948). Within the next 3 years, the chemical structure of serotonin was deduced (Rapport, 1949) and 5-HT (5-hydroxytryptamine; for other abbreviations see Section 16.4) synthesised (Hamlin & Fischer, 1951; Speeter *et al*., 1951). Independently, during the 1930s and 40s, Erspamer and colleagues (Rome, Italy), who were interested in characterising the substance imparting characteristic histochemical properties to the enterochromaffin cells of the gastrointestinal mucosa, extracted a basic gut-stimulating factor and named it "enteramine" (Erspamer, 1954). The chemical identity of enteramine with the natural and synthetic serotonin (Erspamer & Asero, 1952) was soon backed by the similarity of pharmacological profile (contraction of sheep carotid artery, guinea-pig, mouse and rabbit jejunum, rat and cat uterus and cat nictitating membrane, triphasic blood pressure response and antagonism by yohimbine and potentiation by cocaine of the sheep carotid artery contraction) (Erspamer, 1954; Page, 1954). Thus, the scene was set for the characterisation of 5-HT receptors.

In 1957, using the guinea-pig isolated ileum, Gaddum and Picarelli (1957) suggested that the 5-HT-induced contraction was mediated by two different receptors: a neurotropic "M" receptor located on parasympathetic ganglia (effect blocked by morphine and atropine) and a musculotropic "D" receptor located on smooth muscles (effect blocked by dibenzyline, lysergide, 2-bromolysergide and dihydroergotamine). This classification served well for three decades, although, from time to time, it was reported that some 5-HT-induced effects, for example, the vasoconstriction in the canine carotid arterial bed, were not mediated by "M" or "D", but by "special" receptors (Saxena *et al*., 1971; Saxena, 1972), at which methysergide appeared to behave as a partial agonist (Saxena, 1974b).

In 1974, Bennett and Aghajanian (1974) reported the first successful radioligand binding study of 5-HT receptors using [^{3}H]lysergide. Further studies with [^{3}H]5-HT, [^{3}H]spiperone and [^{3}H]lysergide enabled Peroutka and Snyder (1979) to

identify two "receptors", named 5-HT_1 (nM affinity for 5-HT) and 5-HT_2 (nM affinity for spiperone, but µM affinity for 5-HT); lysergide had high (nM) affinities for both. Subsequently, 5-HT_1 "receptors" (recognition sites) were subdivided into 5-HT_{1A} and 5-HT_{1B} subtypes on the basis of spiperone exhibiting a high and low affinity, respectively (Pedigo *et al*., 1981), and 8-OH-DPAT was designated as a selective 5-HT_{1A} ligand (Gozlan *et al*., 1983; Middlemiss & Fozard, 1983). However, the most important tool for probing 5-HT_1 "receptors" proved to be 5-CT, identified by Feniuk *et al.* (1981). It was reported that 5-CT, which potently contracted the dog saphenous vein (Feniuk *et al*., 1981) and inhibited noradrenaline and 5-HT release from sympathetic and central serotonergic neurons, respectively (Feniuk *et al*., 1981; Engel *et al*., 1983), displayed a nM affinity for the 5-HT_1 recognition sites (Engel *et al*., 1983). Furthermore, it was shown that several 5-CT-induced responses were blocked by methiothepin and methysergide, but not by ketanserin, including relaxation of smooth muscle (Feniuk *et al*., 1984), contraction of dog saphenous vein (Feniuk *et al*., 1985), long-lasting hypotension in the rat (Saxena & Lawang, 1985; Martin *et al*., 1987), dilatation of arterioles and contraction of arteriovenous anastomoses in the porcine carotid bed (Saxena & Verdouw, 1985a) and tachycardia in the cat (Saxena *et al*., 1985b). These responses, thus, became associated with 5-HT_1 recognition sites.

Since the 5-HT receptors were being referred to by various names ("D", "M", 5-HT_1, 5-HT_2, S_1, S_2 and others), the need for a uniform terminology was advocated (Humphrey, 1983; Verdouw *et al*., 1984b). Indeed, after several protracted but stimulating deliberations that were aided by the synthesis of several new 5-HT-selective agents, including neuronal "M" 5-HT receptor antagonists, MDL72222 (Fozard, 1984a) and ICS205930 (tropisetron) (Richardson *et al*., 1985), it was agreed to merge "M" and "D" (Gaddum & Picarelli, 1957) and 5-HT_1 and 5-HT_2 (Peroutka & Snyder, 1979) classifications. This effort culminated in the Bradley *et al.* (1986) publication, classifying 5-HT receptors as follows: "5-HT_1-like" (equivalent to some "D" or 5-HT_1), 5-HT_2 (equivalent to most "D" or 5-HT_2) and 5-HT_3 (equivalent to "M") receptors. The authors clearly pointed out that this classification was meant to be a "general framework", which will have to be regularly updated, as new knowledge emerges.

1.2 NC-IUPHAR Classification (1994)

In 1994, the NC-IUPHAR (Serotonin Receptor Nomenclature Committee of the International Union of Pharmacology) reclassified 5-HT receptors into 5-HT_1 ("5-HT_1-like", 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-ht_{1E} and 5-ht_{1F}), 5-HT_2 (5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C}), 5-HT_3, 5-HT_4, recombinant (5-$ht_{5A/5B}$, 5-ht_6, 5-ht_7) and "orphan" receptors (Hoyer *et al.*, 1994). This new classification was, besides the operational characteristics (selective agonists and antagonists and ligand binding affinities), also based on structural (molecular structure) and transductional (intracellular transduction mechanisms) data as additional criteria. To distinguish recombinant receptors from native, functional receptors in whole tissues, lower case letters are used to identify recombinant receptors (Hoyer *et al.*, 1994). This new classification has been updated several times, when new information became available (Hartig *et al.*, 1996; Hoyer & Martin, 1997; Saxena *et al.*, 1998b). The 5-HT receptor classification described below is based on these criteria for classification (see Table 1.1).

1.3 5-HT_1 receptors

According to the Hoyer *et al.* (1994) classification scheme, all 5-HT_1 receptors are by definition negatively coupled to adenylyl cyclase and should be amenable to the agonist action of sumatriptan. This signal transduction system is usually associated with contraction of smooth muscle (Rand *et al.*, 1987) and decrease in neurotransmitter release (Langer, 1980). At the time of the Hoyer *et al.* (1994) classification, 6 subtypes were described, namely 5-HT_1-like, 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-ht_{1E} and 5-ht_{1F}. The 5-HT_{1C} receptor was renamed 5-HT_{2C}, based on operational transductional and structural criteria (Figure 1.1; Hoyer *et al.*, 1994). In view of the rather low affinity displayed by 5-CT at 5-ht_{1E} and 5-ht_{1F} receptors, the characteristic of 5-CT being more potent than 5-HT seems no longer appropriate for all 5-HT_1 receptors. The 5-HT_1 receptor subtypes, with the exception of the 5-HT_1-like receptor, have all been cloned.

Table 1.1 Classification of 5-HT receptors. For details and references, see text.

	5-HT$_{1A}$	**5-HT$_{1B}$**	**rodent 5-HT$_{1B}$**	**5-HT$_{1D}$**
Previous names	-	5-HT$_1$-like 5-HT$_{1X}$ 5-HT$_{1D\beta}$	5-HT$_{1B}$	5-HT$_1$-like 5-HT$_{1X}$ 5-HT$_{1D\alpha}$
Selective agonists	8-OH-DPAT Flesinoxan	Sumatriptan	CP93129	Sumatriptan PNU109291
Selective antagonists	WAY100635	GR127935 SB224289	GR127935 cyanopindolol	GR127935 BRL15572
Transductional properties	Inhibition adenylyl cyclase	Inhibition adenylyl cyclase	Inhibition adenylyl cyclase	Inhibition adenylyl cyclase
Some Responses	Behavioural changes, Central hypotension	Vasoconstriction Trigeminal inhibition	Vasoconstriction Auto/heterore-ceptor	Inhibition noradrenaline release in human atrium
	5-HT$_{2B}$	**5-HT$_{2C}$**	**5-HT$_3$**	**5-HT$_4$**
Previous names	5-HT$_{2F}$	5-HT$_{1C}$	M	-
Selective agonists	BW723C86	RO600175	SR57227 *m*-chlorophe-nylbiguanide	Cisapride BIMU8
Selective antagonists	SB204741	SB242084 RS102221	MDL72222 Ondansetron	GR113808 SB204070
Transductional properties	Induction inositol phosphates	Induction inositol phosphates	Cation channel opening	Stimulation adenylyl cyclase
Some Responses	Constriction rat stomach fundus Endothelium-dependent vasorelaxation	Regulation CSF composition Rat penile erection	Neuronal depolarisation (e.g. von Bezold-Jarisch reflex)	Porcine and human tachycardia Gastrokinetic action

Table 1.1 Continued

	5-ht$_{1E}$	**5-HT$_{1F}$**	**5-HT$_{2A}$**	
Previous names	-	-	D 5-HT$_2$	
Selective agonists	-	LY344864 LY344370	-	
Selective antagonists	-	-	Ketanserin MDL100907	
Transductional properties	Inhibition adenylyl cyclase	Inhibition adenylyl cyclase	Induction inositol phosphates	
Some Responses	-	Trigeminal inhibition	Vasoconstriction Platelet aggregation	
	5-ht$_{5A}$	**5-ht$_{5B}$**	**5-ht$_6$**	**5-HT$_7$**
Previous names	-	-	-	5-HT$_1$-like 5-HT$_{1Y}$ "orphan"
Selective agonists	-	-	-	-
Selective antagonists	-	-	RO046790 RO630563	SB258719
Transductional properties	Inhibition adenylyl cyclase??	-	Stimulation adenylyl cyclase	Stimulation adenylyl cyclase
Some Responses	-	-	-	Vasodilatation Feline tachycardia

1.3.1 5-HT$_1$-like receptor

As described above, according to the Bradley *et al.* (1986) classification 5-HT receptors were classified into three main types, namely "5-HT$_1$-like", 5-HT$_2$ and 5-HT$_3$. At that time, the term "5-HT$_1$-like" receptors was meant to be associated with all 5-HT$_1$ recognition sites and encompassed a broad spectrum of receptors mediating several responses. The operational criteria for this 5-HT$_1$-like receptor were (i) more potent stimulation by 5-CT than 5-HT; (ii) blockade by methiothepin and methysergide; and (iii) resistance to blockade by selective antagonists at 5-HT$_2$ and 5-HT$_3$ receptors.

It was however already recognised by Bradley *et al.* (1986) that the 5-HT$_1$-like receptors were a heterogeneous group of receptors. Indeed, with the availability of selective ligands, several subclasses were identified, namely 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1C}$ and 5-HT$_{1D}$ (see Figure 1.1). Thus, the appellation "5-HT$_1$-like" effectively became restricted to the remaining functional receptors (mediating contraction or relaxation of vascular and non-vascular smooth muscle, sympathetic neuroinhibition and feline tachycardia) and which met operational criteria for admission into this class, but which could not be equated to the aforementioned 5-HT$_1$ subtypes (Leff *et al*., 1987; Martin *et al*., 1987; Saxena & Ferrari, 1989; Feniuk & Humphrey, 1990; Saxena & Villalón, 1990). With continuing evaluation, it became apparent that these receptors were still heterogeneous, since 5-CT was consistently 10-100 times less potent on "5-HT$_1$-like" receptors mediating vascular contraction and sympathetic neuroinhibition than on "5-HT$_1$-like" receptors mediating vascular relaxation and tachycardia in the cat (Feniuk & Humphrey, 1990). More definitive pharmacological distinction was made with the introduction of sumatriptan, which was at that time regarded as a selective "5-HT$_1$-like" receptor agonist (Humphrey *et al*., 1988; 1990). It turned out that sumatriptan only stimulated those "5-HT$_1$-like" receptors mediating vascular contraction and sympathoinhibition, but not those mediating vasodilatation. The two types of "5-HT$_1$-like" receptors were therefore arbitrarily coined 5-HT$_{1X}$ to denote the "proconstrictor" receptor and 5-HT$_{1Y}$ to denote the "prodilator" receptor (see Figure 1.1; Saxena & Ferrari, 1989; Saxena & Villalón, 1990). This operational differentiation was later reinforced by the demonstration that the so-called 5-HT$_{1X}$ receptor was negatively coupled to adenylyl cyclase (Sumner & Humphrey, 1990), while the so-called 5-HT$_{1Y}$ receptor was positively coupled (Sumner *et al*., 1989, see Figure 1.1). In 1994, the Serotonin

Receptor Nomenclature Committee of the IUPHAR decided that the term "5-HT_1-like" should be restricted to the sumatriptan-sensitive (5-HT_{1X}) receptor, while the sumatriptan-insensitive (5-HT_{1Y}) receptor was transferred to the "orphan" category (see Figure 1.1; Hoyer *et al*., 1994).

It is noteworthy that the high affinity of sumatriptan for 5-HT_{1D} recognition sites suggested that the sumatriptan-induced vasoconstriction was mediated by 5-HT_1-like receptors resembling the 5-HT_{1D} subtype (Martin, 1994). The NC-IUPHAR classification scheme also recognised the above similarity, yet this 5-HT_1-like receptor remained a distinct entity (Hoyer *et al*., 1994). The main reason for this distinction was that metergoline, regarded as a potent 5-HT_{1D} receptor ligand, was less active as an antagonist *in vitro* (e.g. canine, human and rabbit saphenous vein, human pial artery and rabbit renal and cerebral arteries; Deckert *et al*., 1994; Hoyer *et al*., 1994) and *in vivo* (porcine cranial arteriovenous anastomoses and canine carotid vascular bed; Den Boer *et al*., 1992b; Villalón *et al*., 1995c) than methiothepin or could be expected by metergoline's affinity at the then defined 5-HT_{1D} recognition sites. However, the experimental conditions used to detect 5-HT_{1D} recognition sites (Waeber *et al*., 1988) may have allowed the inclusion of, at the time unknown, 5-ht_{1E}, 5-ht_{1F} and 5-ht_7 recognition sites. Additionally, molecular biology studies demonstrated the existence of two different 5-HT_{1D} receptors, which were named 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ receptor (see Figure 1.1; Weinshank *et al*., 1992). Moreover, metergoline interacts with a wide variety of 5-HT receptors (see Table 10.2), displays intrinsic efficacy at recombinant 5-HT_{1D} receptors (Schoeffter *et al*., 1988; Walsh *et al*., 1995) and shows variable affinity for 5-HT_{1D} recognition sites in different species (Waeber *et al*., 1988). Thus, there was a clear need for subtype-selective 5-HT_1 receptor antagonists. Indeed, as will be described below several selective ligands were developed.

Apart from the implications discussed below and in the rest of this thesis, it turned out that the group of "5-HT_1-like receptors", as proposed originally by Bradley *et al.* (1986), consists of at least three structurally distinct receptors, the 5-HT_{1B}, 5-HT_{1D} and 5-HT_7 receptors. Consequently, the term "5-HT_1-like receptor" has now become redundant (see Figure 1.1).

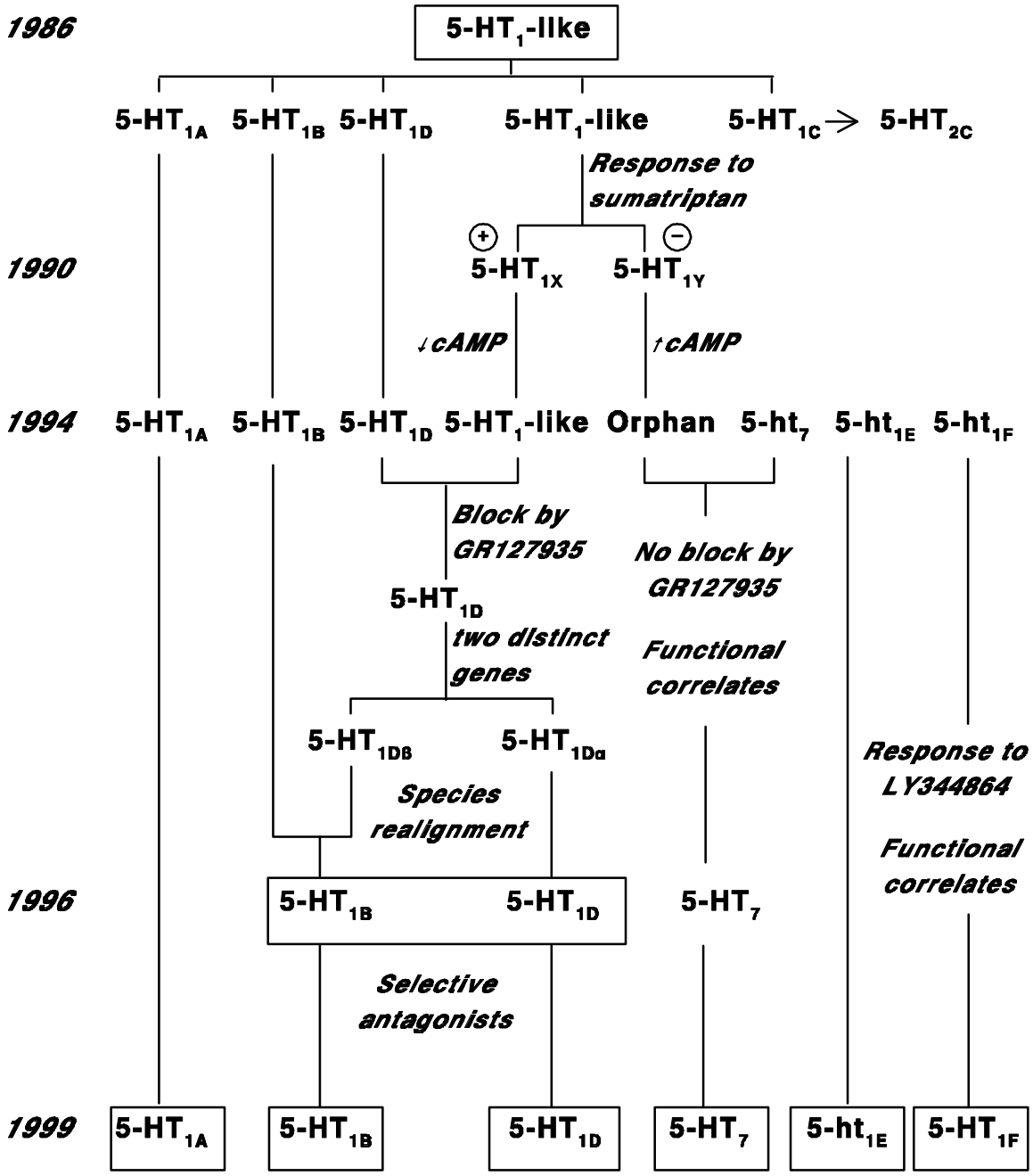

Figure 1.1 The evolution of "5-HT_1-like" receptors into the different sumatriptan-sensitive 5-HT_1 receptor subtypes and the sumatriptan-insensitive 5-HT_7 receptor. For references and further explanations, see text.

Table 1.2 Pharmacological tools to discriminate the different 5-HT_1 receptor subtypes.

	5-HT_{1A}	**5-HT_{1B}**	**Rat 5-HT_{1B}**	**5-HT_{1D}**	**5-ht_{1E}**	**5-HT_{1F}**
Agonists:						
5-HT	9.2[a]	8.5[b]	8.6[b]	8.4[c]	8.0[d]	8.0[d]
8-OH-DPAT	8.7 (pig)[e]	6.6[b]	5.1[b]	7.4[f]	5.5[d]	5.8[d]
5-CT	10.3[a]	8.8[b]	8.9[b]	9.2[c]	5.1[d]	6.1[d]
Sumatriptan	6.4[g]	7.8[g]	7.3[b]	8.5[g]	5.8[g]	7.9[g]
L775606	7.3[h]	7.1[h]	6.1[b]	9.2[h]	<5.0[h]	5.4[h]
PNU109291	6.0[i]	5.2[i]	-	9.0[i]	-	-
LY344864	6.3[j]	6.3[j]	-	6.2[j]	5.8[j]	8.2[j]
LY334370	7.8[k]	6.9[l]	-	6.9[l]	-	8.8[l]
CP93129	5.5 (rat)[m]	6.4[b]	8.1[b]	5.7 (rat)[m]	-	-
CP122288	6.5[n]	8.3[b]	6.8[b]	8.2[n]	6.4[n]	8.5[n]
Antagonists:						
GR127935	7.2[o]	9.0[o]	8.8[b]	8.6[o]	5.4[o]	6.4[o]
SB224289	5.5[p]	8.0[p]	-	6.2[p]	<5.0[p]	<5.0[p]
BRL15572	7.7[o]	6.1[o]	-	7.9[o]	5.2[o]	6.0[o]
Ketanserin	5.5[q]	5.3[r]	<5.1[b]	7.2[r]	<5.0[d]	<5.0[d]
Methiothepin	7.7[q]	7.6[b]	7.2[b]	7.7[f]	6.7[d]	6.2[d]
Metergoline	8.1 (pig)[e]	8.6[b]	8.3[b]	8.7[f]	-	6.5[d]
Cyanopindolol	8.3 (pig)[e]	7.0[b]	9.0[b]	-	-	-
(-)-Propranolol	6.8 (pig)[e]	5.6[b]	7.7[b]	-	-	-
(-)-Pindolol	7.7 (pig)[e]	<5.1[b]	7.6[b]	-	<5.0[d]	<5.0[d]

All data are given as pK_i values at human receptors, except when stated otherwise. Data from: [a], Newman-Tancredi *et al.* (1997); [b], Beer *et al.* (1998); [c], Weinshank *et al.* (1992); [d], Adham *et al.* (1993); [e], Hoyer, (1989); [f], Pauwels *et al.* (1996); [g], Leysen, *et al.* (1996); [h], MacLeod *et al.* (1997), values given as pIC_{50}; [i], Ennis *et al.* (1998); [j], Phebus, *et al.* (1997); [k], Dupuis *et al.* (1998); [l], Johnson *et al.* (1997); [m], Macor *et al.* (1990); [n], Gupta, P. (personal communication); [o], Price *et al.* (1997); [p], Hagan *et al.* (1997); [q], Pauwels, P.J. (personal communication); [r], Zgombick *et al.* (1995).

1.3.2 5-HT_{1A} receptor

The 5-HT_{1A} receptor site, first described by Pedigo *et al.* (1981), has been cloned in several species, including human (Fargin *et al.*, 1988) and rat (Albert *et al.*, 1990). High densities of the 5-HT_{1A} receptor are found in the brain, mainly in areas involved in the modulation of emotion (see Hoyer *et al.*, 1994). In keeping with this, activation

of 5-HT_{1A} receptors induces a wide variety of behavioural changes (see Hoyer *et al*., 1994). The 5-HT_{1A} receptor is an autoreceptor, but also seems to be involved in the central inhibition of noradrenaline release, responsible for the centrally evoked hypotension by 5-HT_{1A} receptor agonists (Doods *et al*., 1988; Dreteler *et al*., 1990). 5-HT_{1A} receptors have not been detected in blood vessels (Ullmer *et al*., 1995). Several selective 5-HT_{1A} receptor agonists are available, such as 8-OH-DPAT, flesinoxan, 5-methylurapidil, buspirone, although it should be noted that 8-OH-DPAT also shows moderate affinity for 5-$HT_{1B/1D}$ receptors (see Table 1.2). WAY100635 is a potent and selective 5-HT_{1A} receptor antagonist (Fletcher *et al*., 1996; Hoyer & Martin, 1997).

1.3.3 5-HT_{1B} receptor

Until recently, the 5-HT_{1B} receptor referred to the binding site found only in rodent brain (Pazos & Palacios, 1985; Hoyer *et al*., 1994). This rodent 5-HT_{1B} receptor was shown to function as auto- and heteroreceptor, mediate vasoconstriction of the rat caudal artery (Craig & Martin, 1993) and inhibit the release of noradrenaline from sympathetic nerve terminals in the rat vena cava (Göthert *et al*., 1986). In the mean time, Weinshank *et al.* (1992) identified two structurally distinct genes encoding human 5-HT_1 receptors with pharmacological profiles most closely resembling the 5-HT_{1D} receptor. Since the operational profiles of these two new receptors were mostly indistinguishable, they were called 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$. It soon became evident that, in spite of fundamental differences in their pharmacology (see below), the 5-$HT_{1D\beta}$ receptor was a human homologue of the rodent 5-HT_{1B} receptor (Hoyer *et al*., 1994). This prompted an important realignment of 5-HT receptor nomenclature that now recognises primacy of human genome (Hartig *et al*., 1996). Consequently, the 5-$HT_{1D\beta}$ receptor was renamed 5-HT_{1B} (subsuming the rodent 5-HT_{1B} receptor), while the 5-$HT_{1D\alpha}$ nomenclature was abandoned in recognition of the fact that this gene product encodes the 5-HT_{1D} receptor (see Figure 1.1; Hartig *et al*., 1996). This new nomenclature for 5-HT_{1B} and 5-HT_{1D} receptors will be used from this point onwards.

As described in section 1.3.1, the 5-HT_1-like receptor mediating smooth muscle contraction and inhibition of noradrenaline release showed close similarities to the 5-HT_{1B} and/or 5-HT_{1D} receptors. However, the lack of selective antagonists at the 5-$HT_{1B/1D}$ receptor hampered further research. Clitherow *et al.* (1994) reported the

properties of several compounds including a piperazinylbenzanilide derivative, GR127935, which showed a high affinity for and selective antagonist activity at 5-$HT_{1B/1D}$ receptors (see Table 1.2). Subsequently, Skingle *et al.* (1996) showed that GR127935 potently blocked several responses elicited by sumatriptan-sensitive 5-HT_1-like receptors, including: (i) contractile effects in the dog saphenous vein and basilar artery; (ii) prejunctional sympathoinhibition in the dog saphenous vein; (iii) (auto)inhibition of 5-HT release from slices of guinea-pig hippocampus and dorsal raphé nucleus and (iv) hypothermic and rotational responses in the guinea-pig. Further studies demonstrated that GR127935 potently blocked sumatriptan-induced contractions in several blood vessels, both *in vitro* (see Pauwels, 1996) and *in vivo* (Villalón *et al.*, 1996). These findings, coupled to the lack of blocking properties of GR127935 at other 5-HT receptors, including the "orphan" receptor mediating smooth muscle relaxation, 5-HT_3 and 5-HT_4 receptors (except 5-HT_{2A}) in several preparations (see Chapter 3), clearly reinforced the view that sumatriptan-sensitive 5-HT_1-like receptors are similar to 5-$HT_{1B/1D}$ receptors subtype (Figure 1.1). Indeed, as will discussed in this thesis, the 5-HT_1-like receptor mediating vasoconstriction of the carotid vasculature in rabbits (Chapter 3) and pigs (Chapters 4 and 6), as well as the inhibition of sympathetic vasopressor outflow in the rat (Chapter 13) were also shown to be GR127935-sensitive. Unfortunately, GR127935 was not capable of distinguishing between the 5-HT_{1B} and 5-HT_{1D} receptor (see Table 1.2). However, *m*RNA for the 5-HT_{1B} receptor was shown to be much more abundantly expressed on vascular smooth muscle cells, compared to 5-HT_{1D} receptor *m*RNA (Branchek *et al.*, 1995; Bouchelet *et al.*, 1996; Sgard *et al.*, 1996). The latter was reinforced by the demonstration of 5-HT_{1B}, but not 5-HT_{1D} receptor immunoreactivity in cranial blood vessels (Longmore *et al.*, 1997). As described in Chapters 8 and 9, as well as by Verheggen *et al.* (1998) in the isolated human temporal artery, using the recently developed, potent and selective antagonists at either the 5-HT_{1B} (SB224289; Hagan *et al.*, 1997; Gaster *et al.*, 1998) or 5-HT_{1D} (BRL15572; Price *et al.*, 1997) receptors (Table 1.2), there is now overwhelming evidence that the 5-HT_{1B}, but not the 5-HT_{1D} receptor mediates the sumatriptan-induced contraction of vascular smooth muscle.

It is important to note that, despite the 96% amino acid sequence homology in the transmembrane regions (Adham *et al.*, 1992), the rodent 5-HT_{1B} receptor displays a distinct pharmacology compared to the 5-HT_{1B} receptor in other species

(Hartig *et al*., 1996). Thus, CP93129 is a selective agonist, whereas some β-adrenoceptor antagonists, such as cyanopindolol, (-)-pindolol and (-)-propranolol, are selective antagonists at the rodent 5-HT_{1B} receptor, but not in other species (see Table 1.2).

1.3.4 5-HT_{1D} receptor

As shown in Table 1.2, the 5-HT_{1D} receptor (previously called 5-$HT_{1D\alpha}$, see above) is potently antagonised by GR127935 (Clitherow *et al*., 1994; Skingle *et al*., 1996) and by the selective 5-HT_{1D} receptor antagonist, BRL15572 (Price *et al*., 1997). Additionally, some 5-HT_2 receptor antagonists (ketanserin and ritanserin) can discriminate this receptor from 5-HT_{1B} and 5-HT_{1F} receptors (Hoyer *et al*., 1994), although this is highly species dependent. Indeed, whereas ketanserin shows a selectivity for the 5-HT_{1D} over the 5-HT_{1B} receptor in the rabbit (20-fold; Bard *et al*., 1996) and human (70-fold; Zgombick *et al*., 1995), this selectivity is absent in the dog (Branchek *et al*., 1995) and guinea-pig (Zgombick *et al*., 1997). In contrast to the rodent 5-HT_{1B} receptor (see above), the rodent 5-HT_{1D} receptor displays similar pharmacology compared to the 5-HT_{1D} receptor found in other species (Hartig *et al*., 1996; Saxena *et al*., 1998b). Sumatriptan and the second-generation triptans (see Chapter 2) are potent agonists at this receptor, but also interact with 5-HT_{1B} and 5-HT_{1F} receptors. Some recently developed compounds, including PNU109291 (Ennis *et al*., 1998) and L775606 (MacLeod *et al*., 1997), have been reported to be selective 5-HT_{1D} receptor agonists (see Table 1.2). It has been demonstrated that the 5-HT_{1D} receptor is located preferentially on neuronal, rather than vascular tissue (Ullmer *et al*., 1995; Sgard *et al*., 1996; Longmore *et al*., 1997). Operationally, 5-HT_{1D} receptors mediate inhibition of noradrenaline release in human atrium (Schlicker *et al*., 1997). Additionally, the 5-HT_{1D} receptor seems to be involved in the inhibition of guinea-pig dural plasma protein extravasation (Waeber *et al*., 1997; Ennis *et al*., 1998) and the central trigeminal inhibitory effects by some antimigraine compounds (see Chapter 2; Mills *et al*., 1995; Goadsby & Knight, 1997; Cumberbatch *et al*., 1998).

1.3.5 5-ht_{1E} receptor

Little information is available on the 5-ht_{1E} receptor. The receptor was cloned by McAllister *et al.* (1992) and seems to be present in several brain regions, as

demonstrated by homogenate-binding (Lowther *et al*., 1992; Miller & Teitler, 1992) and receptor *m*RNA localisation (Bruinvels *et al*., 1994) studies. The 5-ht_{1E} receptor is not present in blood vessels (Ullmer *et al*., 1995). In contrast to the other 5-HT_1 subtypes, sumatriptan displays a very low affinity and, moreover, 5-CT shows little interaction with the 5-ht_{1E} receptor. No selective agonists or antagonists are available and there is no evidence for a physiological role for 5-ht_{1E} receptors (Hoyer *et al*., 1994; Hoyer & Martin, 1997).

1.3.6 5-HT_{1F} receptor

The 5-HT_{1F} receptor can be distinguished from the 5-HT_{1B} and 5-HT_{1D} receptors using the potent 5-HT_{1F} receptor agonists, LY344864 (Phebus *et al*., 1997) and LY334370 (Johnson *et al*., 1997). Additionally, most triptans stimulate 5-HT_{1F} receptors, although rizatriptan (Wainscott *et al*., 1998) and the non-indole compound alniditan (Leysen *et al*., 1996) display low affinities (see also Table 2.2); interestingly, as will be described in Chapter 2, the latter two drugs are at least as effective as sumatriptan in the treatment of migraine (Goldstein *et al*., 1996; Kramer *et al*., 1998). Presently, no selective 5-HT_{1F} receptor antagonists are available. 5-HT_{1F} receptor *m*RNA and the corresponding protein is preferentially expressed in the neuronal tissue rather than vascular smooth muscle (Ullmer *et al*., 1995; Bouchelet *et al*., 1996). Accordingly, 5-HT_{1F} receptor agonists are devoid of vasoconstrictor properties (see Chapter 7; Cohen *et al*., 1998; Bouchelet & Hamel, 1999). The 5-HT_{1F} receptor seems to mediate inhibition of dural plasma protein extravasation following trigeminal ganglion stimulation (Johnson *et al*., 1997; Phebus *et al*., 1997). More recently, it was demonstrated in rats that 5-HT_{1F} receptor stimulation by LY344864 decreased the number of capsaicin-induced C-*fos*-like immunoreactive cells within trigeminal nucleus caudalis (Mitsikostas *et al*., 1999). In view of the demonstration of these functional correlates, it seems that the upper case appellation is appropriate for the 5-HT_{1F} receptor (Figure 1.1).

1.4 5-HT_2 receptors

Three different 5-HT_2 receptor subtypes are known, namely 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C}, which have all been cloned (Hoyer *et al*., 1994). Transductionally, these three receptors are coupled to phospholipase C, which after activation leads to the

conversion of PIP_2 into IP_3 and DAG. Ultimately, this will result in the release of Ca^{2+} from the sarcoplasmatic reticulum and activation of protein kinase C.

1.4.1 5-HT$_{2A}$ receptor

The 5-HT_{2A} receptor mediates contractile responses in several, mainly peripheral, vascular and non-vascular smooth muscle preparations. Additionally, platelet aggregation, increased vascular permeability and several centrally mediated responses are mediated by this receptor (Baxter *et al*., 1994; Hoyer *et al*., 1994). No selective 5-HT_{2A} receptor agonists are available, but ketanserin, spiperone and MDL100907 (Sorensen *et al*., 1993) are quite potent and selective antagonists at the 5-HT_{2A} receptor (Baxter *et al*., 1995). DOI and α-methyl-5-HT are potent agonists, but cannot discriminate between the three 5-HT_2 receptor subtypes. Similarly, ritanserin, mianserin, metergoline, mesulergine and methiothepin are non-selective antagonists at this receptor (see Table 10.2).

Some antimigraine drugs display affinity at 5-HT_{2A} receptors (methysergide, pizotifen, ergotamine, dihydroergotamine; Hoyer *et al*., 1994), but many other such agents (ketanserin, cyproheptadine, mianserin, methiothepin) are not of much use in migraine therapy (Tfelt-Hansen & Saxena, 1993). Ketanserin is approved for the treatment of systemic hypertension. Indeed, it is claimed that this drug decreases blood pressure by blocking 5-HT_{2A} receptors mediating peripheral vasoconstriction. However, as discussed in detail elsewhere (Saxena & Villalón, 1990), it seems that this receptor is not involved to a significant degree in the clinical effects of the drug; ketanserin also exhibits a potent α_1-adrenoceptor antagonist activity, which can adequately explain its antihypertensive effect. In the complex setting of cardiac surgery and cardiopulmonary bypass, several potent mediators are released which, in turn, may produce systemic and/or pulmonary hypertension. One of the mediators may be 5-HT, released from aggregating platelets, causing vasoconstriction by activating 5-HT_{2A} receptors, particularly in patients with an impaired endothelial function e.g. atherosclerosis (Reneman & Van Der Starre, 1990). Ketanserin has also proven effective in the treatment of postoperative pulmonary hypertension (Van Der Starre & Reneman, 1994). It is however far from clear whether 5-HT_{2A} receptor blockade is responsible for the effectiveness of ketanserin, since no selective 5-HT_2 receptor antagonist has been proved to be effective. Perhaps less questionable seems to be the role of 5-HT_{2A} receptors in some cases of portal hypertension, where higher

levels of free plasma 5-HT are found in the portal venous circulation (Robertson, 1991); the above assumption is based on the fact that both ketanserin (which combines 5-HT_{2A} with α_1-adrenoceptor blockade) and ritanserin (at doses without α_1-adrenoceptor blockade) lower portal venous pressure (Lebrec, 1990). Ketanserin has been shown to be effective in the treatment of preeclampsia and eclampsia, pathologies that seem to comprise local release of 5-HT from aggregating platelets in the placental circulation (Weiner, 1990; Robertson, 1991). Although this finding does not establish the involvement of 5-HT_2 receptors, establishing the effectiveness of selective 5-HT_2 receptor antagonists may substantiate this view.

1.4.2 5-HT_{2B} receptor

With the exception of BW723C86 (Kennett *et al*., 1996a), no selective 5-HT_{2B} receptor agonists are available, although 5-MeO-T and α-methyl-5-HT can moderately discriminate this receptor from the other 5-HT_2 receptor subtypes (Baxter *et al*., 1995); however, both compounds can interact with several other 5-HT receptors (Hoyer *et al*., 1994). SB204741 (Forbes *et al*., 1995) is a selective antagonist at the 5-HT_{2B} receptor (Baxter, 1996) and yohimbine, although able to interact with several other receptors, also shows selectivity towards the 5-HT_{2B} receptor over the other two 5-HT_2 receptor subtypes (Ellis *et al*., 1995).

The 5-HT_{2B} receptor has been shown to mediate constriction of smooth muscle in the rat stomach fundus; this receptor was originally named 5-HT_{2F} (Kursar *et al*., 1992). Although at that time there was a lack of compounds discriminating this receptor from the 5-HT_{2C} receptor, the absence of 5-HT_{2C}, but presence of 5-HT_{2B} receptor *m*RNA in the rat stomach fundus (Foguet *et al*., 1992) resolved this issue. The 5-HT_{2B} receptor is also present on several blood vessels, located on the endothelium (Ullmer *et al*., 1995). Activation of this endothelial receptor has been suggested to result in the release of NO, which elicits a strong vasorelaxant activity through activation of soluble guanylate cyclase (Ullmer *et al*., 1995). Indeed, such 5-HT_{2B} receptor mediated endothelium-dependent vasorelaxant responses have been described in the porcine pulmonary artery (Glusa & Roos, 1996) and rat jugular vein (Ellis *et al*., 1995). The contraction of longitudinal smooth muscle in the human small intestine also exhibits a pharmacological profile resembling the 5-HT_{2B} receptor (Borman & Burleigh, 1995).

It has been proposed that 5-$HT_{2B/2C}$ receptors are involved in the initiation of migraine attacks (Fozard & Kalkman, 1994; Kalkman, 1994; Fozard, 1995). Thus, selective antagonism of these receptors, in particular the endothelial vasodilator 5-HT_{2B} receptor, would be effective in migraine prophylaxis (Fozard & Kalkman, 1994; Kalkman, 1994; Fozard, 1995; Schmuck *et al*., 1996). Indeed, the prophylactic effect by the marketed drugs, pizotifen and methysergide, is claimed to involve antagonism of the 5-HT_{2B} receptor (Fozard, 1995). Although the drugs are probably effective, formal evidence is lacking (Ferrari, 1998). Importantly, these compounds are not selective within the 5-HT_2 receptor family and methysergide is also able to interact with 5-HT_1 and 5-HT_7 receptors. Moreover, as already discussed elsewhere (Tfelt-Hansen & Saxena, 1993; Hamel & Saxena, 1999), several 5-$HT_{2B/2C}$ receptor antagonists, including mianserin and cyproheptadine, are not very effective antimigraine agents.

1.4.3 5-HT_{2C} receptor

RO600175 has been reported to be a selective 5-HT_{2C} receptor agonist (Jenck *et al*., 1998; Dekeyne *et al*., 1999); RS102221 (Bonhaus *et al*., 1997) and SB242084 (Bromidge *et al*., 1997; Kennett *et al*., 1997) are selective antagonists at this receptor. Additionally, several compounds, such as SB206553 (Kennett *et al*., 1996b) with antagonist properties at both 5-HT_{2B} and 5-HT_{2C} receptors are available. The 5-HT_{2C} receptor is present in the choroid plexus and has been suggested to regulate the composition and volume of the cerebrospinal fluid (Hoyer *et al*., 1994). Moreover, this receptor plays a role in anxiety disorders and several other psychiatric diseases (Hoyer *et al*., 1994; Baxter *et al*., 1995). Recently, the 5-HT_{2C} receptor was shown to mediate rat penile erections (Millan *et al*., 1997). In contrast to the 5-HT_{2B} receptor, the 5-HT_{2C} receptor is not expressed on blood vessels (Ullmer *et al*., 1995).

1.5 5-HT_3 receptor

The 5-HT_3 receptor is a ligand-gated ion channel, activation of which promotes entry of Na^+ and Ca^{2+} and hence neuronal depolarisation. The receptor is localised in the brain and in peripheral neuronal tissue (Hoyer *et al*., 1994). Activation of the 5-HT_3 receptor leads to bradycardia in the rat via depolarisation of cardiac afferent fibres of the vagal nerve (Von Bezold-Jarisch reflex; see Chapter 3) and in the isolated rabbit heart via the release of acetylcholine (Fozard, 1984b; Saxena & Villalón, 1990; 1991).

In rabbits and dogs, 5-HT_3 receptors mediate tachycardia (Saxena & Villalón, 1990; 1991). Moreover, 5-HT_3 receptors are involved in changes in intestinal tone and secretion (Briejer *et al*., 1995), dermal pain and flare response (Hoyer *et al*., 1994). Additionally, 5-HT_3 receptors mediate the 5-HT-induced initial contraction of the feline urinary bladder (see Chapter 3). 5-HT_3 receptor antagonists are used clinically to reduce chemotherapy-induced nausea and vomiting (Andrews *et al*., 1988; Andrews & Bhandari, 1993; Gregory & Ettinger, 1998).

Some selective 5-HT_3 receptor agonists have been described, such as SR57227 (Bachy *et al*., 1993) and *m*-chlorophenylbiguanide (Hoyer *et al*., 1994). Additionally, this receptor should not be amenable to the agonist action of 5-MeO-T (Hoyer *et al*., 1994). Several potent and selective antagonists are available, including MDL72222, granisetron, ondansetron (Hoyer *et al*., 1994); tropisetron also interacts with 5-HT_4 receptors.

Because no clear-cut positive antimigraine effect has been found with any of the 5-HT_3 receptor antagonists (Ferrari, 1991; Ferrari *et al*., 1991), this receptor does not appear to play a major role in migraine.

1.6 5-HT_4 receptor

The 5-HT_4 receptor, which was cloned by Gerald *et al.* (1995), is coupled positively to adenylyl cyclase. Several splice variants have been described, which, however, are until now pharmacologically indistinguishable (Gerald *et al*., 1995; Claeysen *et al*., 1998). The receptor is located in the brain (Eglen *et al*., 1995), but also in the gastrointestinal tract on neurones, smooth muscle and secretory cells (Hoyer *et al*., 1994). Indeed, several 5-HT_4 receptor (partial) agonists have been marketed as prokinetic drugs (Briejer *et al*., 1995). Moreover, the receptor has been found in the human and porcine cardiac atria, mediating tachycardia and positive inotropy *in vitro* (Kaumann, 1990; Sanders & Kaumann, 1992) as well as *in vivo* (Villalón *et al*., 1990b; 1991). Since 5-HT can induce arrhythmias in the human isolated atrium, it is proposed that 5-HT_4 receptor antagonists could be useful in the treatment of cardiac arrhythmias (Kaumann, 1994). However, the role of 5-HT, if any, in the pathogenesis of cardiac arrhythmias has not been established. On the other hand, the increase in atrial contractility by 5-HT_4 receptor agonists indicated that these drugs might have application in the therapy of heart failure. Any such hope was dashed when it was shown that 5-HT_4 receptors are not present on human ventricles (Jahnel *et al*., 1992;

Schoemaker *et al*., 1993). The 5-HT_4 receptor *m*RNA seems to be expressed on vascular endothelium (Ullmer *et al*., 1995), but no 5-HT_4 receptor-mediated endothelium-dependent vasorelaxations have yet been described. Interestingly, 5-HT_4 receptors have been suggested to mediate endothelium-independent relaxation of the isolated sheep pulmonary vein (Cocks & Arnold, 1992).

The 5-HT_4 receptor is amenable to the agonist action of several compounds (see Hoyer *et al*., 1994; Hoyer & Martin, 1997), including 5-MeO-T, substituted benzamides, such as cisapride and metoclopramide, and BIMU8. GR113808 and SB204070 are potent and selective antagonists at this receptor (Hoyer *et al*., 1994; Hoyer & Martin, 1997).

1.7 5-ht_5 receptors

Two 5-ht_5 receptors, 5-ht_{5A} and 5-ht_{5B}, have been cloned, which are pharmacologically very similar. The gene for the 5-ht_{5A} receptor is found in mouse (Plassat *et al*., 1992), rat (Erlander *et al*., 1993) and human (Rees *et al*., 1994) brain, whereas the 5-ht_{5B} receptor seems to be only present in mice and rats. 5-ht_5 receptors have not been detected in blood vessels (Ullmer *et al*., 1995). 5-ht_5 receptors are the only class of which little is known about the transductional properties. Some evidence suggests that the rat 5-ht_{5A} receptor is negatively coupled to adenylyl cyclase (Carson *et al*., 1996), but others have demonstrated that the (mouse) 5-ht_5 receptor may not inhibit adenylyl cyclase (Plassat *et al*., 1992). Pharmacologically, 5-ht_5 receptors display several similarities with the 5-HT_1 and 5-HT_7 receptors. Thus, 5-CT, ergotamine, methysergide and methiothepin show high affinities at 5-ht_5 receptors (see Tables 10.1 and 10.2). However, the structural characteristics, as well as the localisation of 5-ht_5 receptors are different from these 5-HT receptor classes (Hoyer *et al*., 1994). Moreover, sumatriptan displays low affinity at the 5-ht_5 receptors. No functional correlate has been found for the 5-ht_5 receptors and, hence, according to NC-IUPHAR nomenclature guidelines (Hoyer *et al*., 1994; Hoyer & Martin, 1997), are written in lowercase.

1.8 5-ht_6 receptor

As recently reviewed by Sleight *et al.* (1998a), the 5-ht_6 receptor has been cloned in human (Kohen *et al*., 1996) and rat (Ruat *et al*., 1993a) and is localised in brain tissue; 5-ht_6 receptor *m*RNA was not detected in blood vessels (Ullmer *et al*., 1995). The

5-ht_6 receptor is, like the 5-HT_4 and 5-HT_7 receptor, positively coupled to adenylyl cyclase (Ruat *et al*., 1993a; Schoeffter & Waeber, 1994). The 5-ht_6 receptor has been suggested to be involved in several psychiatric disorders, because of its localisation in specific brain areas and the high affinity of several antipsychotic drugs (e.g. clozapine) displayed at this receptor (Sleight *et al*., 1998a). However, because of the lack of selective ligands, at this moment no functional correlate has been found. Perhaps the recently developed, potent and selective antagonists, RO046790 and RO630563 (Sleight *et al*., 1998b), will aid the full characterisation of this receptor. The 5-ht_6 receptor can be discerned from 5-HT_1 receptors, as well as the 5-ht_5 and 5-HT_7 receptors by the low potency of 5-CT compared to 5-HT. The 5-ht_6 receptor is resistant to blockade by antagonists at 5-HT_2 (ketanserin), 5-HT_3 (MDL72222) and 5-HT_4 (tropisetron) receptors. Moreover, mesulergine, an antagonist at 5-HT_2 and 5-HT_7 receptors, is devoid of interaction with 5-ht_6 receptors (see Table 10.2).

1.9 5-HT_7 receptor

As outlined in section 1.3.1, the appellation "5-HT_1-like" was no longer regarded as appropriate for the sumatriptan-insensitive 5-HT receptors mediating smooth muscle relaxation and feline tachycardia. Consequently, this receptor was transferred to the list of "orphan" 5-HT receptors awaiting further characterisation (see Figure 1.1; Hoyer *et al*., 1994). With the demonstration of novel recombinant receptors positively linked to adenylyl cyclase (5-ht_6 and 5-ht_7), several investigators sought to determine if the above responses mediated by "orphan" 5-HT receptors represented functional correlates of these recombinant receptors. One of the main obstacles that was confronted in characterising these "orphan" receptors was that they also showed some operational characteristics of the 5-HT_1 receptor subtypes, namely, the rank order of agonist potency of some tryptamines (5-CT>5-HT≥5-MeO-T). Fortunately, apart from not being activated by sumatriptan, a remarkable characteristic of these receptors is that they turned out to be resistant to the antagonist action of the 5-$HT_{1B/1D}$ receptor antagonist, GR127935 (see Chapters 10-12; Terrón, 1996b; Villalón *et al*., 1997a). Significantly, this pharmacological profile paralleled that reported at the cloned 5-ht_7 receptor, but differed from that at cloned 5-ht_6 receptors (5-MeO-T>5-HT>5-CT). In agreement with these findings, methiothepin, methysergide, metergoline, mesulergine, lisuride and clozapine, which show either high affinity (methiothepin, methysergide, metergoline, lisuride and clozapine) or relative selectivity (mesulergine) for the cloned

5-ht_7 receptor, behaved as antagonists at the "orphan" receptors mediating relaxation of smooth muscle (see Chapter 10; Carter *et al*., 1995; Leung *et al*., 1996; Terrón, 1996b; Villalón *et al*., 1997a) and feline tachycardia (Chapter 12). Of particular importance is the fact that relatively low doses of mesulergine specifically antagonised these responses, since mesulergine: (i) displays an almost 300-fold selectivity for the cloned 5-ht_7 receptor over the cloned 5-ht_6 receptor; and (ii) does not interact with the 5-HT_1 receptor family. These lines of evidence coupled to the fact that *m*RNA for the putative 5-ht_7 receptors, but not for 5-ht_5 and 5-ht_6 receptors, has been detected in vascular smooth muscle (Ullmer *et al*., 1995; Schoeffter *et al*., 1996), indicate that the "orphan" 5-HT receptors mediating direct smooth muscle relaxation and tachycardia in the cat resemble recombinant 5-ht_7 receptors structurally, transductionally and operationally. Indeed, this has been shown in several preparations, such as guinea-pig ileum (Carter *et al*., 1995), rabbit femoral vein (Martin & Wilson, 1995), dog coronary artery (Terrón, 1996b), monkey jugular vein (Leung *et al*., 1996), dog basilar artery (Terrón & Falcón-Neri, 1999), rabbit pulmonary artery (Morecroft & MacLean, 1998) and the dog external carotid vasculature (Villalón *et al*., 1997c). Additionally, the 5-HT-induced late hypotensive response in rats and tachycardia in the cat were shown to be mediated by 5-ht_7 receptors, as discussed in Chapters 10 and 12, respectively. In view of the above, the search for this "orphan" receptor has successfully ended and the upper case 5-HT_7 receptor appellation is now clearly justified (Eglen *et al*., 1997; Hoyer & Martin, 1997; Saxena *et al*., 1998b).

Although the 5-HT_7 receptor can be characterised using the above criteria, this would be greatly facilitated by selective agonists and antagonists. Unfortunately, no selective agonists are as yet available, but SB258719 has been reported to be a selective antagonists (Forbes *et al*., 1998; Thomas *et al*., 1998).

The 5-HT_7 receptor has been cloned in several species, including human (Bard *et al*., 1993), rat (Lovenberg *et al*., 1993; Ruat *et al*., 1993b; Shen *et al*., 1993), mouse (Plassat *et al*., 1993) and guinea-pig (Tsou *et al*., 1994). As described above the receptor is abundantly expressed on vascular smooth muscle, but the 5-HT_7 receptor is also widely distributed in the brain. Thus, the 5-HT_7 receptor has been implicated in the regulation of the circadian rhythm (Lovenberg *et al*., 1993), depression (Sleight *et al*., 1995) and some psychiatric disorders (Roth *et al*., 1994). Moreover, the 5-HT_7 receptor is localised on cranial blood vessels and may play a role

in cranial vasodilatation, thought to occur during migraine headache. In favour of this suggestion, lisuride (Herrmann *et al*., 1977; Somerville & Herrmann, 1978; Del Bene *et al*., 1983) and methysergide (Herrmann *et al*., 1977; Silberstein, 1998) have been reported to be of use in migraine prophylaxis (see also Section 14.6.3). The latter two compounds display high affinity at the 5-HT_7 receptor, but, admittedly, they are non-selective drugs.

1.10 Orphan 5-HT receptors

1.10.1 5-HT receptor mediating endothelium-dependent vasorelaxation

In several blood vessels 5-HT produces a relaxation dependent on the integrity of the endothelium. In most cases, the operational profile of the receptors involved does not fulfil the current classification criteria. In the case of the dog coronary artery the receptor shows similarities with 5-HT_1 receptors, since the response is antagonised by methiothepin and metergoline, but not by ketanserin and MDL72222 (Houston & Vanhoutte, 1988). A similar pharmacological profile is described in the pig coronary artery (Schoeffter & Hoyer, 1989; Schoeffter & Hoyer, 1990) and guinea-pig jugular vein (Gupta, 1992), where the endothelial vasorelaxant receptor resembles the 5-$HT_{1B/1D}$ receptors, in view of the agonist activity of sumatriptan. More recently, Razzaque *et al.* (1995), suggested that the endothelium-dependent vasodilatation in the guinea-pig jugular vein is mediated by 5-HT_{1D} receptors, in view of the antagonist properties of GR127935 as well as ketanserin. The use of SB224289 and BRL15572 may help resolve this issue.

In the rabbit jugular vein (Leff *et al*., 1987) and pig vena cava (Sumner, 1991), the receptor rather resembles 5-$HT_{2B/2C}$ receptors, since 5-CT displayed low, while α-Me-5-HT high potency. Moreover, ketanserin and MDL72222 were inactive, whereas methiothepin and methysergide acted as antagonists. A similar pharmacological profile was demonstrated in the rat jugular vein (Bodelsson *et al*., 1993) and pig pulmonary artery (Glusa & Richter, 1993). *m*RNA localisation studies and the use of selective ligands seem to indicate that 5-HT_{2B} receptors are involved in the latter blood vessels (Ellis *et al*., 1995; Ullmer *et al*., 1995; Glusa & Roos, 1996).

1.10.2 Novel recognition site in human brain

Castro *et al.* (1997) have described a binding site in human brain showing high affinity for 5-CT. Although 5-CT is known to be able to bind to 5-HT_1, 5-ht_5 and

5-HT_7 receptors, these authors describe that, after pharmacological blockade of these receptors, there is a remaining population that is labelled by [^{3}H]5-CT. Under these experimental conditions, 5-HT and 5-MeO-T bind with high potency, whereas ergotamine also shows a moderate affinity. Additionally, methiothepin seems to be able to interact with this binding site. Sumatriptan, ketanserin, GR127935, mesulergine and ondansetron are virtually devoid of interaction. Some results seem to indicate that the receptor site involves G proteins in its functionality (Castro *et al*., 1997), but further studies are needed to fully characterise the signal transduction. As will be discussed in Chapter 5, the GR127935-resistant part of the 5-HT-induced constriction of carotid arteriovenous anastomoses seems to resemble the pharmacological profile of this binding site, although further studies will be necessary.

1.10.3 Other responses mediated by orphan receptors

According to the Hoyer *et al.* (1994) 5-HT receptor classification the depolarisation in rat motorneurons, inhibition of noradrenaline release in pig coronary artery and the slow depolarisation of myenteric neurones are mediated by an orphan receptor. At this moment, no substantial progress has been made in characterising these responses.

Chapter 2

Migraine

2.1 Epidemiology and diagnostic criteria

Migraine is a syndrome that affects a substantial fraction of world's population, with a higher prevalence in females (15-18%) than in males (6%; Lipton & Stewart, 1997). Migraine is characterised by attacks of intense, pulsatile and throbbing headache, which is typically unilateral and is accompanied by anorexia, nausea, vomiting and photo- and/or phonophobia (see Table 2.1). In about one third of patients, the headache is preceded by aura symptoms, consisting of certain sensory (pins and needle feeling or numbness), motor (weakness or paralysis) and/or focal neurological (characteristically a homonymous, spreading, scintillating scotoma) symptoms (migraine with aura or classic migraine). The majority of patients, however, do not present with such symptoms (migraine without aura or common migraine, for review see Ferrari, 1998).

2.2 Pathophysiology

Based on the clinical features of migraine, three distinct phases can be discerned: an initiating trigger, an aura and, finally, the headache. Although limited information is available about the trigger phase, there is indeed now a better understanding of the pathophysiology of migraine (e.g. Goadsby, 1997; Ferrari, 1998). Some results indicate that the initiating trigger, involving the brainstem as "migraine generator" (Weiller *et al*., 1995), may be linked to a "familial" channelopathy (Ophoff *et al*., 1996; Ferrari, 1998). The subsequent events leading to symptoms observed during the aura and headache phases can be explained on the basis of a neurovascular hypothesis (see Ferrari & Saxena, 1993a; Saxena, 1994; Ferrari, 1998).

Based on:
De Vries, P., Villalón, C.M. & Saxena, P.R. (1999). Pharmacology of triptans. *Emerg. Drugs*, **4**, 107-125.
De Vries, P., Villalón, C.M. & Saxena, P.R. (1999). Pharmacological aspects of experimental headache models in relation to acute antimigraine therapy. *Eur. J. Pharmacol.*, **375**, 61-74.

Table 2.1 Diagnostic criteria proposed by the Headache Classification Committee of the International Headache Society (1988)

Migraine without aura

A. At least five attacks fulfilling B-D
B. Headache attacks lasting 4-72 hours (untreated or unsuccessfully treated)
C. Headache has at least two of the following characteristics
    1. Unilateral location
    2. Pulsating quality
    3. Moderate or severe intensity
    4. Aggravation by walking upstairs or similar routine physical activity
D. During headache at least one of the following
    1. Nausea and/or vomiting
    2. Photophobia and phonophobia

Migraine with aura

A. At least two attacks fulfilling B
B. At least three of the following four characteristics
    1. One or more fully reversible aura symptoms indicating focal cerebral cortical and/or brain stem dysfunction
    2. At least one aura symptom develops gradually over more than 4 min, or two or more symptoms occur in succession
    3. No aura symptoms last more than 60 min
    4. Headache follows aura with a free interval of less than 60 min

As illustrated in Figure 2.1, once the "migraine generator" has been switched on, regional cerebral blood flow decreases, possibly following a wave of cortical spreading depression (see Read & Parsons, 1999). In patients where cerebral blood flow falls below a critical value, corresponding aura symptoms may appear. The reduced cerebral blood flow is then followed by a vasodilatation during the headache phase, probably due to changes in the activity of the neurones innervating cranial extracerebral large arteries and arteriovenous anastomoses (e.g. in the dura mater, base of the skull and scalp). Besides noradrenaline and acetylcholine, immunohistochemical studies have demonstrated the presence of several vasodilator transmitters in perivascular nerve fibres supplying intracranial blood vessels,

including 5-HT, VIP, NO, substance P, neurokinin A and CGRP (for review see Gulbenkian *et al*., 1999). As discussed elsewhere (Olesen *et al*., 1994), NO may play an important role in migraine pathophysiology and inhibition of its synthesis seems to be of therapeutic relevance (Lassen *et al*., 1998). In any case, cranial vasodilatation leads to enhanced blood volume following each cardiac stroke and rapid diastolic runoff, with a consequent augmentation in pulsations within the affected blood vessels. The augmented pulsations can then be sensed by "stretch" receptors in the vessel wall and the resultant increase in perivascular (trigeminal) sensory nerve activity provokes headache and other associated symptoms. This stimulation of the trigeminal nerve may also release neuropeptides, thus reinforcing vasodilatation and perivascular sensory nerve activity (for details and references, see Saxena, 1994).

As shown in Figure 2.1, acutely-acting antimigraine drugs constrict dilated cranial extracerebral blood vessels (Saxena & Ferrari, 1989; Humphrey & Feniuk, 1991; Ferrari & Saxena, 1993a), reduce neuropeptide release and plasma protein extravasation across dural vessels (Moskowitz, 1992; 1993) and inhibit impulse transmission centrally within the trigeminovascular system (Goadsby *et al*., 1991; Goadsby, 1997).

2.3 Acute migraine treatment: sumatriptan and the second-generation triptans

In the last decade there has been a tremendous progress in the acute therapy of migraine, with sumatriptan belonging to a new class of drugs (then known as 5-HT_1-like receptor agonists), providing the lead (Humphrey *et al*., 1988; 1990). This seminal discovery by Humphrey and colleagues that compounds mimicking 5-HT at craniovascular receptors should abort migraine attacks stems from the observations that: (i) urinary excretion of 5-hydroxyindole acetic acid increases, while platelet 5-HT decreases during migraine attacks; (ii) migraine-like symptoms can be precipitated by reserpine and alleviated by 5-HT, which causes carotid vasoconstriction via 5-HT_1-like receptors; and (iii) ergotamine and methysergide elicit a selective carotid vasoconstriction (at least partly via 5-HT_1-like receptors), which is confined to cephalic arteriovenous anastomoses that seem to be involved in migraine pathophysiology (see below). Based on the above, tryptamine derivatives were synthesised to achieve selectivity at the craniovascular 5-HT_1-like receptor and this culminated in the design and development of sumatriptan (Humphrey *et al*., 1990).

Migraine

The success of sumatriptan in migraine therapy undoubtedly resulted in heightened research interest in the field of migraine. This, in turn, led to a better understanding of the pathophysiological processes involved in migraine as well as the development of new triptans (for reviews see Ferrari, 1998; Goadsby, 1998a) and other prospective drugs.

Despite its great utility in migraine treatment, sumatriptan has certain limitations; e.g. low oral bioavailability, high headache recurrence, possibly due to a short $t_{1/2}$ and contra-indication in patients with coronary artery disease. Therefore, a number of pharmaceutical companies decided to develop newer triptans having agonist activity at the craniovascular 5-HT_1-like receptors. Several such compounds (zolmitriptan, rizatriptan and naratriptan) are already on the market, while others (eletriptan, almotriptan, frovatriptan and F11356) are in different stages of clinical development (for chemical structures, see Figure 2.2). These compounds will be referred to as the second-generation triptans, since they are tryptamine derivatives and pharmacologically comparable to sumatriptan. Zolmitriptan (Visser *et al*., 1996b), rizatriptan (Gijsman *et al*., 1997; Kramer *et al*., 1998), naratriptan (Bomhof *et al*., 1998) and eletriptan (Jackson, 1996; Diener, 1997) seem to be at least as effective as sumatriptan in migraine therapy and the outcome of elaborate clinical trials with the other triptans is awaited with interest; preliminary data indicate that frovatriptan (Ryan & Keywood, 1997; McDaris & Hutchison, 1999) and almotriptan (Cabarrocas, 1998; Martinez *et al*., 1999) are also effective in migraine. Despite the efficacy of avitriptan (Ryan *et al*., 1997), BMS181885 (Yocca *et al*., 1997) and the non-triptan alniditan (Goldstein *et al*., 1996) in the treatment of migraine, these compounds are no longer in clinical development.

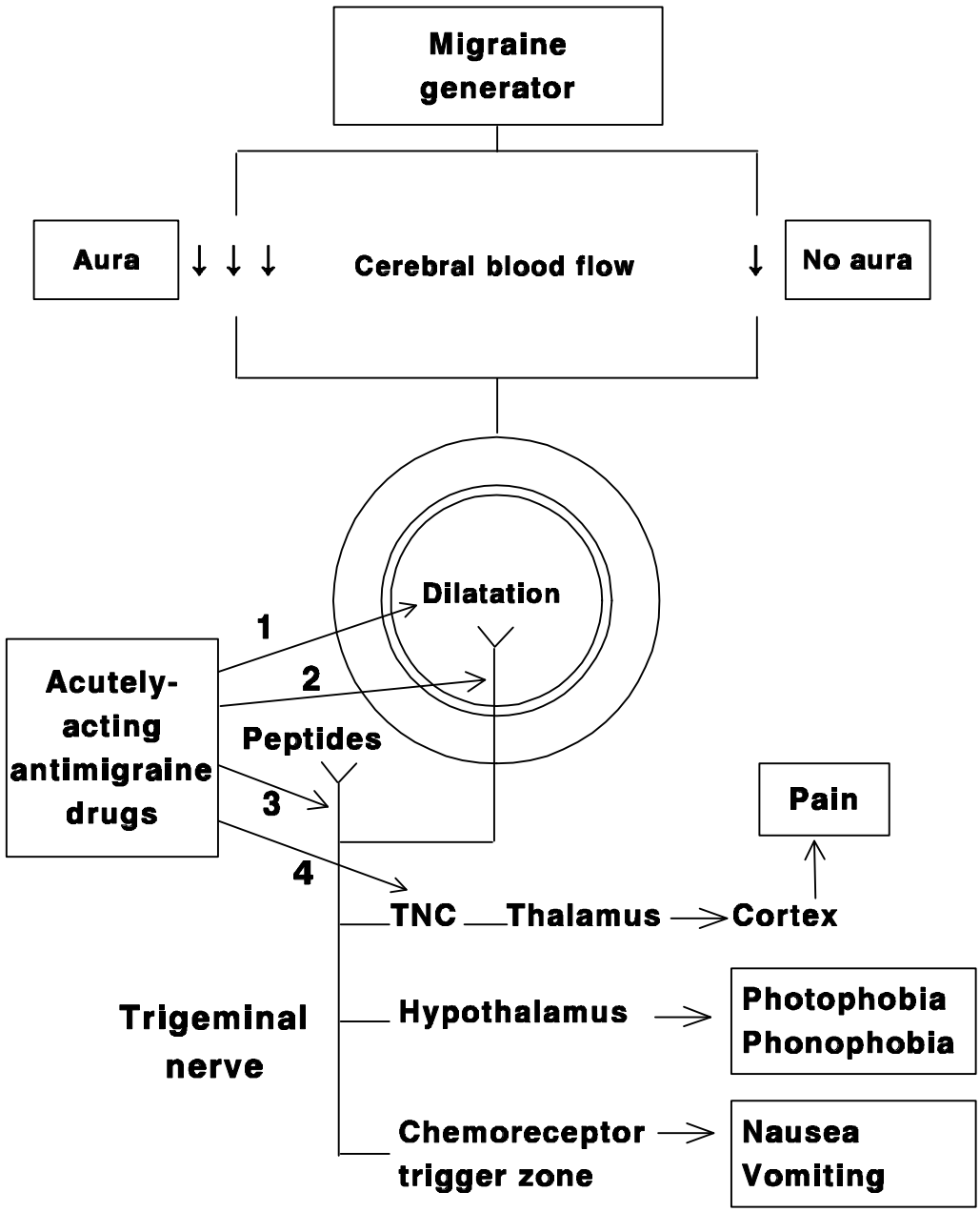

Figure 2.1 Diagram showing putative changes in migraine and the therapeutic targets of acutely acting antimigraine drugs. These drugs are believed to owe their antimigraine efficacy to direct vasoconstriction of dilated cranial blood vessels (1), inhibition of trigeminally-induced cranial vasodilatation (2), plasma protein extravasation (3) and/or central neuronal activity (4). Only lipophilic, brain penetrant triptans (not sumatriptan) exert central trigeminal inhibitory effects. For details see text. Based on Saxena (1994) and De Vries *et al.* (1999a). TNC, trigeminal nucleus caudalis.

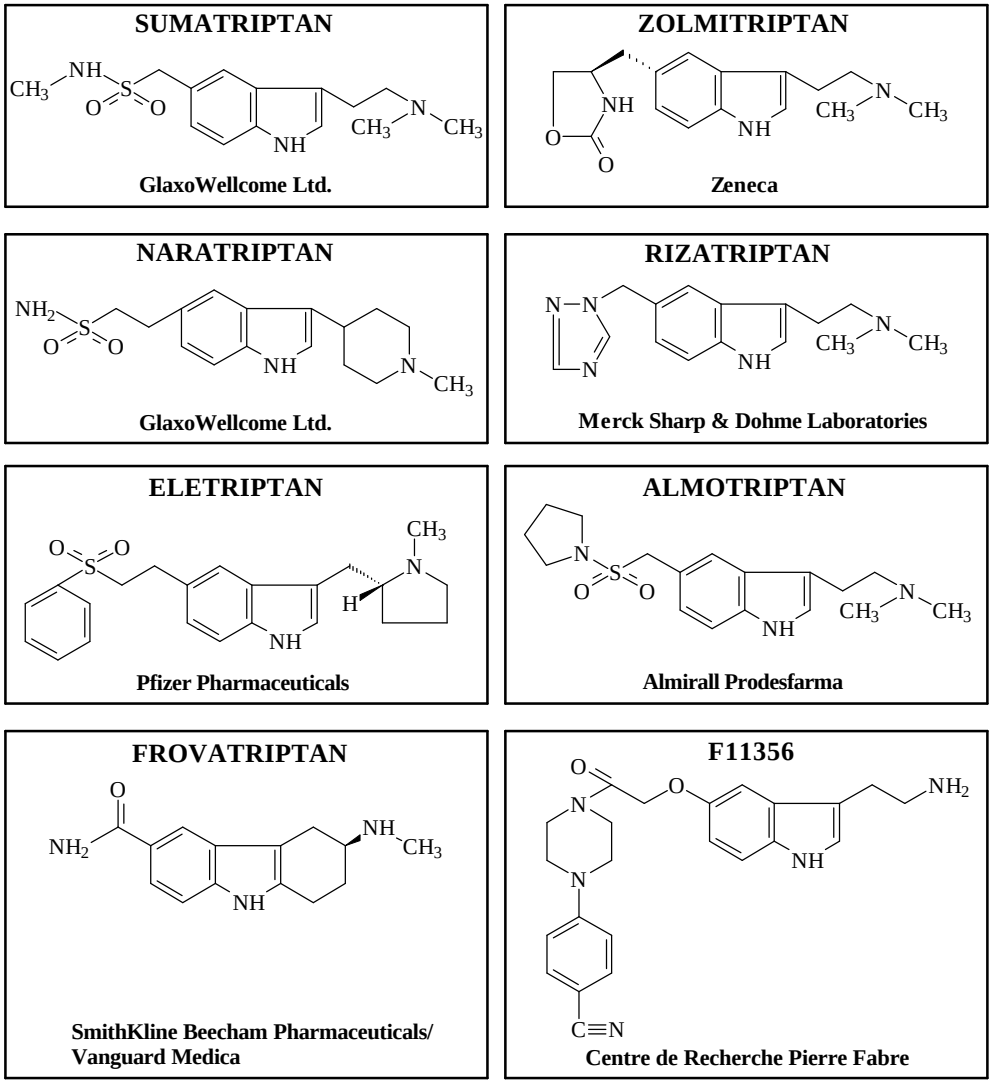

Figure 2.2 Chemical structures of sumatriptan and some second-generation triptans.

2.4 Receptor binding and pharmacokinetics of triptans

Sumatriptan as well as the second-generation triptans display high affinities at 5-HT_1 receptor subtypes, mainly the 5-HT_{1B} and 5-HT_{1D} receptors (Table 2.2). There are no profound differences in affinities, although F11356 is clearly the most potent, while sumatriptan is the weakest at the 5-HT_{1B} receptor. Most compounds have a reasonable affinity at the 5-HT_{1A} and, with the exception of F11356, at the 5-HT_{1F} receptor. Some triptans display a µM affinity at the 5-HT_7 receptor.

The pharmacokinetic characteristics of sumatriptan and most second-generation triptans have been studied in human volunteers and migraine patients (see Table 2.3). Subcutaneous sumatriptan is quickly absorbed and has a high bioavailability. After oral administration of therapeutic doses of sumatriptan, however, the t_{max} is substantially longer and, more importantly, the bioavailability is rather low. Intranasal or rectal administration of sumatriptan does not seem to improve these parameters much. The oral bioavailability of second-generation triptans, especially naratriptan and almotriptan, is much improved. The latter can be partly attributed to the more lipophilic nature of these drugs. Interestingly, the t_{max} after oral administrations of the second-generation triptans does not differ much from that of sumatriptan. When corrected for plasma protein binding, C_{max} values of the new drugs are lower than that of sumatriptan, which is apparently due to two main factors: (i) lower therapeutic concentrations are needed as these drugs have a higher affinity at 5-$HT_{1B/1D}$ receptors (Table 2.2); and (ii) these drugs have been better titrated, thus reducing therapeutic penalty. For the latter reason, low dose formulations of sumatriptan have been marketed in some countries. With the exception of rizatriptan, second-generation triptans are degraded slower than sumatriptan. Especially frovatriptan has a plasma half-life of 26-30 h and, in view of the putative relation of this parameter with headache recurrence, the results of clinical trials with frovatriptan are awaited with interest; preliminary data seem to suggest that frovatriptan results in lower recurrence rates, although only a moderate reduction was observed (Goldstein *et al*., 1999b). In contrast to sumatriptan and naratriptan, active metabolites have been reported for zolmitriptan, rizatriptan and eletriptan. It is not known, whether and if so, to what extent, the metabolites contribute towards the amount and duration of therapeutic activity and recurrence rates. We are not aware whether the metabolism of almotriptan and frovatriptan results in the formation of active metabolites.

Table 2.2 pK_i values of sumatriptan and second-generation triptans at human (except when stated otherwise) 5-HT receptors.

Receptor	Sumatriptan	Zolmitriptan	Naratriptan	Rizatriptan	Eletriptan	Almotriptan	Frovatriptan	F11356
5-HT_{1A}	6.4[a]	6.6[c]	7.6[b]	6.4[c]	7.4[c]	6.3[f]	7.3[g]	7.6[h]
	6.9[b]	6.5[d]	7.1[c]					
	6.0[c]		7.1 (rat)[e]					
5-HT_{1B}	7.8[a]	7.7[c]	8.1[c]	6.9[c]	8.0[c]	8.0[f]	8.6[g]	9.4[h]
	7.4[c]	8.3[d]	8.7[e]	7.7[j]				
	8.3[i]			8.1[k]				
5-HT_{1D}	8.5[a]	8.9[c]	8.4[c]	7.9[c]	8.9[c]	8.0[f]	8.4[g]	9.3[h]
	8.0[c]	9.2[d]	8.3[e]	8.6[k]				
5-ht_{1E}	5.8[a]	7.7[c]	7.7[c]	6.8[c]	7.3[c]		<6.0[g]	5.9[h]
	5.8[c]	8.0[k]						
	5.6[l]							
5-HT_{1F}	7.9[a]	7.5[c]	8.2[c]	6.8[c]	8.0[c]		7.0[g]	5.5[h]
	7.9[c]	7.2[d]						
	7.6[l]							
5-HT_{2A}	<5.5[c]	<5.5[c]	<5.5[c]	<5.5[c]	<5.5[c]		<5.3[g]	6.7[h]
5-HT_{2B}	6.9[m]	7.2[m]		6.6[m]				
5-HT_{2C}	<5.5[c]	<5.5[c]	<5.5[c]	<5.5[c]	<5.5[c]		<5.3[g]	
m5-HT_3	<5.5[c]	<5.5[c]	<5.5[c]	<5.5[c]	<5.5[c]		<6.0[g]	<5.0[h]
gp5-HT_4	<5.5[c]	<5.5[c]	<5.5[c]	<5.5[c]	<5.5[c]			5.7[h]
5-ht_{5A}	<5.5 (rat)[c]	6.4 (rat)[c]	5.5 (rat)[c]	5.3 (rat)[c]	5.8 (rat)[c]			6.1[h]
5-ht_6	<5.5[c]	<5.5[c]	<5.5[c]	<5.5[c]	6.3[c]			5.6[h]
5-HT_7	5.9[c]	7.0[c]	<5.5[c]	5.7[c]	6.7[c]	<6.5[f]	6.7[g]	6.4[h]

Data taken from: [a], Leysen *et al.* (1996); [b], Newman-Tancredi *et al.* (1997); [c], Napier *et al.* (1999); [d], Martin *et al.* (1997); [e], Connor *et al.* (1997); [f], Bou *et al.* (1997); [g], Brown *et al.* (1996); [h], John *et al.* (1999); [i], Beer *et al.* (1998); [j], Wurch *et al.* (1997); [k], Pauwels, P.J., personal communication; [l], Adham *et al.* (1993); [m], Gupta, P., personal communication. m, Mouse; gp, guinea-pig.

Table 2.3 Reported pharmacokinetic parameters for sumatriptan and some second-generation triptans.

Drug	Dose	T_{max} (h)	C_{max} (ng ml^{-1})	Bio-availability (%)	$T_{1/2}$ (h)	Area under curve (ng h ml^{-1})	Active metabolites	Plasma protein binding (%)	CL_R (ml min^{-1})	$LogD_{pH7.4}$
Sumatriptan	6 (s.c.)	0.2	72	96	2.0	90	-	14-21	220	-1.5
	100 (p.o.)	1.5	54	14	2.0	158			260	
	10 (i.n.)	1.5	9		2.0	31				
	20 (i.n.)	1.5	13	16	1.8	48			210	
	25 (rectal)	1.5	27	19	1.8	78			200	
Zolmitriptan	2.5 (p.o.)	1.5	3.3/3.8**	39	2.3/2.6**	18/21**	+	25*		-1.0
	5 (p.o.)	1.5	10	46	3.0	42			193	
	10 (p.o.)	2.5	13	46	2.8	87			179	
Naratriptan	2.5 (p.o.)	2.0	13	63/74**	5.5	98	-	20*	220	-0.2
Rizatriptan	10 (p.o.)	1.0	20	40	2.0	50	+	14*	414	-0.7
Eletriptan	40 (p.o.)	1.8*	82	50*			+	85*	597*	+0.5
	80 (p.o.)	1.4	246	50	6.3	1661				
Almotriptan	12.5 (p.o.)	2.5	50	80	3.1	266				
	25 (p.o.)	2.7	64	69	3.6	443			433	
Frovatriptan	2.5 (p.o.)	3.0	7	30	25.7	94				
	40 (p.o.)	5.0	53	18	29.7	881				
	0.8 (i.v.)		24	100	23.6	104			132	

Data taken from: Fowler *et al.* (1991), Duquesnoy *et al.* (1998), Moore *et al.* (1997) and Lacey *et al.* (1995) for sumatriptan; Dixon *et al.* (1995; 1997), Seaber *et al.* (1998) and Peck *et al.* (1998) for zolmitriptan; Kempsford *et al.* (1997) and Fuseau *et al.* (1997) for naratriptan; Lee *et al.* (1998), Sciberras *et al.* (1997) and Cheng *et al.* (1996) for rizatriptan; Milton *et al.* (1998), Hyland *et al.* (1998) and Morgan *et al.* (1997) for eletriptan; Cabaroccas & Salva (1997), Robert *et al.* (1998) and Fernandez *et al.* (1999) for almotriptan; Buchan *et al.* (1998) for frovatriptan. *, McHarg, A., personal communication; **, Value for men and women, respectively. CL_R: renal clearance; $logD_{pH7.4}$: measure of lipophilicity with increasing numbers indicating greater lipid solubility (see Rance *et al*., 1997).

2.5 Pharmacological aspects of experimental models for acutely-acting antimigraine drugs

2.5.1 Models based on the involvement of cranial vasodilatation in migraine

These models are based on the view that cranial extracerebral vasodilatation is an integral part of the pathophysiology of migraine and that the ergot alkaloids and sumatriptan, which do not readily cross the blood-brain-barrier, owe their therapeutic efficacy primarily to constriction of dilated vessels (Saxena & Ferrari, 1989; Humphrey & Feniuk, 1991; Ferrari & Saxena, 1993a). There are several ways to investigate the effects of antimigraine drugs on cranial blood vessels, both *in vivo* and *in vitro*. Two of these models will be discussed here.

Constriction of carotid arteriovenous anastomoses in anaesthetised animals

Although the factors mediating the initiating events of migraine are still largely unknown, the pulsatile nature of migraine headaches suggests that the pain is a result of dilatation of cephalic blood vessels. A simple cranial vasodilatation is, however, in defiance of the facial paleness and coldness noticed in many migraine sufferers during the headache phase (Drummond & Lance, 1983; 1984). Recognising this clinical feature, Heyck (1969) measured the O_2 content of arterial and jugular venous blood before, during, and after the headache and reported that the arterio-jugular venous O_2 saturation difference (A-V SO_2) was abnormally low during the headache on the ipsilateral side. Moreover, the ipsilateral A-V SO_2 normalised after spontaneous or drug-induced (ergotamine) alleviation of the headache. From these results, he suggested that arteriovenous anastomoses in the carotid vascular bed dilate during the headache phase.

As reviewed by Hales & Molyneux (1988), arteriovenous anastomoses (Figure 2.3) are precapillary communications between arteries and veins and are mainly located in head skin, ears and dura mater in man (Rowbotham & Little, 1965), as well as in pigs (Saxena & Verdouw, 1985b; Den Boer *et al*., 1992a). Opening of carotid arteriovenous anastomoses, leading to shunting of a large amount of blood directly into the venous circulation, can indeed account for facial pallor, lowering of facial skin temperature and increased vascular pulsations. Furthermore, the increased vascular pulsations may be sensed by stretch receptors, resulting in increased activity in perivascular sensory nerve endings. This increased activity in perivascular nerve endings, which is probably of trigeminal origin in view of the innervation of the

arteriovenous anastomoses by Substance P, as well as CGRP containing trigeminal nerves fibres (Molyneux & Haller, 1988), may lead to migraine headache and its associated symptoms (Saxena, 1994; 1995).

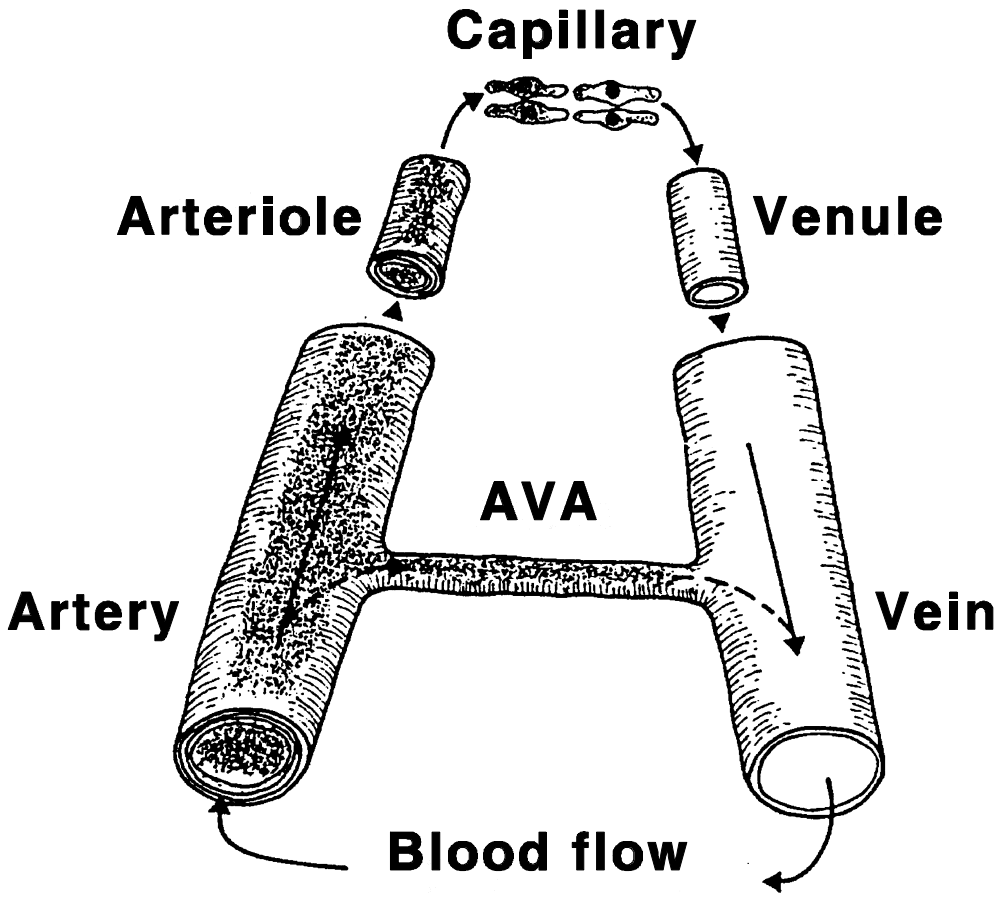

Figure 2.3 A schematic representation of a vascular bed containing an arteriovenous anastomosis (AVA). The porcine carotid AVAs are normally under a tight sympathetic constrictor tone, but under pentobarbital anaesthesia ~80% of total carotid blood flow is shunted through AVAs directly into the jugular venous ciculation (Den Boer *et al.*, 1993). As will be described in Chapter 4, cranial AVA blood flow can be determined using intracarotid adminstered radioactive microspheres.

Based on the above, we developed an animal model in which we can determine arteriovenous anastomotic blood flow, using the radioactive microsphere technique (Saxena, 1990; 1995). Indeed, as will be described below and in the rest of this thesis, 5-HT as well as the antimigraine agents sumatriptan, ergotamine and dihydroergotamine potently and selectively constrict porcine carotid arteriovenous anastomoses (Chapters 4-6 and 9). Due to obvious practical difficulties, the effect of antimigraine agents on human carotid arteriovenous anastomoses has not been investigated. Notwithstanding, sumatriptan has been found to constrict dilated arteriovenous anastomoses in the human forearm (Van Es *et al*., 1995).

The ergot alkaloids, sumatriptan as well as the newer antimigraine agents decrease carotid blood flow in anaesthetised animals (see Chapters 3-9). Consistent with this, sumatriptan evokes a vasoconstrictor action on cephalic arteries during the migraine attack in human volunteers (Friberg *et al*., 1991; Caekebeke *et al*., 1992). As shown in Table 2.4, the apparent rank order of agonist potency in decreasing canine carotid blood flow is: frovatriptan > zolmitriptan > eletriptan = naratriptan ≥ rizatriptan = sumatriptan. Almotriptan and F11356 potently reduce carotid blood flow in the cat and pig, respectively. Using radiolabelled microspheres, it has been shown that the carotid vasoconstriction by sumatriptan (see Chapter 4), zolmitriptan and eletriptan (see Chapter 6) is confined to arteriovenous anastomoses (Table 2.4), which may dilate during migraine headaches (see above). Interestingly, cerebral blood flow does not seem to be affected, as shown with sumatriptan and even with the much more lipophilic, brain-penetrant compounds, zolmitriptan, eletriptan and rizatriptan (see Table 2.4).

Over the years, this vascular model, particularly with the measurement of cephalic arteriovenous anastomotic blood flow, has proven its worth and has been highly predictive of antimigraine activity in the clinic (Saxena, 1995). Another advantage it offers is that one can simultaneously study a number of major vascular beds in order to evaluate craniovascular selectivity of the drugs. It must however be realised that this model will pick up only those putative antimigraine drugs that would be effective by constricting dilated cranial vessels, whatever the mechanism. For example, apart from the ergot alkaloids and triptans, NO synthase inhibitors, which may have antimigraine activity (Olesen *et al*., 1994; Lassen *et al*., 1998), constrict cranial arteriovenous anastomoses (Van Gelderen & Saxena, 1994) and NO donors, which cause headache, can dilate them (Van Gelderen *et al*., 1995).

For details concerning the pharmacology of the canine external carotid vascular bed and the constriction of porcine carotid arteriovenous anastomoses, see Chapters 4-9.

Contraction of isolated cranial blood vessels

As shown in Table 2.5, a number of isolated blood vessels from several species contract in response to acutely acting antimigraine drugs. This effect is undoubtedly more marked on cranial vessels where, contrary to most peripheral arteries, 5-HT_1 rather than 5-HT_2 receptors are predominant (Longmore *et al*., 1997). Consistent with their binding profile, the newer triptans resemble sumatriptan in their action and potency. Interestingly, eletriptan and zolmitriptan seem to behave as partial agonists in the dog (Gupta *et al*., 1999) and rabbit (Martin *et al*., 1997) saphenous vein compared to sumatriptan. The fact that eletriptan (see Chapter 6; Willems *et al*., 1998) and zolmitriptan (MacLennan *et al*., 1998) are as efficacious as sumatriptan in contracting the carotid vasculature could be explained in terms of a larger receptor reserve (Kenakin, 1984) in the carotid circulation compared to that in the isolated saphenous vein.

In a number of isolated blood vessels, such as the canine basilar (Skingle *et al*., 1996) and coronary (Terrón, 1996a) arteries, canine (Skingle *et al*., 1996) and rabbit (Razzaque *et al*., 1995) saphenous vein and human middle meningeal artery (Razzaque *et al*., 1997), the contractile effect of the triptans is antagonised by selective 5-$HT_{1B/1D}$ receptor antagonists. It was recently shown that sumatriptan constricts the isolated human temporal artery via 5-HT_{1B}, but not 5-HT_{1D} receptors (Verheggen *et al*., 1998). Moreover, 5-HT_{1F} receptor agonists seem to be devoid of vasoconstrictor activity in the rabbit saphenous vein (Cohen *et al*., 1998), as well as in human and bovine cerebral arteries (Bouchelet & Hamel, 1999). Further studies using selective 5-HT_{1B}, 5-HT_{1D} and 5-HT_{1F} receptor agonists and antagonists are needed to establish if the 5-HT_{1D} and/or 5-HT_{1F} receptor stimulation are indeed completely devoid of vasoconstrictor action.

Table 2.4 ED_{50} (μg kg^{-1}, i.v.) Values of sumatriptan and some second-generation triptans producing changes in carotid blood flow.

	Sumatriptan	**Zolmitriptan**	**Naratriptan**	**Rizatriptan**	**Eletriptan**	**Almotriptan**	**Frovatriptan**	**F11356**
Total (↓)	39 (dog)[a] 30-100 (pig)[b]	2.3 (dog)[c] 1.0 (cat)[c]	19 (dog)[a]	30 (dog)[d]	12 (dog)[e] 30-100 (pig)[f]	10 (cat)[g]	0.4 (dog)[h]	0.5 (pig)[i]
Arteriovenous anastomotic fraction (↓)	<30 (pig)[b]	<10 (cat)[c]			30-100 (pig)[f]			
Extracerebral fraction	↑ (pig)[b]	↓ (dog)[c]			↑ (pig)[f]			
Cerebral fraction	= (pig)[b]	= (dog)[c]		= (dog)[j]	= (pig)[f]			

Data from: [a], Connor *et al.* (1997); [b], De Vries *et al.* (1996); [c], MacLennan *et al.* (1998); [d], Shepheard *et al.* (1995a); [e], Gupta *et al.* (1996); [f], Willems *et al.* (1998); [g], Bou *et al.* (1997); [h], Parsons *et al.* (1997); [i], John *et al.* (1999); [j], Sperling *et al.* (1995). ↓, decrease; ↑, increase; =, no changes.

Table 2.5 pEC_{50} Values of sumatriptan and some second-generation triptans in producing contraction of isolated blood vessels. If known, the intrinsic relative to 5-HT (5-HT=1) is given between brackets.

	Sumatriptan	**Zolmitriptan**	**Naratriptan**	**Rizatriptan**	**Eletriptan**	**Almotriptan**	**Frovatriptan**	**F11356**
Human BA	6.93 (1.11)[a]					5.46[b]	7.86 (1.25)[a]	
Dog BA	6.16 (0.63)[c]	6.63 (0.61)[c]	6.96 (1.05)[d]		7.20 (0.77)[e]			
	6.44 (1.00)[d]							
	6.80 (0.89)[e]							
Primate BA	6.46 (0.48)[f]	6.92 (0.56)[f]						
Rabbit BA	6.00[g]						7.20[g]	
Dog MCA	6.73 (1.00)[d]		7.15 (1.14)[d]					
	7.80 (1.08)[h]							
Human MMA	7.15 (0.66)[i]	7.37 (0.73)[j]		7.05 (0.83)[i]	7.30 (0.80)[k]	7.52[b]		
	7.05 (0.70)[j]							
	7.28 (0.84)[k]							
Human SV	6.14 (0.54)[l]				5.91 (0.48)[l]			
Dog SV	6.10 (0.85)[e]				6.30 (0.57)[e]			
Rabbit SV	6.48 (0.97)[f]	6.79 (0.77)[f]		6.64 (0.90)[m]				7.10 (0.84)[n]
Human CA	6.14 (0.21)[d]	7.30 (0.37)[f]	6.77 (0.33)[d]	6.35 (0.17)[o]	5.37 (0.33)[k]		7.38 (0.42)[a]	
	6.70 (0.35)[f]	6.32 (0.20)[o]	6.77 (0.17)[o]	5.99 (0.22)[p]				
	5.80 (0.42)[k]							
	6.10 (0.24)[o]							
	6.20 (0.43)[p]							

Data taken from: [a], Parsons *et al.* (1998); [b], Bou *et al.* (1997); [c], Martin *et al.* (1996); [d], Connor *et al.* (1997); [e], Gupta *et al.* (1999); [f], Martin *et al.* (1997); [g], Brown *et al.* (1996); [h], Yocca, F.D., personal communication; [i], Longmore *et al.* (1998); [j], Razzaque *et al.* (1999); [k], MaassenVanDenBrink *et al.* (1999a); [l], Van Den Broek *et al.* (1999); [m], Beer *et al.* (1995); [n], John *et al.* (1999); [o], MaassenVanDenBrink *et al.* (1998); [p], Longmore *et al.* (1996). BA, basilar artery; MCA, middle cerebral artery; MMA, middle meningeal artery; SV, saphenous vein; CA, coronary artery.

2.5.2 Models based on the involvement of trigeminovascular system in migraine

As described above, the contractile effects of sumatriptan and the second-generation triptans in isolated cranial blood vessels are mediated mainly by the 5-HT_{1B} receptor. Given that cranial vasoconstriction is the most important therapeutic mechanism, it implies that agonist action at the 5-HT_{1D} (and 5-HT_{1F}) receptor is not required for the antimigraine action. However, several other mechanisms, which do not seem to be mediated solely by the 5-HT_{1B} receptor, have also been implicated in migraine relief. These mechanisms include inhibition of the trigemino-vascular system either peripherally (Moskowitz, 1992; 1993) or centrally (Goadsby, 1998a).

Inhibition of plasma protein extravasation after stimulation of the trigeminal nerve

In the rat and guinea-pig, electrical or chemical stimulation of the trigeminal ganglion induces plasma protein extravasation in the dura mater, as measured by the increased leakage of iodinated ([^{125}I]) albumin (Markowitz *et al.*, 1987). This plasma extravasation is suggested to be due to the antidromic release of neuropeptides, such as substance P, neurokinin A and CGRP, from sensory trigeminal nerve fibres (see Figure 2.4; Moskowitz, 1992). Indeed, tachykinin NK_1 receptor antagonists, such as GR82334 or CP99994, and the CGRP receptor antagonist αCGRP-(8-37) block this effect (O'Shaughnessy & Connor, 1994; Shepheard *et al.*, 1995b), although the latter was devoid of effect in rats (O'Shaughnessy & Connor, 1994).

Sumatriptan, as well as second-generation triptans inhibit neurogenic plasma protein extravasation (see Table 2.6). This effect by sumatriptan is antagonised by the 5-$HT_{1B/1D}$ receptor antagonist GR127935 in both the rat and guinea-pig, implying the involvement of 5-HT_{1B} and/or 5-HT_{1D} receptors (Yu *et al.*, 1997). In mice, the receptor mediating the sumatriptan-induced effects resembles the 5-HT_{1B} receptor, since sumatriptan loses its activity in mice lacking this receptor (Yu *et al.*, 1996). On the other hand, in the guinea-pig the selective 5-HT_{1D} receptor agonist PNU109291 potently inhibits dural plasma extravasation (Waeber *et al.*, 1997; Ennis *et al.*, 1998). In the rat, mainly based on the lower potency of CP93129 compared to that of sumatriptan, Shepheard *et al.* (1997a) suggested that 5-HT_{1D} rather than 5-HT_{1B} receptors mediate inhibition of plasma protein extravasation.

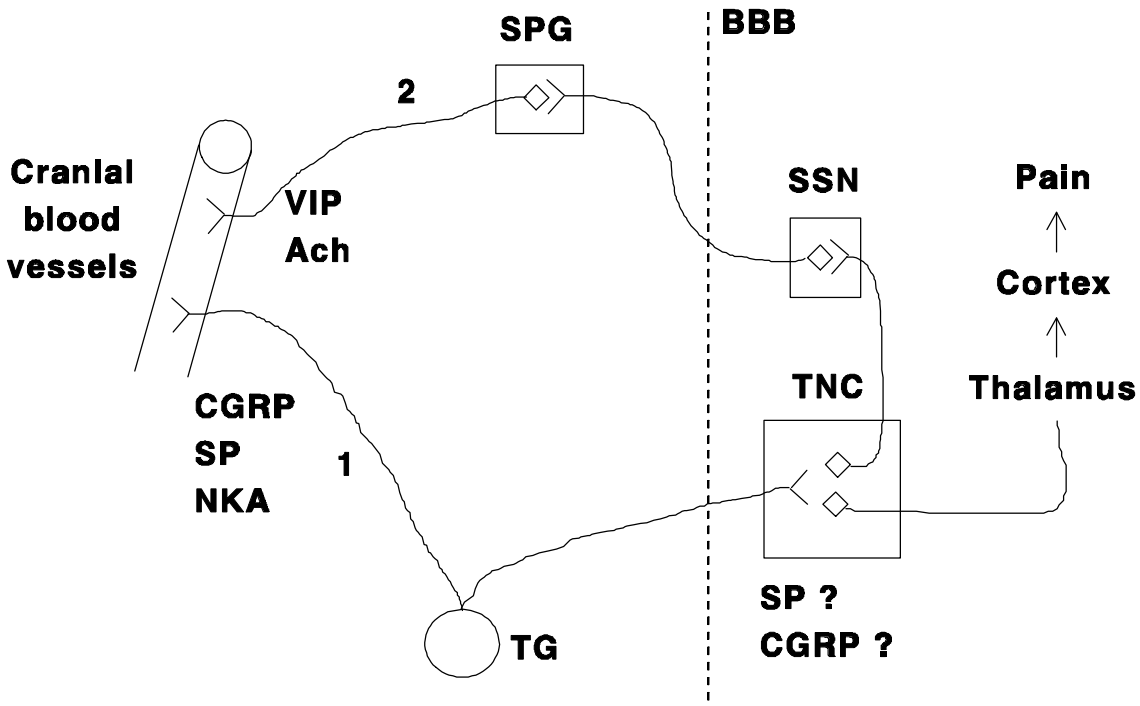

Figure 2.4 Schematic representation of the trigeminal innervation of cranial extracerebral vasculature. Cranial blood vessels are innervated by (1) afferent sensory fibres from the first division (ophthalmic) of the trigeminal nerve; and (2) by efferent parasympathetic fibres from the facial nerve. The trigeminal ganglion (TG) is connected with the facial nerve via a brain stem reflex involving synapses in the trigeminal nucleus caudalis (TNC), superior salivatory nucleus (SSN) and sphenopalatine ganglion (SPG). Redrawn with permission from Goadsby (1997). CGRP, calcitonin gene related peptide; SP, substance P; NKA, neurokinin A; VIP, vasoactive intestinal peptide; Ach, acetylcholine; BBB, blood brain barrier; TG, trigeminal ganglion.

Table 2.6 ED_{50} (μg kg^{-1}, i.v.) Values of sumatriptan and some second-generation triptans in producing trigeminal neuronal inhibition.

	Sumatriptan	Zolmitriptan	Naratriptan	Rizatriptan	Eletriptan	Almotriptan
Inhibition of plasma protein extravasation after trigeminal ganglion stimulation	4 (rat)[a] 31 (rat)[b]	10-30 (guinea-pig)[c]	4.1 (rat)[a]	31 (rat)[d]	30-300 (rat)[e]	200 (guinea-pig)[f]
Inhibition of CGRP release after feline trigeminal stimulation	Yes[g]	Yes[h]				
Inhibition of dural vasodilatation after rat trigeminal ganglion stimulation	1000-10000[b]			1000-3000[d]		
Inhibition of activity in trigeminal nucleus caudalis after stimulation*	Inactive (rat)[i]	100 (cat)[j] 300-1000 (rat)[k]	30-100 (cat)[l]	1000-3000 (rat)[m]	500 (cat)[n]	

Data taken from: [a], Connor *et al.* (1997); [b], Shepheard *et al.* (1997a); [c], Martin *et al.* (1997); [d], Williamson *et al.* (1997b); [e], Gupta *et al.* (1996); [f], Bou *et al.* (1997); [g], Goadsby & Edvinsson (1993); [h], Goadsby & Edvinsson (1994); [i], Shepheard *et al.* (1995b); [j], Goadsby & Hoskin (1996); [k], Cumberbatch *et al.* (1998); [l], Goadsby & Knight (1997); [m], Cumberbatch *et al.* (1997); [n], Goadsby & Hoskin (1999). *, Stimulation of the superior sagittal sinus or dural meninges in rats. No data available for frovatriptan and F11356.

Besides 5-HT_{1B} and 5-HT_{1D} receptors, inhibition of neurogenic plasma protein extravasation can also be effected via other receptor mechanisms. Such inhibitions by 5-CT and CP122288 are observed in 5-HT_{1B} receptor knockout mice (Yu *et al*., 1996) and are not prevented by GR127935 in guinea-pigs (Yu *et al*., 1997). Moreover, CP122288 is more than a thousand times more potent than sumatriptan in rats, which does not correlate with its affinity at the 5-HT_{1B} or 5-HT_{1D} receptor (Shepheard *et al*., 1997a). A part of the action of CP122288 may be via the 5-HT_{1F} receptor where it displays high affinity (see Table 1.1). Indeed, the selective 5-HT_{1F} receptor agonists, LY334370 and LY344864, inhibit plasma protein extravasation in the guinea-pig and rat (Johnson *et al*., 1997; Phebus *et al*., 1997). On the other hand, the response to 5-CT, which displays a very low affinity at this receptor (Hoyer *et al*., 1994), may be mediated by a novel receptor, possibly identical to the novel receptor found in human brain (see section 1.10.2; Castro *et al*., 1997) or that mediating porcine carotid vasoconstriction (see Chapter 5). The neurogenic plasma extravasation can also be inhibited by the $ET_{A/B}$ receptor antagonist bosentan (Brändli *et al*., 1996) as well as a 5-HT_4 receptor antagonist (Connor & Beattie, 1999).

Importantly, it should be noted that activity in this model does not necessarily translate into effectiveness in migraine. For example, antimigraine efficacy was not observed with CP122288 (at doses devoid of vasoconstrictor action) (Roon *et al*., 1997), the $ET_{A/B}$ receptor antagonist bosentan (May *et al*., 1996) as well as the tachykinin NK_1 receptor antagonists, lanipetant (Goldstein *et al*., 1997) and RPR100893 (Diener, 1995). The results of the trials with selective 5-HT_{1D} or 5-HT_{1F} receptor agonists are also of major interest. Interestingly, as discussed in detail in section 14.4.1, the 5-HT_{1D} receptor agonist PNU109291 is ineffective in the treatment of migraine (McCall, 1999), whereas the 5-HT_{1F} receptor agonist LY334370 did show antimigraine properties (Goldstein *et al*., 1999a). May *et al.* (1998) recently questioned the involvement of plasma extravasation in migraine, based on the lack of retinal permeability changes during migraine attacks. Finally, it may be questioned whether the effectiveness of triptans in this model is due to presynaptic inhibition of neuropeptide release or via a physiological antagonism of vasodilatation (Humphrey & Goadsby, 1994).

Inhibition of cranial vasodilatation (carotid, dural, cortical) induced by trigeminal stimulation

It has been shown in the cat (Lambert *et al*., 1984), monkey (Goadsby *et al*., 1986) and guinea-pig (Beattie & Connor, 1994) that electrical stimulation of the trigeminal ganglion decreases carotid vascular resistance. In contrast to plasma protein extravasation (see above), the carotid vasodilatation is not amenable to blockade by CGRP or tachykinin receptor antagonists (Beattie & Connor, 1994; Raval *et al*., 1999). This vasodilatation seems to involve the release of VIP, since a blockade is observed with the VIP antagonist, [p-Cl-D-Phe^{6}, Leu^{17}]-VIP (Beattie & Connor, 1994), and VIP antiserum (Goadsby & MacDonald, 1985). As depicted in Figure 2.4, VIP can be released upon trigeminal stimulation from facial nerve parasympathetic fibres, which are connected with the trigeminal nerve via a brain stem reflex (Lambert *et al*., 1984; Goadsby, 1997). Interestingly, the ganglion blocking agent hexamethonium abolishes carotid vasodilatation in the cat (Lambert *et al*., 1984), but not in the guinea-pig (Beattie & Connor, 1994). The latter implies that the nerve pathways differ in these two species. In any case, as discussed by Beattie & Connor (1994), it is unlikely that the effects in the guinea-pig involve sensory afferent trigeminal fibres, in view of the lack of immunohistochemical evidence for the presence of VIP in these neurones. The vasodilatation within the feline carotid vascular bed following trigeminal ganglion stimulation is inhibited by endothelin ET_B receptor antagonists, such as BQ788 (Raval *et al*., 1999). Whether the latter is a result of inhibition of the vascular effects of endogenously released endothelins or modulation of the release of vasorelaxant neuropeptides from parasympathetic or trigeminal sensory nerve remains to be clarified.

It is important to note that sumatriptan fails to inhibit carotid vasodilatation evoked by trigeminal stimulation, as shown in several species (Spokes & Middlefell, 1995; Lambert & Michalicek, 1996; Raval *et al*., 1999). Additionally, the endothelin $ET_{A/B}$ antagonist bosentan (May *et al*., 1996), the tachykinin NK_1 antagonists lanipetant (Goldstein *et al*., 1997) and RPR100893 (Diener, 1995) are ineffective in the acute treatment of migraine.

The dural vasculature also dilates in response to trigeminal stimulation, as demonstrated using Doppler flowmetry in the cat (Lambert & Michalicek, 1993) and rat (Kurosawa *et al*., 1995). Recently, Shepheard *et al.* (1997a) have developed a technique in the anaesthetised rat enabling them to determine dural blood vessel

diameter through a closed cranial window. Electrical stimulation of trigeminal afferents through the cranial window elicits a dilatation of meningeal blood vessels (Shepheard *et al.*, 1997a; Williamson *et al.*, 1997a; 1997b). In contrast to the neurogenically-induced increase in carotid blood flow (mainly via VIP release, see above), this dural vasodilatation involves CGRP. Thus, αCGRP-(8-37) potently inhibits the effect, whereas the tachykinin NK_1 receptor antagonist RP67580 is ineffective (Williamson *et al.*, 1997a; 1997b). It should be noted that in this model the increases in vessel diameter were evoked by short, low intensity electrical stimulation, which primarily stimulate trigeminal sensory Aδ-fibres containing only CGRP. In contrast, the dural plasma protein extravasation evoked by longer, higher intensity stimulation, amenable to blockade by tachykinin NK_1 receptor antagonists (see above), seems to involve primarily C-fibres containing substance P (see Shepheard *et al.*, 1997a). In this context, it should be noted that stimulation of the trigeminal nerve in humans results in increased levels of CGRP as well as substance P in jugular venous blood (Goadsby *et al.*, 1988), but during migraine only elevated levels of CGRP are found (Goadsby *et al.*, 1990). Interestingly, species differences seem to exist, since in the cat the trigeminally-induced dural vasodilatation predominantly involves entirely different mechanisms (Lambert *et al.*, 1997).

The neurogenic dural vasodilatation in the rat is dose-dependently reduced by sumatriptan and rizatriptan, probably by inhibiting the release of CGRP (Williamson *et al.*, 1997a; 1997b). Sumatriptan and rizatriptan affect neither vessel diameter *per se* nor the dilatation produced by exogenous CGRP or substance P (Williamson *et al.*, 1997a). The 5-HT_1 receptor subtype mediating this effect is likely to be the 5-HT_{1B} receptor, in view of the higher potency of CP93129 compared to sumatriptan (Shepheard *et al.*, 1997a). Stimulation of the trigeminal ganglion also increases blood flow to facial skin (Escott *et al.*, 1995a) and brain (Goadsby *et al.*, 1997) via the release of CGRP (Goadsby, 1993; Escott *et al.*, 1995b). Recently, it was demonstrated that trigeminal stimulation in the cat leads to an increase in nucleus trigeminal caudalis blood flow, being inhibited by intravenously administered sumatriptan (McCall, 1997).

Taken together, it appears that vasodilatation mediated via CGRP release and involving trigeminal sensory Aδ-fibres appears to be a good model for investigating prospective antimigraine drugs. However, it must be realised that the triptans have a low affinity at the rodent 5-HT_{1B} receptor. It is, therefore, of great interest to assess

whether this neurogenic dural vasodilatation occurs in other species and, if so, which receptor mechanism is involved.

Central trigeminal neuronal inhibition

Goadsby and colleagues have shown that i.v. administration of zolmitriptan (Goadsby & Hoskin, 1996), naratriptan (Goadsby & Knight, 1997) and eletriptan (Goadsby & Hoskin, 1999) inhibits action potentials generated in the trigeminal nucleus caudalis after superior sagittal sinus stimulation in the cat (see Table 2.6). Similarly, in the rat rizatriptan (i.v.) inhibits such potentials evoked by dural stimulation (Cumberbatch *et al*., 1997). Thus, these drugs exhibit a central inhibitory effect within the trigeminal system and this may partly contribute to their therapeutic effect in migraine (see Figure 2.4). However, due to its poor central penetration, i.v. sumatriptan did not affect C-*fos m*RNA expression in the trigeminal nucleus caudalis following trigeminal ganglion stimulation in rats (Shepheard *et al*., 1995b). This raises the question whether central trigeminal inhibition is predictive of antimigraine potential. On the other hand, it has been argued, though there is little evidence, that the blood brain barrier gets disrupted during migraine. Indeed, after disruption of blood brain barrier by infusion of hyperosmolar mannitol, sumatriptan can inhibit C-*fos m*RNA expression (Shepheard *et al*., 1995b).

The central trigeminal inhibitory effects of naratriptan in the cat, being susceptible to blockade by GR127935, are mediated by 5-$HT_{1B/1D}$ receptors (Goadsby & Knight, 1997). Since ketanserin displaced zolmitriptan from its binding sites in the cat brain stem, the involvement of 5-HT_{1D} receptors is likely (Mills *et al*., 1995). Also, in rats the central trigeminal antinociceptive action of zolmitriptan is mediated by 5-HT_{1D}, but not 5-HT_{1B} receptors (Cumberbatch *et al*., 1998). Interestingly, CP99994 blocks C-*fos m*RNA expression in the nucleus caudalis in the rat (Shepheard *et al*., 1995b), but GR205171 does not affect the central trigeminal activity as measured electrophysiologically or by C-*fos* expression in the cat (Goadsby *et al*., 1998). Since both compounds are lipophilic tachykinin NK_1 antagonists, the latter results seem to indicate a species dependent involvement of substance P in the central effects. In any case, the non-lipophilic tachykinin NK_1 receptor antagonists lanipetant (Goldstein *et al*., 1997) and RPR100893 (Diener, 1995) are ineffective in the acute treatment of migraine, but the clinical efficacy of brain penetrant tachykinin NK_1 receptor antagonists will hopefully provide further

insights. It has yet to be established whether CGRP is involved in the central trigeminal inhibition and whether CGRP antagonists are effective in acute migraine therapy.

2.6 Conclusion

The seminal discovery of sumatriptan led to the development of new second-generation triptans, which show much improved pharmacokinetics (e.g. higher oral bioavailability, longer half-life) as compared to sumatriptan and this will hopefully contribute to a better tolerability and clinical efficacy. The pharmacodynamics of these second-generation triptans, however, do not seem to differ much from sumatriptan. Moreover, the introduction of sumatriptan has improved the understanding of the disease pathophysiology and led to a growing number of experimental models for migraine. These experimental models aim at achieving drugs that (i) counteract continued cranial extracerebral vasodilatation either by vasoconstriction or by decreasing neuropeptide release at neurovascular synapse; and (ii) inhibit impulse transmission within the trigeminovascular system.

The models to study direct carotid vasoconstriction, particularly involving arteriovenous anastomoses have been highly predictive of therapeutic efficacy in migraine (for details see Chapters 3-9). This cannot be said for the inhibition of neurogenic plasma protein extravasation (mainly involving antidromic release of neurokinins), since several such compounds later proved ineffective in migraine. At least in the rat, triptans do inhibit neurogenic vasodilatation, involving the release of CGRP from Aδ fibres. However, it is yet to be confirmed that CGRP antagonists are effective in acute migraine therapy. Similarly, the antimigraine efficacy of VIP receptor antagonists, which block trigeminally-induced carotid vasodilatation, has not been assessed. Both ergot alkaloids and triptans can interfere with impulse transmission centrally within the trigeminovascular system, but we do not yet know if this property is linked with antimigraine efficacy. For these reasons, detailed results of the ongoing clinical trials with selective 5-HT_{1D} and 5-HT_{1F} receptor antagonists, which are claimed to be devoid of vasoconstrictor activity, are awaited with considerable interest (see Chapter 14).

In view of the research efforts being devoted in developing selective and novel ligands and the use of experimental models incorporating the knowledge of the disease pathophysiology, it is undeniable that acute migraine therapy will continue to

evolve in the next millennium. Selective agonists at the different 5-HT_1 receptor subtypes will be valuable tools in probing the putative antimigraine mechanisms clinically and provide further insights in the pathophysiology of migraine, as will be discussed in Chapter 14. Notwithstanding, we must also make efforts to advance prophylactic drug therapy in migraine.

2.7 Aims of the thesis

Taking Chapters 1 and 2 into account the aims of the present thesis were as follows:

1. To establish the selectivity towards 5-$HT_{1B/1D}$ receptors of the newly developed putative 5-$HT_{1B/1D}$ receptor antagonist, GR127935, in several *in vivo* experimental models.

2. To investigate, using selective agonists and antagonists, whether 5-HT_{1B}, 5-HT_{1D} and/or other receptors resemble the 5-HT_1-like receptors mediating (i) vasoconstriction of porcine carotid arteriovenous anastomoses; (ii) decreases in canine external carotid blood flow; and (iii) inhibition of sympathetic vasopressor outflow in the rat.

3. To investigate whether the 5-HT_1-like or "orphan" 5-HT receptors mediating blood pressure decreases in the rat and heart rate increases in the cat are identical to the recombinant 5-ht_7 receptor.

Chapter 3

Interactions of GR127935, a 5-$HT_{1B/1D}$ receptor antagonist, with functional 5-HT receptors

Summary GR127935 has recently been introduced as an experimental tool to antagonise 5-$HT_{1B/1D}$ receptor-mediated functional responses. The compound indeed exhibits a very high affinity and selectivity for 5-$HT_{1B/1D}$ binding sites and has already shown to antagonise a number of 5-$HT_{1B/1D}$ receptor-mediated responses. The present experiments were performed to investigate the selectivity of GR127935 against functional responses mediated by 5-HT_1-like, 5-HT_2, 5-HT_3, 5-HT_4 or 5-HT_7 receptors in several *in vivo* preparations. Intravenous (i.v.) treatment with GR127935 (300 µg kg^{-1}) potently antagonised decreases in total carotid blood flow as well as hypotensive responses induced by the 5-HT_1-like receptor agonist, sumatriptan, in rabbits. I.v. bolus injections of GR127935 (up to 500 and/or 1500 µg kg^{-1}) did not significantly modify 5-HT-induced: (i) tachycardia in the pig (5-HT_4 receptor-mediated) and cat (5-HT_7 receptor-mediated); (ii) depressor effects in the rat and cat (5-HT_7 receptor-mediated); (iii) von Bezold-Jarisch reflex in the rat or the early phase of the urinary bladder contraction in the cat (both 5-HT_3 receptor-mediated). In contrast, high doses (500-1500 µg kg^{-1}) of GR127935 suppressed 5-HT-induced pressor responses in the rat and cat and urinary bladder contractions (secondary phase) in the cat as well as the DOI-induced pressor responses in the rat, which are all mediated by 5-HT_{2A} receptors. Lastly, 0.5 mg kg^{-1} of GR127935 produced a methiothepin (3 mg kg^{-1})-resistant vasoconstriction of carotid arteriovenous anastomoses in pigs. In conclusion, the present study demonstrates that GR127935 is a potent and selective 5-$HT_{1B/1D}$ receptor antagonist devoid of interactions at 5-HT_3, 5-HT_4 and 5-HT_7 receptors. However, GR127935 possesses a moderate 5-HT_{2A} receptor blocking property, which is consistent with its binding profile (pK_i: 7.4) and seems to display intrinsic activity in the porcine carotid vascular bed. Lastly, in view of the potent antagonist action of GR127935, the sumatriptan-induced hypotension in rabbits seems to be mediated by 5-$HT_{1B/1D}$ receptors.

3.1 Introduction

The present decade has indisputably witnessed a remarkable progress in the classification of 5-HT receptors; this achievement is due not only to the adoption of structural and transductional criteria, but also to the discovery of compounds acting selectively at 5-HT receptors (Hoyer *et al*., 1994; Hoyer & Martin, 1997). For

Based on: De Vries, P., Apaydin, Ş., Villalón, C.M., Heiligers, J.P.C. & Saxena, P.R. (1997). Interactions of GR127935, a 5-$HT_{1B/D}$ receptor ligand, with functional 5-HT receptors. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **355**, 423-430.

example, it is nowadays clear that the cardiovascular effects of 5-HT are mediated by several different receptor types, including 5-HT_1-like, 5-HT_2, 5-HT_3, 5-HT_4 and, as described recently, 5-HT_7 receptors (see Chapter 1). Notwithstanding, the exact identity of 5-HT_1-like receptor mediating vascular contraction is in debate, although several lines of pharmacological evidence (see Hoyer *et al*., 1994) suggest a close resemblance with the 5-$HT_{1B/1D}$ receptor subtypes, formerly designated as 5-$HT_{1Dß}$ and 5-$HT_{1Dα}$, respectively (Hartig *et al*., 1996). This notion has been hampered because of the lack of compounds acting selectively at 5-$HT_{1B/1D}$ receptor subtypes, but the advent of GR127935, a piperazinyl-benzanilide derivative with high affinity for and selective antagonist activity at 5-$HT_{1B/1D}$ receptors (Clitherow *et al*., 1994), will hopefully overcome this obstacle. Indeed, some studies already show that GR127935 potently antagonises: (i) contralateral turning in guinea-pigs induced by the 5-$HT_{1B/1D}$ receptor agonist GR56764 (Skingle *et al*., 1996); (ii) sumatriptan-evoked inhibition of 5-HT release in the guinea-pig dorsal raphe nucleus (Clitherow *et al*., 1994; Starkey & Skingle, 1994); and (iii) sumatriptan-induced contraction of porcine carotid arteriovenous anastomoses (Chapter 4), canine external carotid vessels (Villalón *et al*., 1996), rabbit isolated saphenous vein (Valentin *et al*., 1996) and canine basilar artery (Skingle *et al*., 1996). Several of these responses have been previously described as being mediated by 5-HT_1-like receptors (see Saxena & Villalón, 1990; 1991).

In view of the pharmacological relevance that GR127935 has been acquiring as an experimental tool to identify functional 5-$HT_{1B/1D}$ receptors, we have considered it most crucial to ascertain the selectivity of the drug. For this purpose, the effect of GR127935 has been studied on several functional responses mediated by different 5-HT receptors: (i) sumatriptan-induced decrease in carotid blood flow in the rabbit (5-HT_1-like); (ii) 5-HT and/or DOI-induced pressor responses in the rat and cat as well as the secondary phase of urinary bladder contraction in the cat (5-HT_{2A}); (iii) 5-HT-induced bradycardia (von Bezold-Jarisch reflex) in the rat and the early phase of urinary bladder contraction in the cat (5-HT_3); (iv) 5-HT-induced tachycardia in pigs (5-HT_4); and (v) 5-HT-induced hypotension in the rat and tachycardia in the cat (5-HT_7) (for references see Saxena & Villalón, 1990; 1991; Hoyer *et al*., 1994; Choppin & O'Connor, 1996, Chapter 10 and 12). Lastly, we have studied the effects of GR127935 *per se* on porcine carotid haemodynamics.

3.2 Methods

3.2.1 Experiments in rabbits

Experiments were carried out in 7 male New Zealand White rabbits (2.5-3 kg). After initial anaesthesia with sodium pentobarbital (45 mg kg^{-1}, i.v.) and tracheal cannulation, the rabbits were artificially ventilated with a mixture of oxygen and room air using a respiratory pump (Infant ventilator MK3, HoekLoos, The Netherlands) at a rate of 30 strokes min^{-1} (15 ml kg^{-1}). At this point a continuous infusion of pentobarbital sodium (18 mg kg^{-1} h^{-1}, i.v.) was started. The left femoral artery and vein were dissected free and cannulated for blood pressure measurement with a pressure transducer (Combitrans disposable pressure transducer; Braun, Melsungen, Germany) and the administration of drugs, respectively. Heart rate was measured with a tachograph (CRW, Erasmus University, Rotterdam, The Netherlands) triggered by ECG signals. After cutting both vagosympathetic trunks, the right common carotid artery was dissected free. Blood flow was measured in the right common carotid artery with a flow probe (internal diameter: 1.4 mm) connected to a sine-wave electromagnetic flow meter (Transflow 601-system, Skalar, Delft, The Netherlands). Blood pressure and heart rate were continuously monitored on a polygraph (CRW, Erasmus University, Rotterdam, The Netherlands). After a stable haemodynamic condition for about 30 min, all animals received consecutive bolus injections of sumatriptan (1, 3, 10, 30, 100 µg kg^{-1}, i.v. every 5-15 min). These responses were elicited again 15 min after a bolus injection of physiological saline (0.5 ml, i.v.) and, subsequently, 15 min after an i.v. infusion of GR127935 (300 µg kg^{-1}) over a period of 1 min.

3.2.2 Experiments in rats

Experiments were carried out in 39 male Wistar rats (300-350 g). After initial anaesthesia with ether, the trachea was cannulated and a catheter was placed in the left external jugular vein. At this point, ether anaesthesia was stopped and, subsequently, the animals received i.v. bolus injections of sodium pentobarbital (30-40 mg kg^{-1}). Hereafter, the left carotid artery was cannulated for the recording of blood pressure, using a pressure transducer (Combitrans disposable pressure transducer, Braun, Melsungen, Germany) and the rats were artificially ventilated with a mixture of oxygen and room air using a respiratory pump (Infant ventilator MK3, HoekLoos, The Netherlands) at a rate of 40 strokes min^{-1} (volume: 20 ml kg^{-1}). Heart rate was

measured with a tachograph (CRW, Erasmus University, Rotterdam, The Netherlands) triggered from electrocardiogram signals. Both blood pressure and heart rate were recorded simultaneously on a polygraph (CRW, Erasmus University, Rotterdam, The Netherlands). It should be noted that three experiments were carried out simultaneously; because of limited channels for heart rate, this variable was determined in only one out of three rats.

At this point, the animals were divided into three groups. In the first group both vagus nerves were left intact (n=12), whilst the second and third group underwent sectioning of vagosympathetic trunk bilaterally (n=12 and 15, respectively) to avoid the bradycardia and hypotension caused by 5-HT via the von Bezold Jarisch reflex (Paintal, 1973). After a stable haemodynamic condition for at least 30 min, baseline values of mean blood pressure and heart rate were determined. Then, the first and second group of animals received intravenous (i.v.) bolus injections of 5-HT (3, 10 and 30 μg kg^{-1}) every 5-10 min, while the third group received i.v. bolus injections of DOI (1, 3, 10 and 30 μg kg^{-1}) and the changes produced in mean blood pressure and heart rate were noted. At this point, all groups of animals were subdivided again into two treatment groups, which received i.v. bolus injections of either GR127935 (20, 100 and 500 μg kg^{-1}) or the corresponding volumes of saline (control animals); at the end, the control animals received an i.v. infusion of ritanserin (50 μg kg^{-1}) over a period of about 5 min to establish that the hypertensive responses were amenable to blockade by this 5-HT_2 receptor antagonist. Before each antagonist or saline treatment, the animals received small i.v. bolus injections of pentobarbital (20-30 mg kg^{-1}), necessary to establish equal baseline values of blood pressure. Fifteen to twenty min after each antagonist or saline treatment, the responses to 5-HT and DOI were elicited again.

3.2.3 Experiments in cats

Two cats (4 and 6 kg) were fasted overnight and anaesthetised with ketamine (50 mg kg^{-1}, i.m.) and sodium pentobarbital (30 mg kg^{-1}, i.v.). Anaesthesia was maintained with a continuous infusion of sodium pentobarbital (15-20 mg kg^{-1} h^{-1}, i.v.). Subsequently, the animals were artificially ventilated with a mixture of oxygen and room air using a respiratory pump (Infant ventilator MK3, HoekLoos, The Netherlands). The left femoral vessels were catheterised for the i.v. injection of drugs and measurement of arterial blood pressure with a pressure transducer (Combitrans

disposable pressure transducer, Braun, Melsungen, Germany). Heart rate was measured with a tachograph (CRW, Erasmus University, Rotterdam, The Netherlands) triggered from electrocardiogram signals. Subsequently, the spinal cord (at the level of C_1-C_2) and both vagosympathetic trunks were sectioned, as previously reported (Saxena *et al*., 1985b). Hereafter, the urinary bladder was cannulated with a suitable polyethylene tube inserted into its cavity via a cut in the urethra. Intravesical pressure was monitored by connecting the bladder cannula to another pressure transducer. Blood pressure, heart rate and intravesical pressure were continuously monitored on a polygraph (CRW, Erasmus University, Rotterdam, The Netherlands).

After the animals had been in a stable haemodynamic condition for at least 30 min, bolus injections of 5-HT (3, 10 and 30 μg kg^{-1}, i.v.) were given at intervals of 10-15 min and the changes produced in mean blood pressure, heart rate and urinary bladder pressure were noted. Subsequently, GR127935 (500 μg kg^{-1}, i.v.) was slowly administered over a period of 5 min and the responses to 5-HT were elicited again after 15 min. The responses induced by 5-HT (30 μg kg^{-1}) on the urinary bladder pressure were also analysed after subsequent administrations of ketanserin and MDL72222 (both 0.5 mg kg^{-1}, i.v.) at 15 min interval.

3.2.4 Experiments in pigs

After an overnight fast, 15 domestic pigs (Yorkshire X Landrace; 10-15 kg) were anaesthetised with azaperone (160 mg, i.m.), midazolan hydrochloride (5 mg, i.m.) and metomidate (200 mg, i.v.), intubated and connected to a respirator (BEAR 2E, BeMeds AG, Baar, Switzerland) for intermittent positive pressure ventilation with a mixture of room air and oxygen. Respiratory rate, tidal volume and oxygen supply were adjusted to keep arterial blood gas values within physiological limits (pH: 7.35-7.48; pCO_2: 35-48 mmHg; pO_2: 100-120 mmHg). Anaesthesia was maintained with a continuous i.v. infusion of pentobarbitone sodium at 20 mg kg^{-1} h^{-1} and both vagi and the accompanying cervical sympathetic nerves were cut to avoid possible reflex changes. Catheters were placed in the inferior vena cava via the left femoral vein for the administration of drugs and in the aortic arch via the left femoral artery for the measurement of arterial blood pressure (Combitrans disposable pressure transducer; Braun, Melsungen, Germany). Heart rate was measured with a tachograph (CRW, Erasmus University, Rotterdam, The Netherlands) triggered from electrocardiogram signals. Blood pressure and heart rate were continuously

monitored on a polygraph (CRW, Erasmus University, Rotterdam, The Netherlands). During the experiment, body temperature was kept at about 37°C and the animals were continuously infused with saline to compensate for fluid losses.

After the animals had been in a stable haemodynamic condition for at least 30 min, the first group (n=5) was given methiothepin (500 µg kg^{-1}, i.v.) in order to block 5-HT-induced changes in arterial blood pressure without affecting heart rate responses (Bom *et al*., 1988). After 10 min, i.v. bolus injections of 5-HT (10 and 30 µg kg^{-1}) were administered at intervals of 10-15 min and the changes produced in heart rate were noted (for further details see Villalón *et al*., 1990b; 1991). Subsequently, GR127935 (500 µg kg^{-1}, i.v.) was slowly administered over a 5 min period and the responses to 5-HT were elicited again.

In the second and third groups (n=5 each) baseline values of heart rate, mean arterial blood pressure, total carotid blood flow and its distribution into the non-nutrient (arteriovenous anastomotic) and nutrient fractions were measured, using the radioactive microsphere technique (for details, see Chapter 4). Subsequently, the second group received 3 mg kg^{-1} (i.v.) of methiothepin, whereas the third group received the corresponding volume (5 ml) of physiological saline. Thereafter, in both groups 0.5 mg kg^{-1} (i.v.) of GR127935 was administered over a period of 5 min. All haemodynamic parameters were reassessed 10 min after the administration of GR127935. The protocols of the investigation were approved by the joint Ethical Committee of the Erasmus University Rotterdam and the University Hospital Rotterdam "Dijkzigt" dealing with the use of animals in scientific experiments.

3.2.5 Data presentation and statistical evaluation

All data in the text and the illustrations are presented as the mean±s.e.mean. The changes and percent changes from baseline in blood pressure, heart rate and blood flows by the different doses of the agonists/antagonists were calculated in each experiment. The changes from baseline values within one group by the different doses of the drugs used were evaluated using Duncan's new multiple range test, once an analysis of variance (randomised block design) had revealed that the samples represented different populations (Steel & Torrie, 1980). The changes caused by the drugs after administration of an antagonist dose were compared to the corresponding changes before antagonist administration by the use of Student's unpaired or paired

t-test, where appropriate. A P-value of 0.05 or less (two-tailed) was considered statistically significant.

3.2.6 Drugs

Apart from the anaesthetics, azaperone, metomidate (both from Janssen Pharmaceutica, Beerse, Belgium), midazolan hydrochloride (Hoffmann La Roche b.v., Mijdrecht, The Netherlands) and pentobarbitone sodium (Apharmo, Arnhem, The Netherlands), the drugs used in this study (obtained from the sources indicated) were: GR127935 and sumatriptan succinate (both from Glaxo Group Research, Ware, UK); 5-HT creatinine sulphate (Sigma Chemical Company, St. Louis, MO, USA); methiothepin maleate (Hoffmann La Roche b.v., Mijdrecht, The Netherlands); DOI and ritanserin (both from RBI, Natick, USA); ketanserin tartrate (Janssen Pharmaceutica, Beerse, Belgium); MDL72222 (Merrell Dow Research Institute, Strasbourg, France) and heparin sodium (Leo Pharmaceutical Products, Weesp, The Netherlands) to prevent clotting of the catheters. GR127935 was solubilised according to the instructions of the supplier by heating the dispersion in distilled water to about 70ºC for 10 s and then allowing to cool down to room temperature. Ritanserin was dissolved in distilled water (30% methanol). The other drugs were dissolved in physiological saline. All doses refer to the respective salts, whereas those of 5-HT and ritanserin refer to the free base.

3.3 Results

3.3.1 Effect of sumatriptan on total carotid blood flow, heart rate and mean arterial blood pressure in rabbits before and after GR127935

As depicted in Figure 3.1, sumatriptan (1-100 µg kg^{-1}, i.v.) elicited dose-dependent decreases in the total carotid blood flow (maximum change 32±5%; baseline 53.9±7.8 ml min^{-1}) and concomitant increases in carotid vascular resistance (maximum change 13±3%, baseline 1.6±0.2 mmHg ml^{-1} min^{-1}). Moreover, sumatriptan produced dose-dependent decreases in mean arterial blood pressure (maximum change 25±5%; baseline 74.7±2.4 mmHg); baseline heart rate (275±16 beats min^{-1}) was not altered by sumatriptan. The percent changes in these variables (pre-saline values of carotid blood flow, carotid vascular resistance and blood pressure were 46.0±9.3 ml min^{-1}, 1.9±0.3 mmHg ml^{-1} min^{-1} and 76.7±3.9 mmHg, respectively) induced by the same doses of sumatriptan about 15 min after the administration of physiological saline were not significantly different, except for a small change in the response to 30 µg kg^{-1} sumatriptan on carotid blood flow and arterial blood pressure. Subsequent treatment with GR127935 (300 µg kg^{-1}, i.v.) potently antagonised the sumatriptan-induced changes in common carotid blood flow (maximum change -5±3%), vascular resistance (maximum change -1±1%) and mean arterial blood pressure (maximum change -6±3%) (Figure 3.1). Post-GR127935 values of carotid blood flow, carotid vascular resistance and blood pressure were 45.8±8.9 ml min^{-1}, 1.9±0.3 mmHg ml^{-1} min^{-1} and 72.3±3.3 mmHg, respectively and, thus, contrary to results obtained in the porcine carotid vascular bed (see below) and some earlier reports (Pauwels & Colpaert, 1995; Watson *et al*., 1996), GR127935 did not show any intrinsic activity in rabbits.

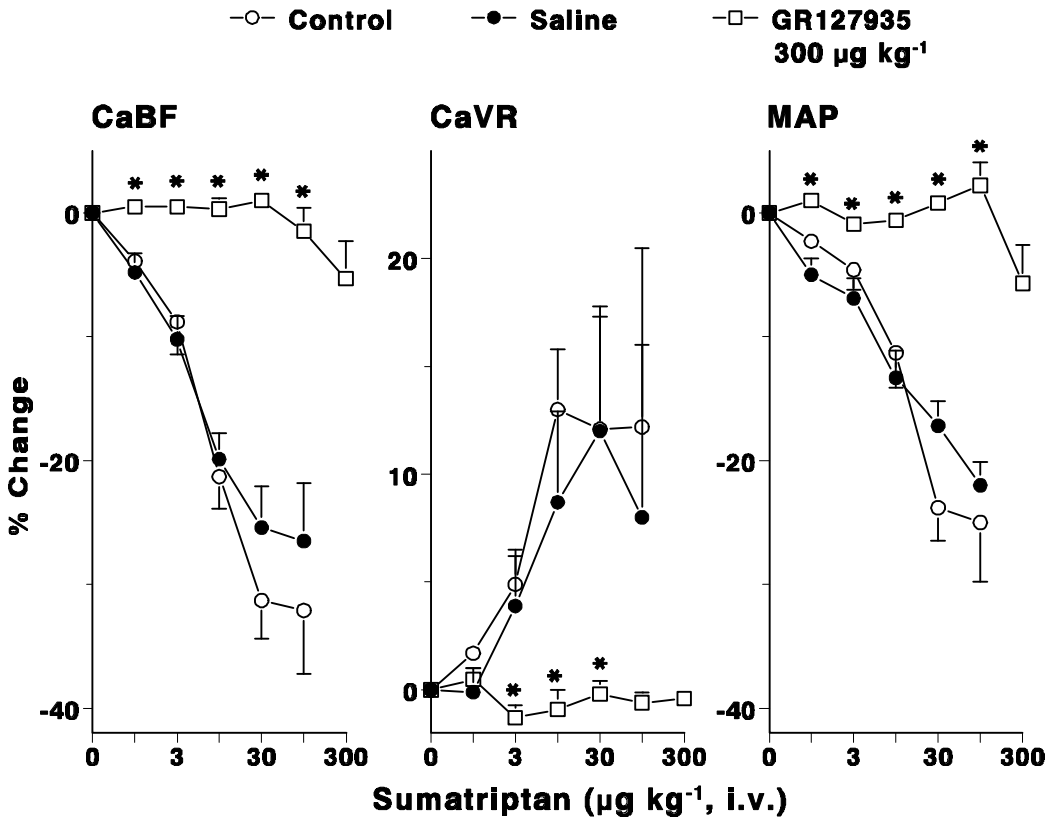

Figure 3.1 Percentage changes from baseline values by sumatriptan in carotid blood flow (CaBF), vascular resistance (CaVR) and mean arterial blood pressure (MAP) in rabbits (n=7), obtained before and after treatment with saline and GR127935. *, P<0.05 vs the corresponding dose of sumatriptan before treatments.

3.3.2 Cardiovascular effects of 5-HT in intact and vagosympathectomised rats and of DOI in vagosympathectomised rats before and after GR127935

Baseline values

The baseline values of mean arterial blood pressure and heart rate in the three groups of rats (see Methods section) were, respectively, 111±2, 101±2 and 86±2 mmHg and 265±13, 298±18 and 216±29 beats min^{-1}. These variables were not significantly modified by the subsequent injection of GR127935 (20, 100, 500 and 1500 µg kg^{-1}) or the corresponding volumes of physiological saline (data not shown).

Animals with intact vagus

As depicted in Figure 3.2, 5-HT produced a triphasic effect on arterial blood pressure in rats with intact vagus, comprising of an initial hypotension associated with a brief, but intense, bradycardia (not shown in the Figure) via the von Bezold-Jarisch reflex, followed by a pressor effect and, finally, a longer-lasting hypotension. These responses were reproducible and remained essentially unchanged in control animals receiving 3 doses of saline (Figure 3.2, *upper panel*). Treatment with ritanserin (50 µg kg^{-1}, i.v.) completely antagonised the pressor responses, while the early and late hypotensive responses were significantly potentiated (data not shown).

GR127935 (100 and 500 µg kg^{-1}), which showed no agonist effect, significantly attenuated the pressor effects induced by the highest dose of 5-HT. When compared to the respective responses in the saline-treated control animals, only at 500 µg kg^{-1} of GR127935 a significant attenuation of the response to the highest dose of 5-HT was observed. Additionally, the pressor effects induced by 3 µg kg^{-1} of 5-HT were slightly, but significantly potentiated by GR127935 (20 and 100 µg kg^{-1}). Similarly, GR127935 (100 and 500 µg kg^{-1}) slightly enhanced the late depressor responses induced by the lowest dose of 5-HT (Figure 3.2, *lower panel*).

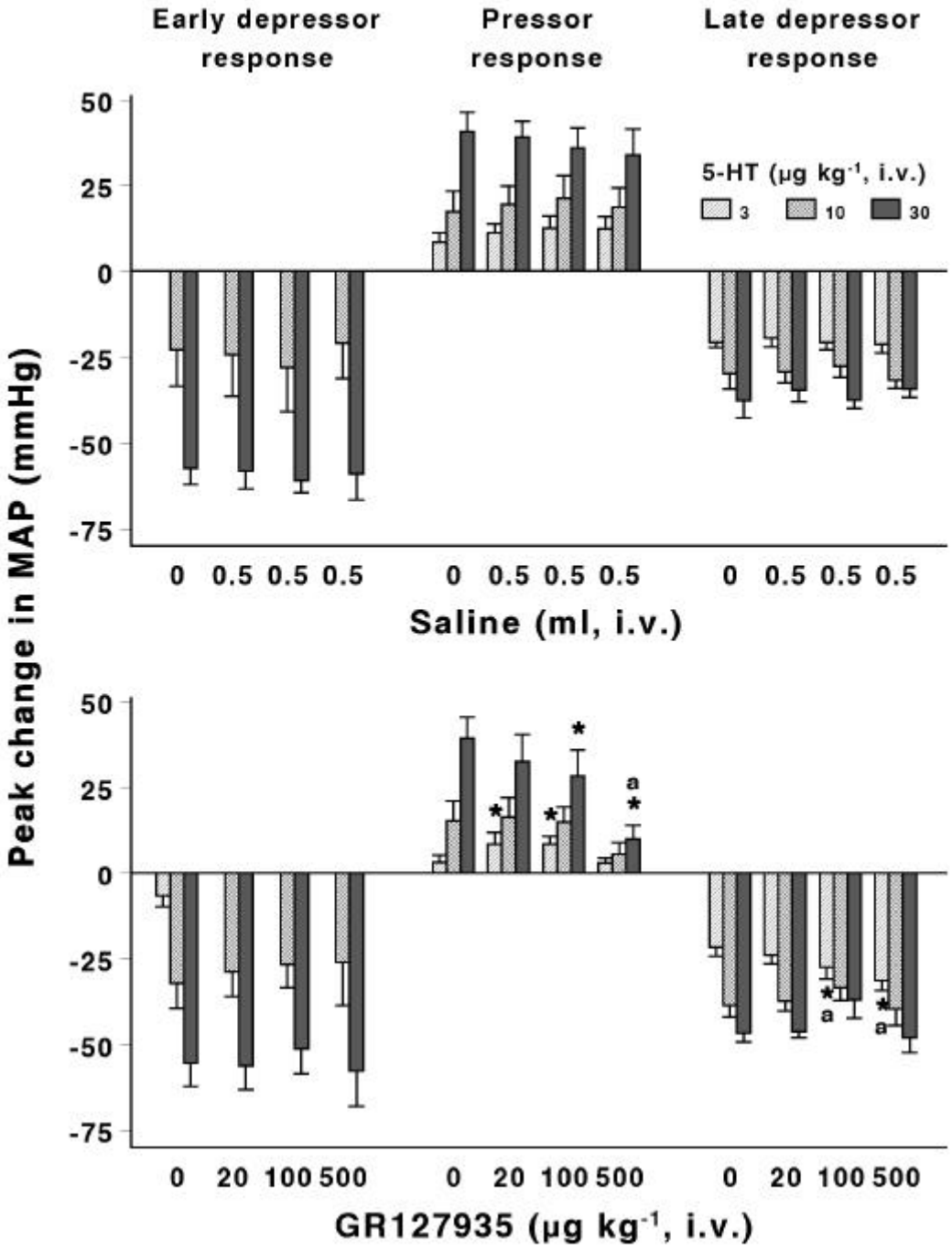

Figure 3.2 Effects of either saline (*upper panel*; control; n=6) or GR127935 (*lower panel*; n=6) on the triphasic blood pressure response to 5-HT in rats with intact vagi. *, P<0.05 vs first curve to 5-HT; a, P<0.05 vs the response by corresponding dose in control animals.

Vagotomised animals

After section of both vagosympathetic trunks, 5-HT no longer produced bradycardia or the associated initial hypotensive response, but the pressor responses, which became more pronounced, and the late hypotensive responses were present. These responses were reproducible and remained essentially unchanged in control animals receiving 3 subsequent doses of saline (Figure 3.3, *upper panels*); after additional treatment with ritanserin (50 µg kg^{-1}, i.v.) the pressor response was completely abolished, while the late depressor response was significantly potentiated (data not shown). As shown in the *lower panel* of Figure 3.3, under these experimental conditions, GR127935 (500 and, primarily, 1500 µg kg^{-1}) produced a significant blockade of the pressor responses to 5-HT.

As depicted in Figure 3.4, the selective 5-HT_{2A} receptor agonist DOI exclusively produced dose-dependent pressor responses in vagosympathectomised rats. These responses were reproducible, but slightly (though significantly) decreased in control animals receiving 3 subsequent doses of saline (Figure 3.4, *upper panel*). The responses to DOI, particularly to 3 and 10 µg kg^{-1}, i.v., were significantly more attenuated by treatment with GR127935 (100 and 500 µg kg^{-1}, i.v.) than was the case in the saline-treated animals (Figure 3.4, *lower panel*).

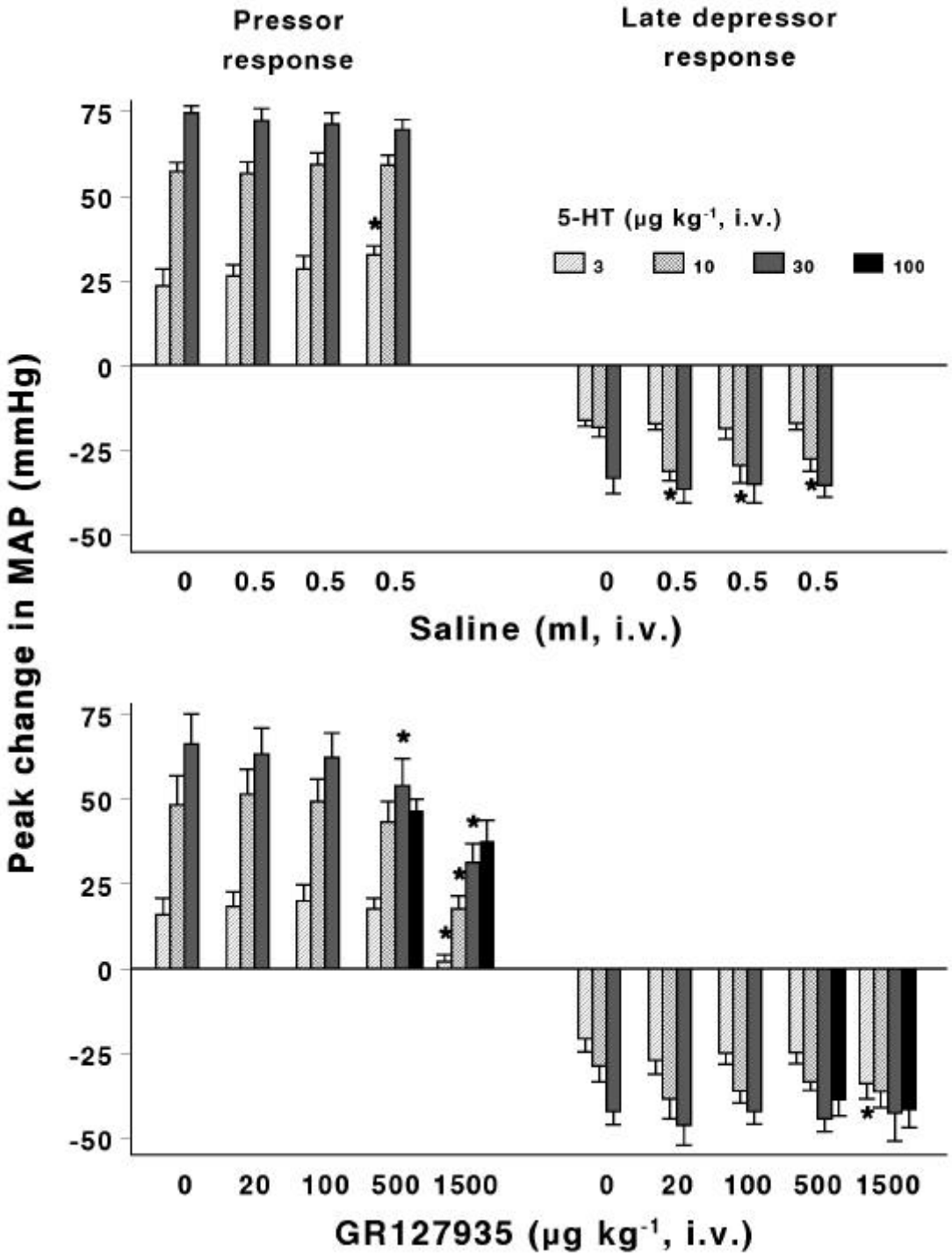

Figure 3.3 Effects of either saline (*upper panel*; control; n=6) or GR127935 (*lower panel*; n=6) on the blood pressure response to 5-HT in vagotomised rats. *, P<0.05 vs first curve to 5-HT; a, P<0.05 vs the response by corresponding dose in control animals.

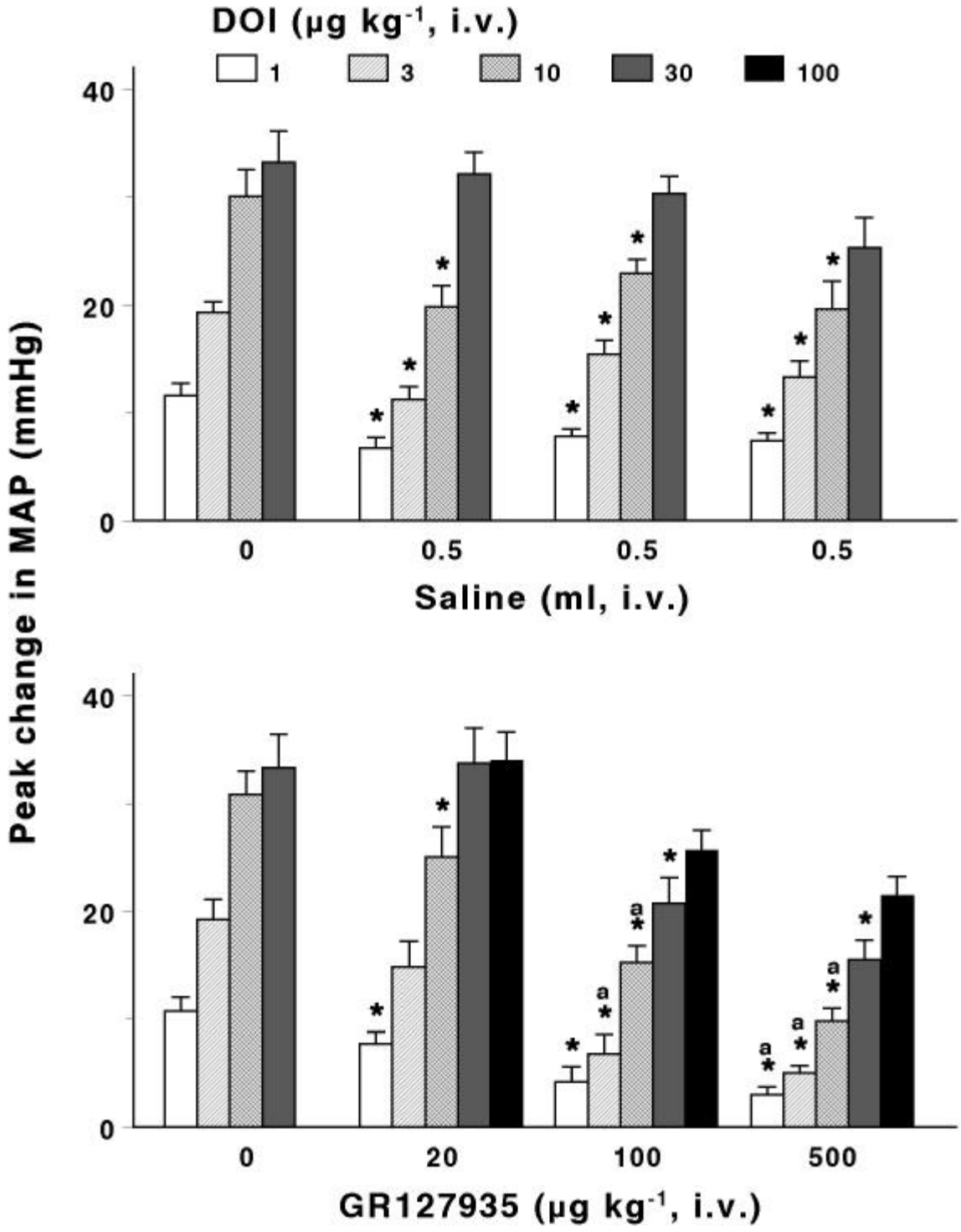

Figure 3.4 Effects of either saline (*upper panel*; control; n=6) or GR127935 (*lower panel*; n=6) on the pressor response to DOI in vagotomised rats. *, P<0.05 vs first curve to 5-HT; a, P<0.05 vs the response by corresponding dose in control animals.

3.3.3 Effects of 5-HT on heart rate, mean arterial blood pressure and intravesical pressure in spinal cats before and after GR127935

I.v. administration of 5-HT (3, 10 and 30 µg kg^{-1}) produced dose-dependent increases in heart rate, mean arterial blood pressure and intravesical pressure. After GR127935 (500 µg kg^{-1}) the increases in heart rate (26, 55 and 72 beats min^{-1} for cat 1 and 7, 31, 49 beats min^{-1} for cat 2) remained unaltered (23, 49 and 66 beats min^{-1} for cat 1 and 4, 30 and 47 beats min^{-1} for cat 2), but the increases in mean arterial blood pressure (10, 21 and 42 mmHg for cat 1 and 1, 15 and 40 mmHg for cat 2) seemed to be attenuated (9, 11 and 5 mmHg for cat 1 and 5, 4 and 12 mmHg for cat 2).

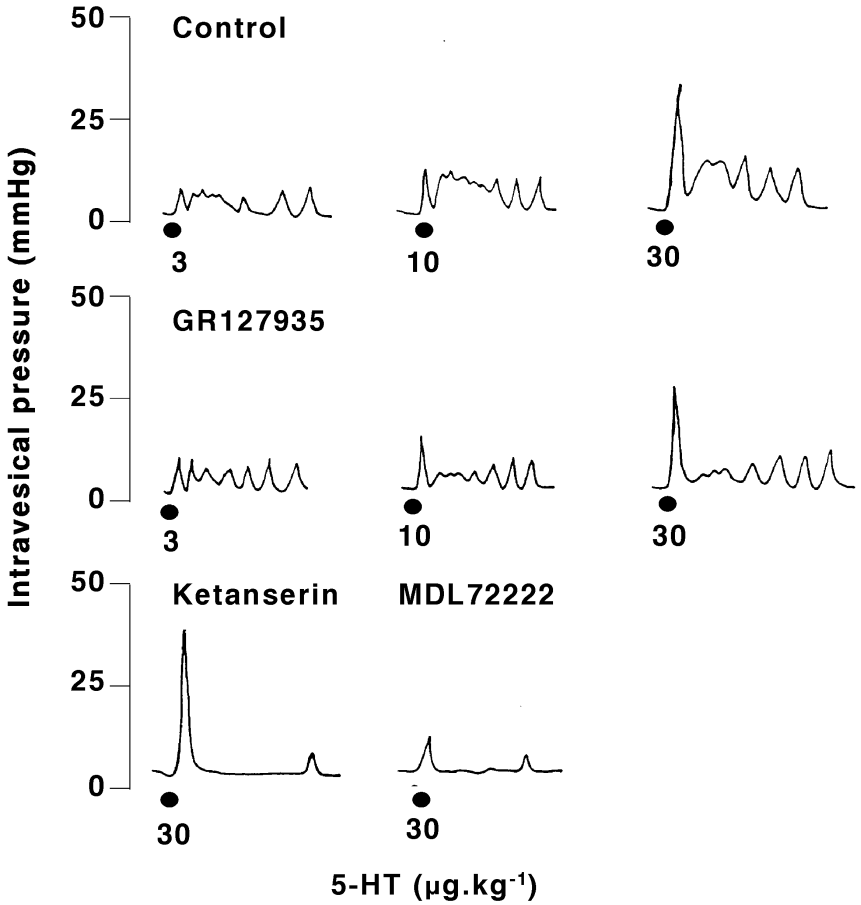

Figure 3.5 Tracings illustrating the effects by 5-HT on feline intravesical pressure before (Control) and after 0.5 mg kg^{-1} of GR127935. Note that 5-HT produced a biphasic response and that only the secondary phase was attenuated by GR127935. Ketanserin (0.5 mg kg^{-1}) and MDL72222 (0.5 mg kg^{-1}) blocked the second and third phase, respectively.

As has been previously reported (Saxena *et al.*, 1985a), the contractile responses of the cat urinary bladder induced by 5-HT (3, 10 and 30 µg kg-1) were biphasic, consisting of an initial spike followed by a longer-lasting (secondary) increase in intravesical pressure (Figure 3.5). 0.5 mg kg-1 of GR127935 did not modify the first phase of the increase in intravesical pressure, but seemed to attenuate the second contractile phase. This remaining response to 5-HT (30 µg kg-1) was changed after the administration of ketanserin (500 µg kg-1, i.v.) into a monophasic (spike) response, which was then markedly antagonised by the subsequent administration of MDL 72222 (Figure 3.5).

3.3.4 Tachycardiac responses to 5-HT in pigs

Consistent with previous findings (Villalón *et al.*, 1990b; Villalón *et al.*, 1991), i.v. bolus injections of 5-HT (10 and 30 µg kg^{-1}) resulted in dose-dependent increases in heart rate of, respectively, 45±8 and 70±9 beats min^{-1}. These tachycardiac responses to 5-HT were not blocked after administration of 500 µg kg^{-1} of GR127935 (59±7 and 73±9 beats min^{-1}, respectively).

3.3.5 Effects of GR127935 per se on carotid haemodynamic in pigs

As shown in Figure 3.6, 0.5 mg kg^{-1} (i.v.) of GR127935 decreased total carotid and arteriovenous anastomotic blood flow, without significantly affecting the nutrient fraction. Additionally, the drug slightly decreased mean arterial blood pressure (10±2%), heart rate (4±1%) and increased the arterio-jugular venous oxygen saturation difference (A-V SO_2) by 82±13%. In animals pretreated with methiothepin (3 mg kg^{-1}), the GR127935-induced decreases in total carotid and arteriovenous anastomotic blood flows were not modified. After methiothepin, GR127935 increased mean arterial blood pressure (13±3%) and decreased heart rate (-8±1%; both $P<0.05$ vs before methiothepin; the change in A-V SO_2 was not modified.

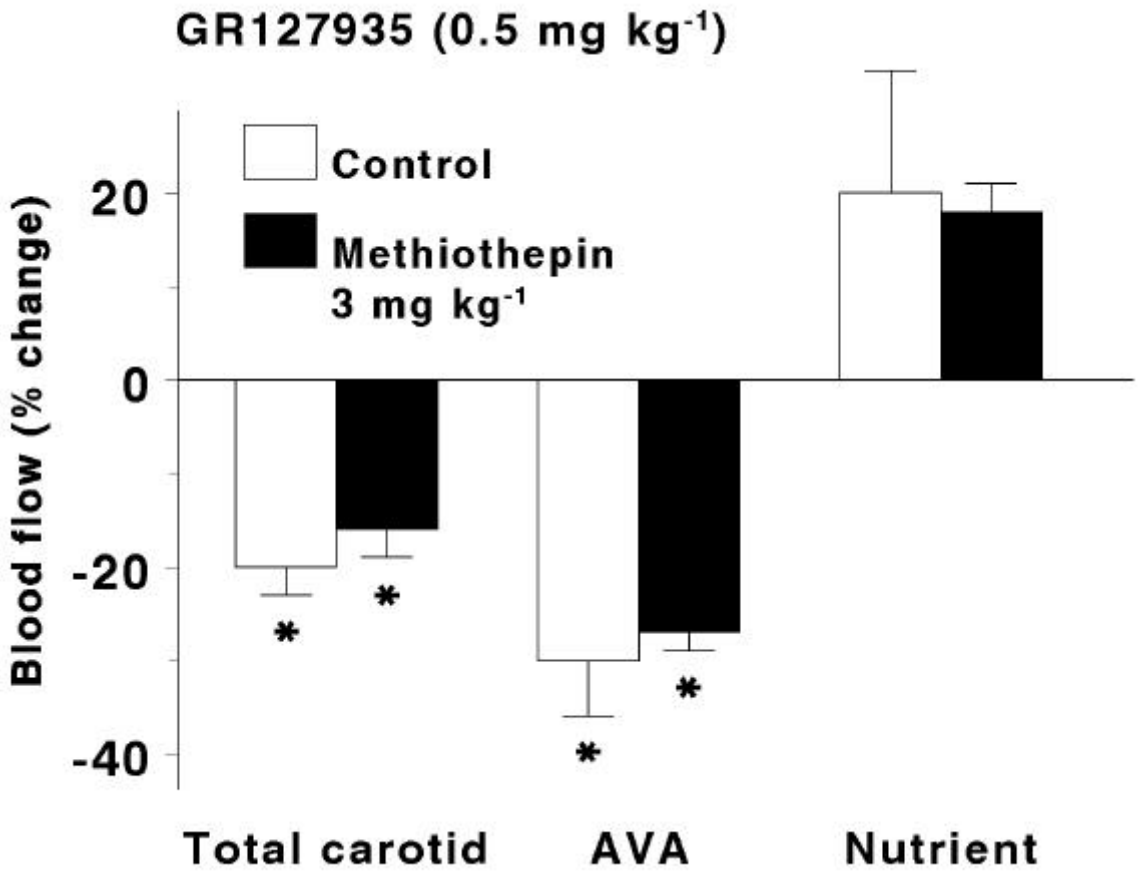

Figure 3.6 Effect of GR127935 on total carotid, arteriovenous anastomotic (AVA) and nutrient blood flows in pigs pretreated (i.v.) with either saline (Control) or methiothepin. *, P<0.05 vs baseline.

3.4 Discussion

3.4.1 General

The lack of availability of selective 5-$HT_{1B/1D}$ receptor antagonists has been felt as an impediment in investigating the relationship between the 5-HT_1-like and 5-$HT_{1B/1D}$ receptors (see Saxena & Villalón, 1990; Den Boer *et al*., 1992b). Thanks to the advent of GR127935, it is becoming increasingly evident that sumatriptan-sensitive 5-HT_1-like receptors do indeed resemble the 5-$HT_{1B/1D}$ subtype, with the remaining 5-HT_1-like receptors resembling the pharmacological profile of the 5-ht_6 or 5-HT_7 receptors or even conforming the operational characteristics of "orphan" 5-HT receptors (Hoyer *et al*., 1994).

Binding studies show that GR127935 displays high affinities for human 5-HT_{1D}, human 5-HT_{1B} and rat 5-HT_{1B} receptors (pK_i: 8.9, 9.9 and 8.5, respectively), moderate affinity for 5-HT_{2A} receptors (pK_i: 7.2) and very low affinity for 5-HT_3, 5-HT_4 and 5-ht_5 receptors (pK_i < 5.2) (see Pauwels, 1996). There is only limited information available concerning the selectivity of GR127935 in functional studies and, therefore, the present investigation deals with this issue. However, when interpreting the results, one should keep in mind that the activity of GR127935 at different 5-HT receptors is based only on binding data for human and rat 5-HT receptors; binding data of GR127935 for the cat, pig and rabbit 5-HT receptors are, at this moment, not available.

3.4.2 Effect of sumatriptan on total carotid blood flow, heart rate and mean arterial blood pressure in rabbits before and after GR127935

As described by Choppin and O'Connor (1996), sumatriptan dose-dependently decreased total carotid blood flow in anaesthetised rabbits. These authors showed that this effect was antagonised by methiothepin, suggestive for the involvement of 5-HT_1-like receptors. Taking into account the high affinity of sumatriptan, but also of methiothepin for 5-$HT_{1B/1D}$ receptors (Beattie *et al.*, 1994), it is argued that these 5-HT_1-like receptors are identical to 5-$HT_{1B/1D}$ receptors. Indeed, we have recently shown that the sumatriptan-induced decreases in porcine carotid blood flow are abolished by GR127935 and, therefore, mediated by 5-$HT_{1B/1D}$ receptors (see Chapter 4). In agreement with this, the present results demonstrate that the sumatriptan-induced reduction in the rabbit carotid blood flow, which was amenable to blockade by GR127935, involves 5-HT_1-like receptors resembling 5-$HT_{1B/1D}$ receptors. Interestingly, sumatriptan produced dose-dependent hypotensive responses and, in view of the potent antagonist action of GR127935, the involvement of 5-$HT_{1B/1D}$ receptors is likely.

3.4.3 Effects of GR127935 on 5-HT-induced cardiovascular responses in the rat

As previously described by Saxena and Lawang (1985), i.v. administration of 5-HT produced a triphasic response on the arterial blood pressure of rats with intact vagus nerves. This response consists of an initial hypotension associated with a brief, but intense, bradycardia via the von Bezold-Jarisch reflex (mediated by 5-HT_3 receptors), followed by a vasopressor effect (mediated by 5-HT_{2A} receptors) and, finally, a

longer-lasting hypotension. As described in detail in Chapter 10, the late hypotension is mediated by 5-HT_7 receptors, previously classified as 5-HT_1-like or "orphan" receptors (see Chapter 1; Saxena & Villalón, 1990; 1991). After bilateral resection of both vagosympathetic trunks, the initial hypotension due to reflex bradycardia was abolished, consistent with the concept that the von Bezold-Jarisch reflex is elicited by stimulation of 5-HT_3 receptors located on sensory vagal nerve endings in the heart (Saxena & Villalón, 1990; 1991). Our finding that only the vasopressor response to 5-HT elicited in intact as well as vagosympathectomised rats was significantly antagonised by 500 and 1500 µg kg^{-1} of GR127935, suggests that the drug produces a moderate blockade of vascular 5-HT_{2A} receptors. Moreover, the use of DOI, a compound acting selectively at 5-HT_2 receptors, enabled us to study the pressor response without the influence of the initial and late hypotensive responses. Indeed, GR127935 dose-dependently antagonised DOI-induced pressor responses. The latter blockade is in keeping with the affinity of GR127935 for 5-HT_{2A} binding sites (see above). However, it may be remarked that Skingle *et al.* (1996) have reported that GR127935 (1-10 mg kg^{-1}, s.c.) did not affect wet dog shakes induced by DOI (3 mg kg^{-1}, s.c.) in the guinea-pig, a response mediated by 5-HT_2 receptors (Skingle *et al.*, 1991).

The fact that GR127935 failed to antagonise both the initial transient hypotensive (in intact animals) and the late hypotensive responses to 5-HT in intact and vagosympathectomised rats implies that the drug does not interact with, respectively, 5-HT_3 and 5-HT_7 receptors.

3.4.4 Effects of 5-HT on the urinary bladder of spinal cats before and after GR127935

Saxena *et al.* (1985a) have previously shown in anaesthetised cats that intra-arterial administration of 5-HT elicits a biphasic contractile response of the urinary bladder, consisting of an initial spike followed by a longer-lasting increase in the intravesical pressure. These early and late phases of the cat urinary bladder contraction elicited by 5-HT are mediated, respectively, by 5-HT_3 receptors (amenable to blockade by MDL72222) located on parasympathetic ganglia and 5-HT_2 receptors (amenable to blockade by ketanserin, cyproheptadine or methysergide) located on smooth muscle (Saxena *et al.*, 1985a). The results of the present study confirm that the contractile response of the cat urinary bladder to 5-HT is biphasic in nature (Figure 3.5). Since

GR127935 did not significantly antagonise the early contractile phase, it is concluded that the drug does not interact with 5-HT_3 receptors located on parasympathetic ganglia. In contrast, GR127935 moderately attenuated the 5-HT-induced secondary phase of urinary bladder contraction. In conformity with its moderate affinity for 5-HT_{2A} binding sites (Skingle *et al.*, 1996) as well as the attenuation of pressor responses to 5-HT and DOI (see above), these results show that high doses of GR127935 can antagonise functional 5-HT_{2A} receptors located on the cat urinary bladder smooth muscle. Indeed, for this reason we decided to confirm the involvement of both 5-HT_2 and 5-HT_3 receptors by using their respective antagonists ketanserin and MDL72222 (Figure 3.5) after treatment with GR127935. Thus, the fact that a monophasic response was obtained after ketanserin, and that this latter response was markedly blocked after MDL72222 unequivocally demonstrates the involvement of both 5-HT_2 and 5-HT_3 receptors.

3.4.5 Effects of 5-HT on heart rate and mean blood pressure of spinal cats before and after GR127935

It has previously been shown that the increases in heart rate produced by 5-HT in the cat, being mimicked by 5-carboxamidotryptamine, are mediated by 5-HT_1-like receptors (Saxena *et al.*, 1985b; Saxena, 1988). Recently, however, it has been demonstrated that these 5-HT_1-like receptors closely resemble the recombinant 5-HT_7 receptor (Chapter 12). Thus, our finding demonstrating that the drug failed to antagonise 5-HT-induced tachycardia in cats implies that GR127935 does not interact with cardiac 5-HT_7 receptors in the cat. In keeping with this, the drug displays low affinity (pK_i: 5.5) at the 5-HT_7 receptor and did not affect the late hypotensive response in rats (see above; Chapter 10).

The antagonism of the pressor responses by GR127935 is in keeping with the findings described above, that GR127935 displays moderate affinity for 5-HT_{2A} receptors.

3.4.6 Effects of GR127935 on 5-HT-induced porcine tachycardia

It has been well characterised that the 5-HT-induced tachycardia in the pig is mediated by 5-HT_4 receptors (Duncker *et al.*, 1985; Bom *et al.*, 1988; Villalón *et al.*, 1990b; 1991), which are positively coupled to adenylyl cyclase (Chapter 1). In the present investigation, the animals were deliberately vagosympathectomised (to avoid reflex

bradycardia) and pretreated with methiothepin (500 µg kg^{-1}) to block 5-HT_1, 5-HT_2 and 5-HT_7 receptors, which may mediate vasopressor and depressor responses. Under these experimental conditions, i.v. bolus injections of 5-HT produced dose-dependent increases in heart rate which were resistant to antagonism by GR127935. Therefore, we conclude that GR127935 does not interact with cardiac 5-HT_4 receptors in the pig.

3.4.7 The effects of GR127935 per se in the porcine carotid vascular bed

The present results demonstrate that GR127935 by itself constricts the porcine carotid vascular bed by a selective action on the arteriovenous anastomoses. Several studies have described the partial agonist properties of GR127935 at h5-HT_{1B} and h5-HT_{1D} receptors *in vitro* (Walsh *et al*., 1995; Pauwels, 1996; Watson *et al*., 1996) and, *in vivo*, at (unknown) receptors mediating inhibition of neurogenic plasma extravasation in the guinea-pig (Yu *et al*., 1997). Interestingly, the 5-$HT_{1B/1D}$ receptors do not seem to be involved in the porcine carotid vasculature, since this effect was resistant to the antagonist action of methiothepin, using a dose capable of abolishing the sumatriptan-induced carotid vascular effects (Den Boer *et al*., 1991b). Therefore, it seems that the intrinsic activity of the compound is not related to 5-HT_2 or 5-HT_1 receptors, including partial agonist properties at the 5-$HT_{1B/1D}$ receptor subtypes and this requires further investigation.

In conclusion, the present study demonstrates that the piperazinyl-benzanilide derivative, GR127935, is a selective 5-$HT_{1B/1D}$ receptor antagonist devoid of interactions at 5-HT_3, 5-HT_4 and 5-HT_7 receptors. However, GR127935 possesses moderate 5-HT_{2A} blocking properties which are consistent with its binding profile.

Chapter 4

Blockade of porcine carotid vascular responses to sumatriptan by GR127935, a selective 5-$HT_{1B/1D}$ receptor antagonist

Summary It has previously been shown that the antimigraine drug sumatriptan, a putative 5-$HT_{1B/1D}$ receptor agonist, decreases porcine common carotid and arteriovenous anastomotic blood flows, but slightly increases the arteriolar (nutrient) blood flow to the skin and ears. Interestingly, such responses, being mediated by 5-HT_1-like receptors, are resistant to blockade by metergoline, which, in addition to displaying a very high affinity for (and occasionally intrinsic efficacy at) the 5-$HT_{1B/1D}$ receptor subtypes, blocks (with lower potency than methiothepin) some 5-$HT_{1B/1D}$ receptor-mediated vascular responses. These findings raise doubts whether sumatriptan-sensitive 5-HT_1-like receptors mediating changes in the distribution of porcine carotid blood flow are identical to cloned 5-$HT_{1B/1D}$ receptors. With the recent advent of the potent and selective 5-$HT_{1B/1D}$ receptor antagonist, GR127935, the present study has now analysed if the carotid vascular effects of sumatriptan in the pig are amenable to blockade by GR127935. In animals pretreated with saline, sumatriptan (30, 100 and 300 μg kg^{-1}, i.v.) reduced the total carotid and arteriovenous anastomotic blood flows in a dose-dependent manner. In contrast, sumatriptan increased blood flow to the skin, ears and fat, although the total capillary fraction was not significantly affected. While GR127935 (0.25 or 0.5 mg kg^{-1}) itself slightly reduced the total carotid and arteriovenous anastomotic blood flows, carotid vasoconstrictor responses to sumatriptan were either partly (0.25 mg kg^{-1}) or completely (0.5 mg kg^{-1}) blocked by the compound. In GR127935-pretreated animals, the sumatriptan-induced increases in blood flow to the skin, ears and fat were also attenuated. Taken together, the above results suggest that arteriovenous anastomotic constriction and, possibly, arteriolar dilatation in the skin, ears and fat by sumatriptan are mediated by 5-$HT_{1B/1D}$ receptors. Therefore, vascular 5-HT_1-like receptors in the porcine carotid bed appear to be identical to 5-$HT_{1B/1D}$ receptors.

4.1 Introduction

Sumatriptan is a 5-HT_1-like receptor agonist (Humphrey *et al*., 1988; 1990; Hoyer *et al*., 1994) effective in the acute treatment of migraine headaches (The Subcutaneous Sumatriptan International Study Group, 1991; Ferrari & Saxena, 1993b). Several studies have shown that the drug produces constriction of large cerebral and extracerebral blood vessels (e.g. Feniuk *et al*., 1989; Caekebeke *et al*., 1992; Villalón *et al*., 1995c), including porcine carotid arteriovenous anastomoses

Based on: De Vries, P., Heiligers, J.P.C., Villalón, C.M. & Saxena, P.R. (1996). Blockade of porcine carotid vascular response to sumatriptan by GR127935, a selective 5-HT_{1D} receptor antagonist. *Br. J. Pharmacol.*, **118**, 85-92.

(Den Boer *et al*., 1991b; 1992b), as also shown for the antimigraine drugs, ergotamine and dihydroergotamine (Den Boer *et al*., 1991a; Villalón *et al*., 1992). The constriction of porcine cranial arteriovenous anastomoses by sumatriptan and, partly, by the ergot alkaloids is mediated via the 5-HT_1-like receptor because these effects are antagonised, either partially (ergot alkaloids) or fully (sumatriptan), by methiothepin, but not by ketanserin (Den Boer *et al*., 1991a; 1991b). The 5-HT_1-like receptor mediating vasoconstriction has not yet been cloned and, therefore, its exact identity is in debate. In view of the high affinity (see Table 4.1) of sumatriptan, but also of methiothepin, for 5-HT_{1B} and 5-HT_{1D} receptors (previously called 5-$HT_{1D\beta}$ and 5-$HT_{1D\alpha}$, respectively; see Chapter 1), it is argued that sumatriptan-induced vasoconstriction is mediated by 5-$HT_{1B/1D}$ receptors (e.g. Hamel & Bouchard, 1991), implying that 5-HT_1-like and 5-$HT_{1B/1D}$ receptors are identical. In contrast, the IUPHAR 5-HT receptor classification scheme (Hoyer *et al*., 1994) recognises the 5-HT_1-like receptor as a distinct entity, separate from any of the 5-HT_1 receptor subtypes, including 5-HT_{1B} and 5-HT_{1D} receptors, identified by radioligand binding and cloning techniques. One of the main reasons for this distinction is that metergoline, which has an even higher affinity than methiothepin for 5-$HT_{1B/1D}$ receptors (see Table 4.1), does not or only weakly antagonises sumatriptan-induced vasoconstrictor responses, both *in vivo* (Villalón *et al*., 1990a; Perren *et al*., 1991; Den Boer *et al*., 1992b; Villalón *et al*., 1995c) and *in vitro* (Hamel & Bouchard, 1991; Perren *et al*., 1991; Bax *et al*., 1992a; Deckert *et al*., 1994).

Recently, a series of piperazinylbenzanilide derivatives with high affinity for and antagonist activity at 5-$HT_{1B/1D}$ receptors has been described (Clitherow *et al*., 1994). One such derivative, GR127935, potently inhibited contralateral turning induced by unilateral infusion of the 5-HT_1 receptor agonist GR56764 into the guinea-pig substantia nigra as well as sumatriptan-evoked inhibition of 5-HT release in the guinea-pig dorsal raphe nucleus (Clitherow *et al*., 1994; Starkey & Skingle, 1994) and canine basilar artery contraction (Skingle *et al*., 1996). The present study has been designed to analyse whether the 5-HT_1-like receptor mediating changes in the distribution of common carotid artery blood flow by sumatriptan in the pig are amenable to blockade by GR127935.

Table 4.1 pK_i values of sumatriptan, GR127935, methiothepin, metergoline and ketanserin at human cloned 5-HT_1 receptor subtypes.

	5-HT_{1A}	**5-HT_{1B}**	**5-HT_{1D}**	**5-ht_{1E}**	**5-HT_{1F}**
Sumatriptan[a]	6.4	7.8	8.5	5.8	7.9
GR127935[b]	7.2	9.0	8.6	5.4	6.4
Methiothepin	7.7*	7.6[c]	7.7[d]	6.7[e]	6.2[e]
Metergoline	8.1[f] (pig)	8.6[c]	8.7[d]	6.0[g]	6.5[e]
Ketanserin	5.5*	5.3[h]	7.2[h]	<5.0[e]	<5.0[e]

[a], Leysen *et al.* (1996); [b], Price *et al.* (1997); [c], Beer *et al.* (1998); [d], Pauwels *et al.* (1996); [e], Adham *et al.* (1993); [f], Hoyer (1988); [g], McAllister *et al.* (1992); [h], Adham *et al.* (1992). *, P.J. Pauwels, personal communication.

4.2 Methods

4.2.1 General

After an overnight fast, 14 domestic pigs (Yorkshire X Landrace; 10-15 kg) were anaesthetised with azaperone (160 mg, i.m.), midazolan hydrochloride (5 mg, i.m.) and metomidate (200 mg, i.v.). After tracheal intubation, the animals were connected to a respirator (BEAR 2E, BeMeds AG, Baar, Switzerland) for intermittent positive pressure ventilation with a mixture of room air and oxygen. Respiratory rate, tidal volume and oxygen supply were adjusted to keep arterial blood gas values within physiological limits (pH: 7.35-7.48; pCO_2: 35-48 mmHg; pO_2: 100-120 mmHg). Anaesthesia was maintained with a continuous i.v. infusion of sodium pentobarbital (20 mg kg^{-1} h^{-1}). It may be pointed out that this anaesthetic regimen, together with bilateral vagosympathectomy (see below), leads to an increase in heart rate and vasodilatation of arteriovenous anastomoses due to a loss of parasympathetic and sympathetic tone, respectively. Indeed, basal arteriovenous anastomotic blood flow is considerably higher in sodium pentobarbital-anaesthetised pigs (70-80% of carotid blood flow) than in those under fentanyl/thiopental anaesthesia (~19% of carotid blood flow) (Den Boer *et al.*, 1993). A high basal carotid arteriovenous anastomotic blood flow is particularly useful for investigating the effects of drugs that constrict arteriovenous anastomoses.

A catheter was placed in the inferior vena cava via the left femoral vein for infusion of antagonists and sodium pentobarbital. Another catheter was placed in the

aortic arch via the left femoral artery for the measurement of arterial blood pressure (Combitrans disposable pressure transducer; Braun, Melsungen, Germany) and arterial blood withdrawal for the measurement of blood gases (ABL-510; Radiometer, Copenhagen, Denmark). Subsequently, the right common carotid artery and the external jugular vein were dissected free and the accompanying vagosympathetic trunks were cut between two ligatures in order to prevent a possible influence via baroreceptor reflexes on agonist-induced carotid vascular responses. Another catheter was placed in the right external jugular vein for the withdrawal of venous blood samples for determining blood gases (ABL-510; Radiometer, Copenhagen, Denmark). A hub-less needle, connected to a polyethylene tube, used for the administration of radioactive microspheres was inserted into the right common carotid artery against the direction of blood flow for uniform mixing. In the case of intracarotid administrations of drugs (such as 5-HT; see Chapter 5), a second needle was placed into the right common carotid artery.

Blood flow was measured in the right common carotid artery with a flow probe (internal diameter: 2.5 mm) connected to a sine-wave electromagnetic flow meter (Transflow 601-system, Skalar, Delft, The Netherlands). Heart rate was measured with a tachograph (CRW, Erasmus University, Rotterdam, The Netherlands) triggered by electrocardiogram signals. Arterial blood pressure, heart rate and right common carotid blood flow were continuously monitored on a polygraph (CRW, Erasmus University, Rotterdam, The Netherlands). During the experiment, body temperature was kept about 37°C and the animal was continuously infused with saline to compensate fluid losses.

4.2.2 Distribution of carotid blood flow

The distribution of common carotid blood flow was determined with 15.5±0.1 μm (S.D.) diameter microspheres labelled with ^{141}Ce, ^{113}Sn, ^{103}Ru, ^{95}Nb or ^{46}Sc (NEN Dupont, Boston, USA). For each measurement, about 200,000 microspheres, labelled with one of the radioisotopes, were mixed and injected into the right common carotid artery. At the end of the experiment, the animal was killed by an overdose of sodium pentobarbital and the heart, lungs, kidneys and all ipsilateral cranial tissues were dissected out, weighed and put in vials. The radioactivity in these vials was counted for 10 min in a γ-scintillation counter (Packard, Minaxi autogamma 5000), using suitable windows to discriminate the different isotopes (^{141}Ce: 120-167, KeV, ^{113}Sn:

355-435 KeV, ^{103}Ru: 450-548 KeV, ^{95}Nb: 706-829 KeV and ^{46}Sc: 830-965 KeV). All data were processed by a set of specially designed programs (Saxena *et al*., 1980).

The fraction of carotid blood flow distributed to the different tissues was calculated by multiplying the ratio of tissue and total radioactivity of each radioisotope by the total common carotid blood flow at the time of the injection of the microspheres labelled with the respective isotope. Since little or no radioactivity was detected in the heart and kidneys, all microspheres trapped in lungs reached this tissue from the venous side after escaping via carotid arteriovenous anastomoses. Therefore, the amount of radioactivity in the lungs was used as an *index* of the arteriovenous anastomotic fraction of the common carotid blood flow (Saxena & Verdouw, 1982). Vascular conductance (10^{-2} ml min^{-1} mmHg^{-1}) was calculated by dividing blood flow (ml min^{-1}) by blood pressure (mmHg), multiplied by hundred.

4.2.3 Experimental protocol

The experiments were started after a stabilisation period of about 1 h. At baseline, heart rate, mean arterial blood pressure, carotid blood flow and its distribution as well as arterial and jugular venous blood gases were measured. Thereafter, the animals were divided into three groups which received i.v. infusions of either saline (5 ml; n=5), 0.25 mg kg^{-1} (n=4) or 0.5 mg kg^{-1} of GR127935 (n=5) over a period of 4-5 min. All variables were reassessed about ten min after the end of the infusion. Subsequently, all three groups of animals received cumulative i.v. doses of sumatriptan (30, 100 and 300 µg kg^{-1}) every twenty min. Fifteen min after each dose of sumatriptan the haemodynamic variables were assessed again.

4.2.4 Data presentation and statistical analysis

All data have been expressed as the mean±s.e.mean. The significance of the changes induced by saline, GR127935 or the different doses of sumatriptan within one group was evaluated with Duncan's new multiple range test, once an analysis of variance (randomised block design) had revealed that the samples represented different populations (Steel & Torrie, 1980). The changes (from baseline values) caused by sumatriptan (30, 100 or 300 µg kg^{-1}) in the two groups of animals pretreated with GR127935 (0.25 or 0.5 mg kg^{-1}) were compared with the corresponding changes in the saline-pretreated group using a Student's unpaired *t*-test. Statistical significance was accepted at $P<0.05$ (two-tailed).

4.2.5 Drugs

Apart from the anaesthetics, azaperone, metomidate (both from Janssen Pharmaceuticals, Beerse, Belgium), midazolan hydrochloride (Hoffmann La Roche b.v., Mijdrecht, The Netherlands) and pentobarbitone sodium (Apharmo, Arnhem, The Netherlands), the drugs used in this study were: sumatriptan succinate, GR127935 (both from Glaxo Group Research, Ware, UK) and heparin sodium (Leo Pharmaceutical Products, Weesp, The Netherlands) to prevent clotting of the catheters. GR127935 was solubilised according to the instructions of the supplier by heating the dispersion in distilled water to about 70°C for 10 s and then allowing to cool down to room temperature. Sumatriptan was dissolved in physiological saline. All doses refer to the respective salts.

4.3 Results

4.3.1 Effects of GR127935

The changes in systemic and carotid haemodynamics and in arterio-jugular venous oxygen saturation difference elicited by saline and GR127935 are shown in Table 4.2. Pretreatment with saline did not affect any of the variables measured. At the highest dose (0.5 mg kg^{-1}), GR127935 slightly, but significantly, reduced heart rate (4±1%) and mean arterial blood pressure (10±2%). While nutrient blood flow did not change, both doses (0.25 and 0.5 mg kg^{-1}) of GR127935 decreased total carotid blood flow (13±1% and 20±3%, respectively) by a selective action on its arteriovenous anastomotic fraction, which was decreased by 22±5% and 30±6%, respectively. The decreases in arteriovenous anastomotic blood flow were accompanied by significant decreases in the corresponding conductance. In keeping with these findings, the difference in arterial and jugular venous oxygen saturation was significantly increased by the highest dose of GR127935. However, it has to be remarked that the baseline value in this group was lower than in the other two groups (Table 4.2).

Table 4.2 Changes in heart rate (HR; beats min^{-1}), mean arterial blood pressure (MAP; mmHg), difference in arterial and jugular venous oxygen saturation (A-V SO_2; %), total carotid, arteriovenous anastomotic (AVA) and nutrient blood flows (ml min^{-1}) and AVA conductance (AVACon; 10^{-2} ml min^{-1} $mmHg^{-1}$) caused by either saline or GR127935.

	Saline (5 ml; n=5)		**GR127935 (0.25 mg kg^{-1}; n=4)**		**GR127935 (0.5 mg kg^{-1}; n=5)**	
	Before	*After*	*Before*	*After*	*Before*	*After*
HR	99±5	97±4	96±5	96±5	93±2	89±3*
MAP	105±3	102±4	97±1	99±5	103±5	93±4*
A-V SO_2	8.9±3.2	8.3±3.1	8.2±3.4	9.2±3.2	5.2±1.9	9.6±3.8*
Total	132±11	133±14	121±2	106±3*	125±13	101±11*
AVA	107±12	106±16	91±6	71±7*	98±11	68±8*
Nutrient	24±4	27±4	30±4	35±4	27±3	32±5
AVACon	102±9	102±12	94±7	71±6*	96±12	73±8*

All values have been presented as the mean±s.e.mean. *, P<0.05 vs before.

4.3.2 Systemic haemodynamic effects of sumatriptan in saline- and GR127935-pretreated groups

Bolus injections of sumatriptan (30-300 µg kg^{-1}, i.v.) elicited a slight, but significant, decrease in heart rate in both saline- and GR127935-pretreated animals. Mean arterial blood pressure was not changed by sumatriptan, except in the animals pretreated with 0.25 mg kg^{-1} GR127935 where the highest dose of sumatriptan decreased arterial pressure by 13±1% (Table 4.3). In the saline-pretreated animals, sumatriptan (100 and 300 µg kg^{-1}, i.v.) increased the arterio-jugular venous oxygen saturation difference by 147±63% and 229±96%, respectively. Table 4.3 shows that in animals pretreated with GR127935 this effect was either markedly reduced (0.25 mg kg^{-1}) or completely blocked (0.5 mg kg^{-1}).

Table 4.3 Values of heart rate, mean arterial blood pressure and difference in arterial and jugular venous oxygen saturation at baseline and after cumulative doses of sumatriptan in animals pretreated (i.v.) with either saline (Control) or GR127935.

	Sumatriptan (μg kg^{-1}, i.v.)			
	Baseline	*30*	*100*	*300*
Heart rate (beats min^{-1})				
Control (0.5 ml)	97±4	94±4	94±4*	92±5*
GR127935 (0.25 mg kg^{-1})	96±5	94±5	93±5*	92±5*
GR127935 (0.5 mg kg^{-1})	89±3	88±3*	86±3*	85±3*
Mean arterial blood pressure (mmHg)				
Control (0.5 ml)	102±4	103±6	103±6	96±6
GR127935 (0.25 mg kg^{-1})	99±5	97±5	93±6	86±4*
GR127935 (0.5 mg kg^{-1})	93±4	91±4	91±3	87±3
Arterial-jugular venous oxygen saturation difference (%)				
Control (0.5 ml)	8.3±3.1	10.7±3.2	15.9±3.2*	20.1±3.7*
GR127935 (0.25 mg kg^{-1})	9.2±3.2	9.8±2.1	9.6±3.2	12.5±2.4*
GR127935 (0.5 mg kg^{-1})	9.6±3.8	9.2±3.6	11.6±4.4	12.8±5.2

All values have been presented as the mean±s.e.mean. *, P<0.05 vs baseline.

4.3.3 Carotid haemodynamic effects of sumatriptan in saline- and GR127935-pretreated groups

As shown in Figures 4.1 and 4.2, sumatriptan (30, 100 and 300 μg kg^{-1}, i.v.) elicited a dose-dependent decrease in both the total carotid and arteriovenous anastomotic blood flows, but the total capillary fraction was not significantly increased. The decreases in total carotid and arteriovenous anastomotic blood flows by sumatriptan (maximal decreases 52±6% and 76±4%, respectively) were attenuated by 0.25 mg kg^{-1} of GR127935 (maximal decreases 15±2% and 27±4%, respectively) or abolished by 0.5 mg kg^{-1} of GR127935 (maximal decreases 9±10% and 14±12%, respectively).

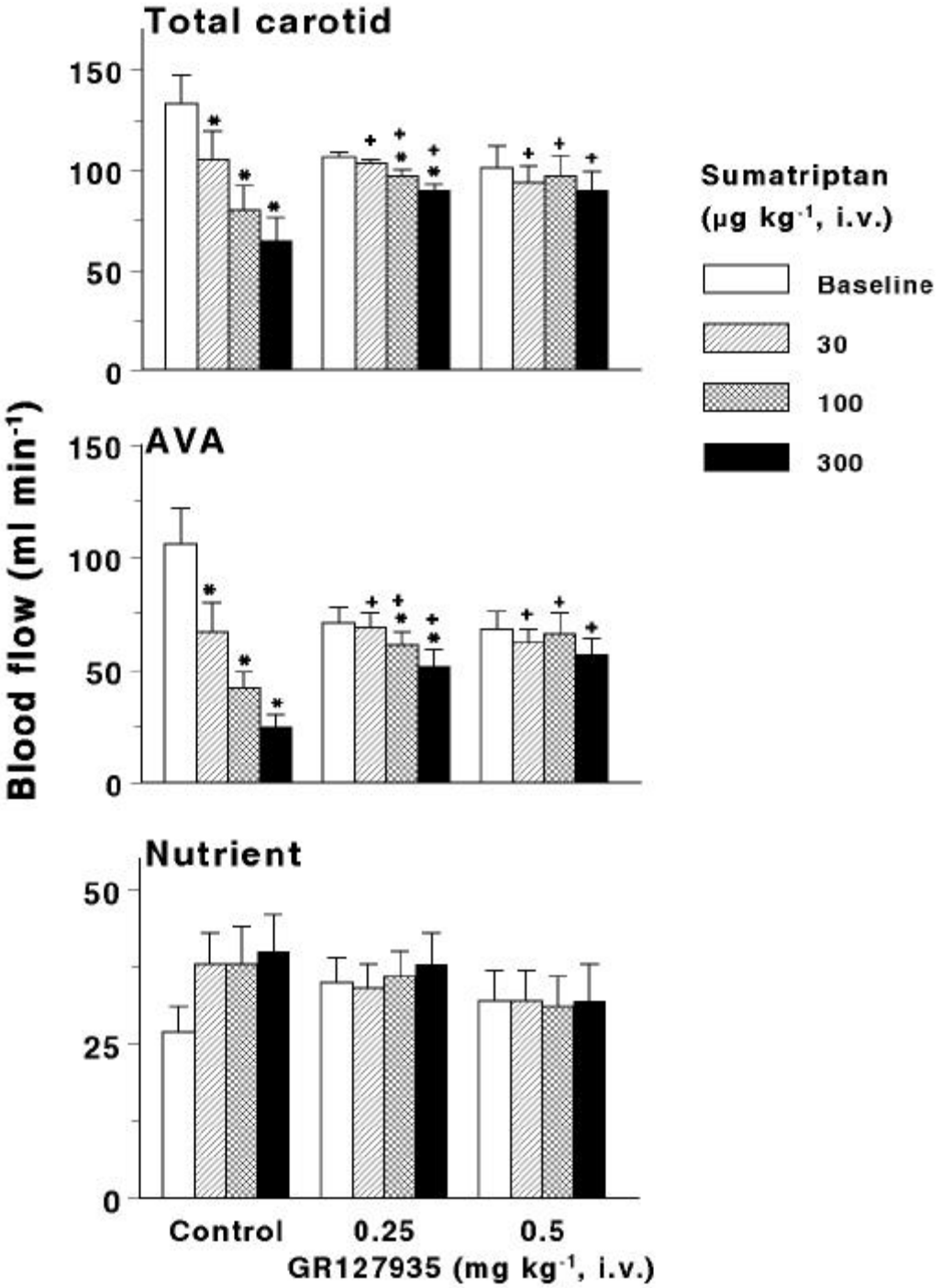

Figure 4.1 Effects of sumatriptan on the distribution of total carotid blood flow into its arteriovenous anastomotic (AVA) and nutrient fractions in pigs pretreated with either saline (Control; n=5) or GR127935 (0.25 or 0.5 mg kg^{-1}; n=4 or 5, respectively). All values are presented as the mean±s.e.mean. *, $P<0.05$ vs baseline; +, $P<0.05$ vs response by corresponding dose in control animals.

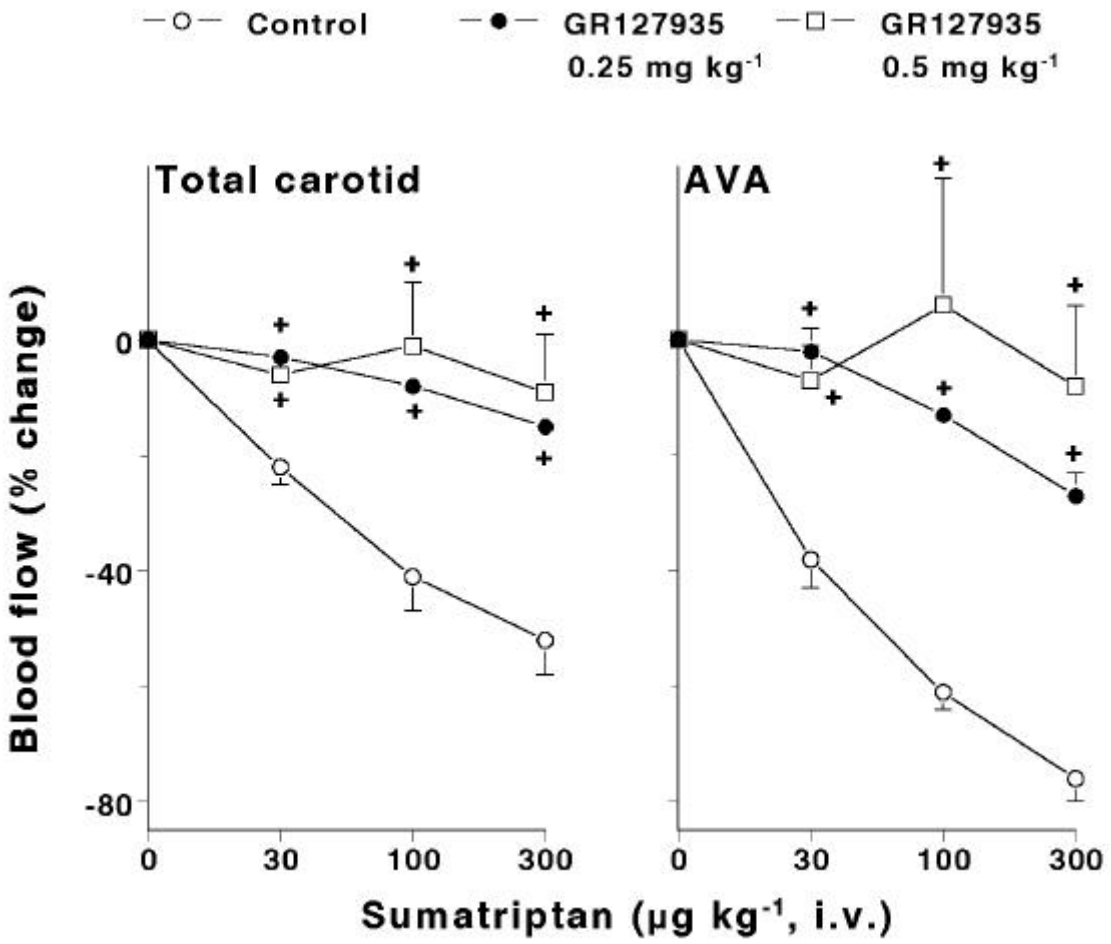

Figure 4.2 Percent changes from baseline values sumatriptan in total carotid and arteriovenous anastomotic (AVA) blood flow in pigs pretreated with either saline (Control) or GR127935 (0.25 or 0.5 mg kg^{-1}). All values are presented as the mean±s.e.mean. +, P<0.05 vs control.

The distribution of carotid blood flow to the head tissues in the three groups of animals is depicted in Figure 4.3. Sumatriptan did not significantly modify the fraction of carotid blood flow distributed to the skeletal muscle, bone, salivary gland, eye, tongue, brain and dura mater. In contrast, sumatriptan markedly increased blood flow to the skin (maximum increase 166±99%) and ears (maximum increase 234±111%) and, slightly, to the fat (maximum increase 96±42%); these effects of sumatriptan were absent in animals pretreated with GR127935.

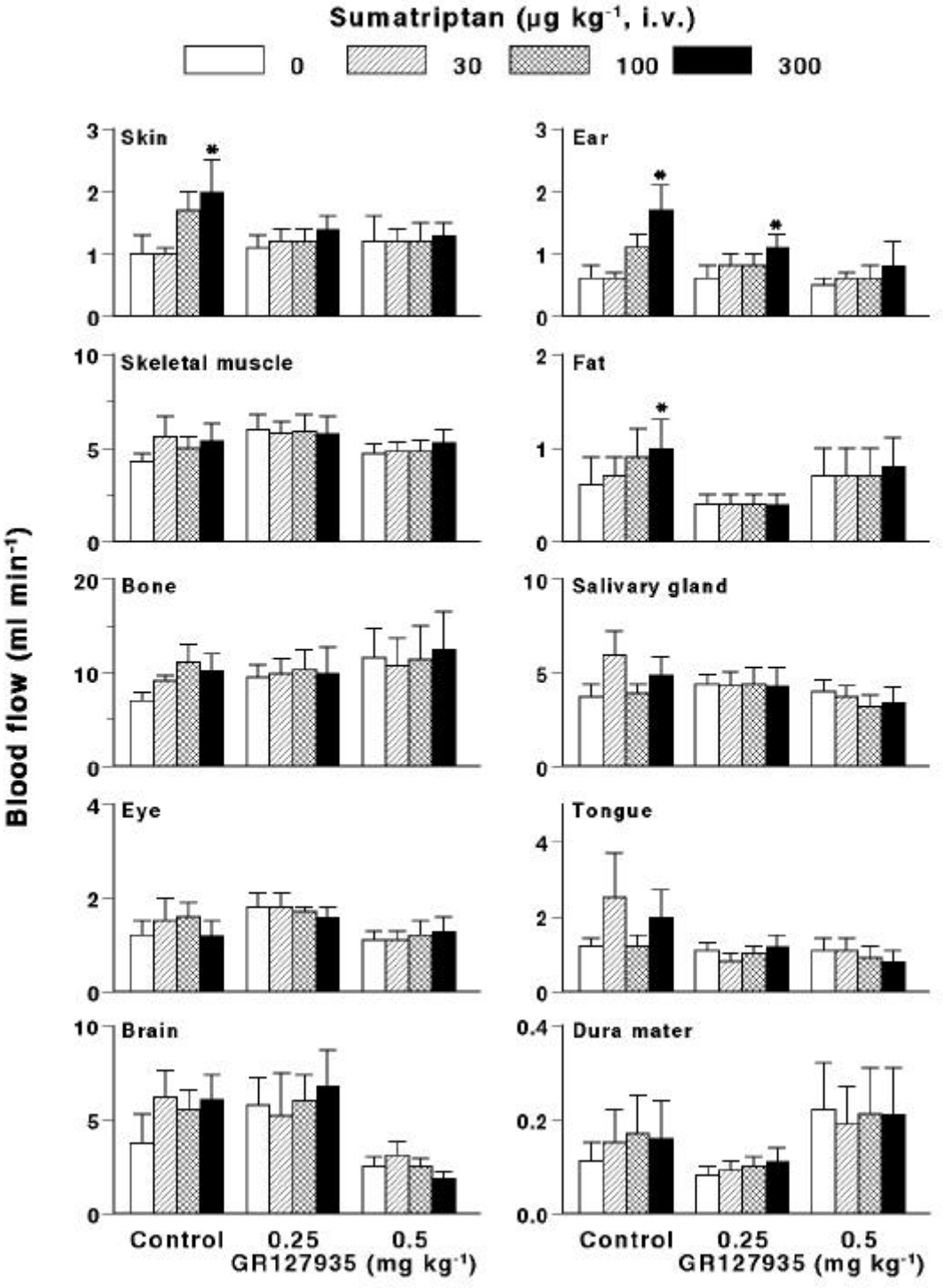

Figure 4.3 Effects of sumatriptan on the distribution of total carotid blood flow to the different cranial tissues in pigs pretreated with either saline (Control) or GR127935 (0.25 or 0.5 mg kg^{-1}). All values are presented as the mean±s.e.mean. *, $P<0.05$ vs baseline.

4.4 Discussion

4.4.1 General

The mechanisms involved in vascular constriction and blood flow reduction by 5-HT are complex and can be mediated by 5-HT_1-like and/or 5-HT_2 receptors depending on, amongst other factors, the species, the blood vessel under study and the degree of sympathetic vascular tone (Saxena & Villalón, 1990; 1991). Unlike 5-HT, sumatriptan, which has a negligible affinity for 5-HT_2 receptors (Humphrey *et al*., 1988; Peroutka & McCarthy, 1989; Humphrey *et al*., 1990), reduces porcine common carotid and arteriovenous anastomotic blood flows exclusively by 5-HT_1-like receptors (Den Boer *et al*., 1991b).

Previous findings obtained by the use of several 5-HT receptor agonists and antagonists suggested that 5-HT_1-like receptors were unrelated to the 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1C} (now 5-HT_{2C}) subtypes (for references see Saxena & Villalón, 1990). In this context it is important to note that until 1996 (Hartig *et al*., 1996) the 5-HT_{1B} receptor exclusively referred to the rodent 5-HT_{1B} receptor, which displays a distinct pharmacology compared to the human homologue. Thus, the 5-HT_{1B} receptor was excluded to be identical to the 5-HT_1-like receptors mediating constriction of porcine carotid arteriovenous anastomoses, based on the lack of antagonism by (±)pindolol (Bom *et al*., 1989b), a compound displaying high affinity at the rodent 5-HT_{1B} receptor (Adham *et al*., 1992), but not in other species (see Table 1.2). Additionally, Den Boer *et al*. (1992b) contended that the sumatriptan-sensitive 5-HT_1-like receptor mediating constriction of porcine arteriovenous anastomoses was apparently unrelated to the 5-HT_{1D} subtype, mainly because of the resistance to antagonism by metergoline, which displays the highest affinity for 5-HT_{1D} receptors (Waeber *et al*., 1988). Notwithstanding, this "5-HT_{1D}" receptor, is in fact encoded by two different receptors, namely the 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ receptors (Weinshank *et al*., 1992). In 1996, the 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ were renamed 5-HT_{1D} and 5-HT_{1B} receptors, respectively, based on their molecular homologies. These receptors are pharmacologically difficult to distinguish, and therefore, will be termed 5-$HT_{1B/1D}$ in this chapter. Although metergoline is capable of antagonising some 5-$HT_{1B/1D}$ receptor-mediated vascular responses (e.g. Schoeffter & Hoyer, 1990; Hamel & Bouchard, 1991; Bax *et al*., 1992b; Jansen *et al*., 1993; Deckert *et al*., 1994; Villalón & Terrón, 1994a), it is important to note that in these studies, the antagonist potency of metergoline did not correlate with its affinity at 5-$HT_{1B/1D}$ receptors (Waeber *et al*., 1988) and, in some

cases, metergoline showed a non-competitive antagonism (e.g. Hamel & Bouchard, 1991) or even intrinsic efficacy at 5-$HT_{1B/1D}$ receptors (Schoeffter *et al*., 1988; Miller *et al*., 1992). The advent of GR127935, which is a potent and selective ligand for 5-$HT_{1B/1D}$ receptors and antagonises a number of responses elicited by 5-$HT_{1B/1D}$ receptor agonists (Clitherow *et al*., 1994; Pauwels, 1996; Skingle *et al*., 1996), offers us the possibility of investigating its effect on sumatriptan-induced changes. Indeed, in addition to the implications discussed below, our results show that GR127935 antagonised sumatriptan-induced changes in porcine carotid haemodynamics, implying a common site of action.

4.4.2 Effect of GR127935 on systemic and carotid haemodynamics

GR127935 elicited a slight reduction in heart rate and mean arterial blood pressure, but we have no clear-cut explanation for it. The compound also decreased by itself the total carotid blood flow and, as observed with sumatriptan (see below), this decrease was exclusively confined to the arteriovenous anastomotic fraction. Since these effects were observed with a concomitant decrease in the arteriovenous anastomotic conductance (Table 4.2), GR127935 may act as an agonist at the receptors mediating contraction of arteriovenous anastomoses. This is supported by the fact that in cells with human cloned receptors GR127935 can behave as an agonist at 5-HT_{1B} and 5-HT_{1D} receptor subtypes (Pauwels & Colpaert, 1995; Pauwels, 1996; Watson *et al*., 1996). However, as described in Chapter 3, the effects of GR127935 are not affected by methiothepin, at a dose capable of abolishing sumatriptan-induced porcine carotid vascular effects.

4.4.3 Systemic haemodynamic changes after sumatriptan

As previously reported by other authors (Feniuk *et al*., 1989; Den Boer *et al*., 1991b; 1992b), sumatriptan produced a slight, but significant, decrease in heart rate; this is likely to be an effect of the drug, since in similar experiments no changes in heart rate were observed after four consecutive bolus injections of saline (Den Boer *et al*., 1991a). The mechanism involved in the rather small decrease in heart rate by sumatriptan is not clear, but may be related to inhibition of sympathetic neurons (Humphrey *et al*., 1988; Dreteler *et al*., 1989; Humphrey *et al*., 1990; Saxena & Villalón, 1990; Pagniez *et al*., 1998). In any case, bradycardia following

the use of sumatriptan in patients seems to be of little clinical relevance (Saxena & Tfelt-Hansen, 1993).

Significantly, the fact that sumatriptan did not produce important changes in mean arterial blood pressure in the saline- or GR127935-pretreated groups (Table 4.2) implies that the drug has a more selective vasoconstrictor action on cranial blood vessels than, for example, ergotamine, which elicits a hypertensive response (Den Boer *et al*., 1991a).

4.4.4 Carotid haemodynamic changes after sumatriptan

Sumatriptan elicited a dose-dependent reduction in the total carotid blood flow, which was exclusively due to a decrease in its arteriovenous anastomotic fraction. Consistent with this finding, sumatriptan also increased the arterio-jugular venous oxygen saturation difference. The reductions in the total and arteriovenous anastomotic blood flows as well as the accompanying increase in the arterio-jugular venous oxygen saturation difference by sumatriptan were potently attenuated (0.25 mg kg^{-1}) or abolished (0.5 mg kg^{-1}) in animals pretreated with GR127935. Although it cannot be entirely ruled out that sumatriptan and GR127935 both act on an "unknown" receptor, taking into account that both sumatriptan and GR127935 have high affinities for 5-$HT_{1B/1D}$ receptors (Table 4.1), our results suggest that the sumatriptan-induced vasoconstriction of carotid arteriovenous anastomoses is mediated by 5-$HT_{1B/1D}$ receptors. Thus, these findings reinforce the view that 5-HT_1-like receptors mediating vascular smooth muscle contraction are identical to 5-$HT_{1B/1D}$ receptors.

Nevertheless, it may be recalled that sumatriptan also displays considerable affinity for the cloned human 5-HT_{1F} receptor (Table 4.1). However, the involvement of the 5-HT_{1F} receptor seems not very likely because (i) GR127935 has a substantially lower affinity for 5-HT_{1F} than for 5-HT_{1D} or 5-HT_{1B} receptors (Table 4.1); (ii) sumatriptan is several folds less potent than ergotamine (not more potent as may be expected from their affinities for the 5-HT_{1F} receptor) on porcine arteriovenous anastomoses (Chapter 5; Den Boer *et al*., 1991a; 1991b), and (iii) sumatriptan is more potent at the 5-HT_{1B} or 5-HT_{1D} than at the 5-HT_{1F} receptor (Table 4.1). The use of 5-HT_{1F} receptor agonists will help confirm the latter. Indeed, in the dog carotid circulation, the selective 5-HT_{1F} receptor agonist, LY344864 (Phebus *et al*., 1997) is devoid of vasoconstrictor properties (Chapter 7).

As reported earlier from our laboratory (Den Boer *et al.*, 1991b), sumatriptan conspicuously increased blood flows to the skin and ears, without any alteration in the total capillary blood flow. Although the increase in the skin and ear blood flow was largely attenuated in animals pretreated with GR127935, it can be argued that the dilatation of the skin and ear arterioles is an indirect consequence of the closure of arteriovenous anastomoses by sumatriptan. On the other hand, Schoeffter & Hoyer (1990) have reported 5-HT receptors similar to 5-$HT_{1B/1D}$ receptor subtypes mediate endothelium-dependent relaxations of porcine isolated coronary artery.

4.4.5 Nature of 5-$HT_{1B/1D}$ receptors mediating constriction of porcine carotid arteriovenous anastomoses

Based on the lack of 5-HT_{1D} *m*RNA, but the abundant expression of 5-HT_{1B} receptor *m*RNA in human or bovine cerebral arteries (Hamel *et al.*, 1993; Bouchelet *et al.*, 1996) it is likely that the 5-HT_{1B} receptor mediates contractile responses in these vessels. The use of GR127935, which has similar affinities at 5-HT_{1B} and 5-HT_{1D} receptors (Table 4.1), does not allow us to infer if one or both 5-HT receptor subtypes are involved in the vasoconstrictor effect of sumatriptan on porcine cranial arteriovenous anastomoses (and, possibly, in the vasodilatation of arterioles). For a more definitive evidence, one must await the development of subtype selective 5-$HT_{1B/1D}$ receptor agonists and antagonists (see Chapter 8 and 9).

In conclusion, the results of the present experiments imply that the constriction of porcine carotid arteriovenous anastomoses by the 5-HT_1-like receptor agonist sumatriptan, being antagonised by the 5-$HT_{1B/1D}$ receptor ligand GR127935, is mediated by 5-$HT_{1B/1D}$ receptors. It would, therefore, appear that vascular 5-HT_1-like receptors, which are yet to be cloned, are identical to 5-$HT_{1B/1D}$ receptors. In view of the putative pathophysiological role of arteriovenous anastomotic dilatation in migraine (Chapter 1), the constriction of these non-nutrient vessels by sumatriptan via a 5-$HT_{1B/1D}$ receptor mechanism may be, at least partly, responsible for the therapeutic effect of the drug in migraine.

Chapter 5

Effects of GR127935 on the 5-HT-, ergotamine- and dihydroergotamine-induced porcine carotid vascular changes

Summary It was previously shown that porcine cranial arteriovenous anastomoses constrict to 5-HT, ergotamine, dihydroergotamine and sumatriptan; sumatriptan acts exclusively via 5-$HT_{1B/1D}$ receptors. The present study was devoted to establish the contribution of 5-$HT_{1B/1D}$ receptors in the constriction of arteriovenous anastomoses elicited by 5-HT (in presence of 0.5 mg kg^{-1} ketanserin), ergotamine and dihydroergotamine in anaesthetised pigs. Intracarotid infusion of 5-HT (2 µg kg^{-1} min^{-1}) and intravenous doses of ergotamine (2.5-20 µg kg^{-1}) and dihydroergotamine (3-100 µg kg^{-1}) reduced arteriovenous anastomotic and increased nutrient blood flows and vascular conductances. The vasodilator response to 5-HT, observed mainly in the skin and ear, was much more prominent than that of the ergot alkaloids. Treatment with the 5-$HT_{1B/1D}$ receptor antagonist GR127935 (0.5 mg kg^{-1}, i.v.) significantly attenuated both ergot-induced arteriovenous anastomotic constriction and arteriolar dilatation, whereas GR127935 only slightly affected the carotid vascular effects by 5-HT. The results suggest that 5-HT constricts carotid arteriovenous anastomoses primarily via receptors, which seem to differ from those (5-$HT_{1B/1D}$) stimulated by sumatriptan. The ergot alkaloids produce arteriovenous anastomotic constriction for a substantial part via 5-$HT_{1B/1D}$ receptors, but also invoke unidentified receptors. Both these non-5-$HT_{1B/1D}$ receptors may be targets for the development of novel antimigraine drugs. The moderate vasodilator response to the ergot derivatives seems to be mediated, at least in part, by 5-$HT_{1B/1D}$ receptors, whereas the arteriolar dilatation caused by 5-HT may be mediated by other, possibly 5-HT_7 receptors.

5.1 Introduction

In previous studies in vagosympathectomised, anaesthetised pigs, we have shown that intracarotid infusions of 5-HT lead to a redistribution of carotid blood flow towards tissue arterioles at the expense of arteriovenous anastomoses (Saxena & Verdouw, 1982; Saxena *et al*., 1986; Den Boer *et al*., 1992b). These changes are dose-dependent and recover within minutes of stopping 5-HT infusions (Saxena & Verdouw, 1982). The vasoconstrictor action of 5-HT on arteriovenous anastomoses is mainly mediated by 5-HT_1-like receptors with some contribution from 5-HT_2 receptors (Saxena *et al*., 1986). The constriction of porcine arteriovenous

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anastomoses by the antimigraine drugs sumatriptan and, partly, by the ergot alkaloids (ergotamine and dihydroergotamine) is also mediated by 5-HT_1-like receptors, because these effects are antagonised, either partially (ergot alkaloids) or fully (sumatriptan), by methiothepin, but not by ketanserin (Den Boer *et al*., 1991a; 1991b).

It is now recognised that the term 5-HT_1-like includes several different receptor subtypes. The vascular 5-HT_1-like receptor appears to be identical to recombinant 5-$HT_{1B/1D}$ receptors, since the vasoconstrictor responses to sumatriptan, which has a high affinity for 5-$HT_{1B/1D}$ receptors (Peroutka & McCarthy, 1989; Beattie *et al*., 1994), are antagonised by GR127935 (see Chapter 4), a selective 5-$HT_{1B/1D}$ receptor antagonist (Chapter 3; Clitherow *et al*., 1994; Pauwels, 1996; Skingle *et al*., 1996). Using subtype selective antagonists, we have shown that the sumatriptan-induced cranial vasoconstriction is most probably mediated by the 5-HT_{1B} receptor (see Chapters 8 and 9). Indeed, *m*RNA for the 5-HT_{1B} but not 5-HT_{1D} receptor has been located in cranial blood vessels (Hamel & Bouchard, 1991; Hamel *et al*., 1993; Bouchelet *et al*., 1996).

On the basis of above, the present investigation was undertaken to establish the contribution of 5-$HT_{1B/1D}$ receptors in the reduction of porcine carotid arteriovenous anastomotic blood flow induced by the endogenous ligand, 5-HT, and by the ergot alkaloids, ergotamine and dihydroergotamine, both potent antimigraine agents. For this purpose, we analysed the carotid vasoconstrictor effects of intracarotid infusions of 5-HT and i.v. doses of the ergot compounds before and after treatment with GR127935 or equivalent volumes of physiological saline. To eliminate 5-HT_2 receptor-mediated arteriovenous anastomotic constriction (Verdouw *et al*., 1984b), the animals receiving 5-HT were systematically pretreated with ketanserin (0.5 mg kg^{-1}).

5.2 Methods

5.2.1 General

The methods used for anaesthesia, surgical preparations and determination of systemic and carotid haemodynamics are described in detail in Chapter 4.

5.2.2 Experimental protocols

After a stabilisation period of about 1 h, the animals (n=36) were divided into two groups. The first group (n=12) was systematically pretreated with ketanserin

(0.5 mg kg^{-1}, i.v.) and subsequently subdivided into two subgroups. After measuring baseline values of heart rate, mean arterial blood pressure, carotid blood flow and its distribution and arterial and jugular venous blood gases, the first subgroup (n=5), which will be referred to as the control group, received an intracarotid infusion of 5-HT (2 µg kg^{-1} min^{-1}), lasting 10 min. All variables were reassessed at the end of this infusion and, again, 30 min after terminating the infusion (recovery). Then, the animals received an i.v. infusion of physiological saline (1 ml min^{-1} for 5 min). Ten min after the end of this infusion and at the end of a second 10-min 5-HT infusion, all variables were collated again. In the second subgroup of animals (n=7) the same protocol was used, but instead of physiological saline, GR127935 (0.5 mg kg^{-1}, i.v.; 1 ml min^{-1} for 5 min) was administered. This dose of GR127935 completely blocks the carotid vascular effects of sumatriptan (see Chapter 4).

The second group of animals (n=24; untreated with ketanserin) was divided into four subgroups (n=6 each). The first two groups received an i.v. infusion of physiological saline (5 ml), whereas the last two groups received an i.v. infusion of GR127935 (0.5 mg kg^{-1}); both were administered over a period of 4-5 min. Ten minutes after the end of these infusions, baseline values of heart rate, mean arterial blood pressure, carotid blood flow and its distribution, as well as arterial and jugular venous blood gases were measured. Then, the first and third group received sequential i.v. bolus injections of ergotamine (2.5, 5, 10 and 20 µg kg^{-1}), every 20 min, whereas the second and fourth group received sequential i.v. bolus injections of dihydroergotamine (3, 10, 30 and 100 µg kg^{-1}). Fifteen min after each dose of ergotamine or dihydroergotamine all haemodynamic variables were reassessed.

5.2.3 Data presentation and statistical analysis

All data have been expressed as the mean±s.e.mean. The significance of the difference between the variables within one group was evaluated with Duncan's new multiple range test, once an analysis of variance (randomised block design) had revealed that the samples represented different populations (Steel & Torrie, 1980). Differences between variables of two groups were evaluated using Student's unpaired *t*-test. Statistical significance was accepted at $P<0.05$ (two-tailed).

5.2.4 Drugs

Apart from the anaesthetics (see Chapter 4), the drugs used in this study were: GR127935 (Glaxo Group Research, Ware, UK; courtesy Dr. H.E. Connor), 5-HT creatinine sulphate (Sigma Chemical Company, St. Louis, MO, USA), ketanserin tartrate (Janssen Pharmaceutica, Beerse, Belgium), ergotamine tartrate and dihydroergotamine mesylate (both from Sandoz Pharma Ltd., Basel, Switzerland) and heparin sodium (Leo Pharmaceutical Products, Weesp, The Netherlands) to prevent clotting of the catheters. Ketanserin, ergotamine and dihydroergotamine were dissolved in distilled water; 5-HT was dissolved in physiological saline. GR127935 was solubilised according to the instructions of the supplier by heating the dispersion in distilled water to about 70°C for 10 s and then allowing to cool down to room temperature. All doses refer to the respective salts whereas that of 5-HT refers to the free base.

5.3 Results

5.3.1 Effects of intracarotid 5-HT infusions before and after physiological saline or GR127935 on systemic haemodynamics

The effects of 5-HT on the systemic haemodynamics are depicted in Table 5.1. Intracarotid infusion of 5-HT (2 μg kg^{-1} min^{-1}) produced a slight increase in heart rate (7±2%, $P<0.05$) in control animals; mean arterial blood pressure (-5±4%, $P>0.05$) was not affected. In the second subgroup of animals also, only small changes in heart rate (+7±2%, $P<0.05$) and blood pressure (-8±2%, $P<0.05$) were observed with the initial 5-HT infusion. Heart rate returned to baseline values after a recovery period of 30 min, but blood pressure remained slightly lower in the second subgroup. The subsequent administrations of saline or GR127935 did not cause significant systemic haemodynamic changes. In control animals, the second infusion of 5-HT did not change heart rate, but produced a slight (though significant) hypotensive effect. In the other subgroup, after treatment with GR127935, the hypotension was not affected, but the tachycardia by 5-HT was attenuated.

No conspicuous changes were observed by 5-HT in the A-V SO_2; in both groups, however, the response to the second infusion of 5-HT was significantly different compared to that by the first 5-HT infusion (Table 5.1).

Table 5.1 Values of heart rate, mean arterial blood pressure and difference in arterial and jugular venous oxygen saturation at baseline, during a 10-min intracarotid infusion of 5-HT, after 30 min of recovery, after saline or GR127935 and during a second infusion of 5-HT.

	Baseline	*5-HT (1^{st})*	*Recovery*	*Saline or GR127935*	*5-HT (2^{nd})*
Heart rate (beats min^{-1})					
Control	92±2	98±4*	93±3	93±3	95±4[c]
GR127935	93±3	100±3*	93±3	92±2	95±2[bc]
Mean arterial blood pressure (mmHg)					
Control	106±3	101±5	102±2	97±2	88±4[bc]
GR127935	86±6	80±7*	81±6*	77±5	71±5[b]
Arterial-jugular venous oxygen saturation difference (%)					
Control	6.1±1.2	4.3±0.7	7.7±1.9	9.0±2.9	12.9±4.1[c]
GR127935	10.1±2.3	7.9±1.8	11.3±2.2	13.9±3.5	14.5±3.7[c]

All values have been presented as the mean±s.e.mean. 5-HT was infused at 2 μg kg^{-1} min^{-1}; after 30 min of recovery, 5 ml saline (n=5) or 0.5 mg kg^{-1} of GR127935 (n=7) was administered. *, P<0.05 vs baseline; [a], P<0.05 vs recovery value; [b], P<0.05 vs values after saline or GR127935; [c], P<0.05 vs first response to 5-HT.

5.3.2 Effects of intracarotid 5-HT infusions before and after physiological saline or GR127935 on carotid haemodynamics

As shown in the *upper panel* of Figure 5.1, in control animals a 10-min intracarotid infusion of 5-HT (2 μg kg^{-1} min^{-1}) produced an increase in the total carotid blood flow (31±6%), a decrease in the arteriovenous anastomotic blood flow (79±4%) and a marked increase in nutrient (capillary) blood flow (482±71%). These variables returned to baseline values after a 30 min recovery period and remained unaltered after the subsequent administration of saline. During a second infusion of 5-HT no changes in the total carotid blood flow were observed (P<0.05 vs first response to 5-HT), while the decrease in the arteriovenous anastomotic blood flow was more pronounced (-93±3%; P<0.05 vs first response to 5-HT) and the increase in nutrient blood flow was less marked (173±71%; P<0.05 vs first response to 5-HT).

In the second subgroup of animals (Figure 5.1, *lower panel*) no changes in the total carotid blood flow were observed during the initial infusion of 5-HT ($P<0.05$ vs response to 5-HT in control animals), whereas 5-HT produced a decrease in arteriovenous anastomotic blood flow (84±6%) and an increase in nutrient blood flow (238±74%), of which the latter response was significantly less as compared to control animals. These variables returned to baseline values after a 30 min recovery period. The subsequent administration of GR127935 ($0.5\ mg\ kg^{-1}$, i.v.) produced a non-significant decrease in the total carotid blood flow (-17±3%); this change was significantly different from that observed in control animals (-6±2%) infused with saline. GR127935 caused a decrease in arteriovenous anastomotic blood flow (-27±3%; $P<0.05$ vs recovery and $P<0.05$ vs response to saline in control animals), without changing nutrient blood flow. In the presence of GR127935, a second infusion of 5-HT did not change the total carotid blood flow, but decreased arteriovenous anastomotic (-65±12%; $P<0.05$ vs values after GR127935) and increased nutrient (76±26%; $P<0.05$ vs values after GR127935) blood flows. Both these 5-HT-induced changes were significantly less compared to the changes induced by the initial infusion with 5-HT. Compared to control animals (Figure 5.1, *upper panel*), however, the decrease in arteriovenous anastomotic, as well as the increase in nutrient blood flow, induced by the second 5-HT infusion, was *not* significantly different after GR127935 treatment (Figure 5.1, *lower panel*).

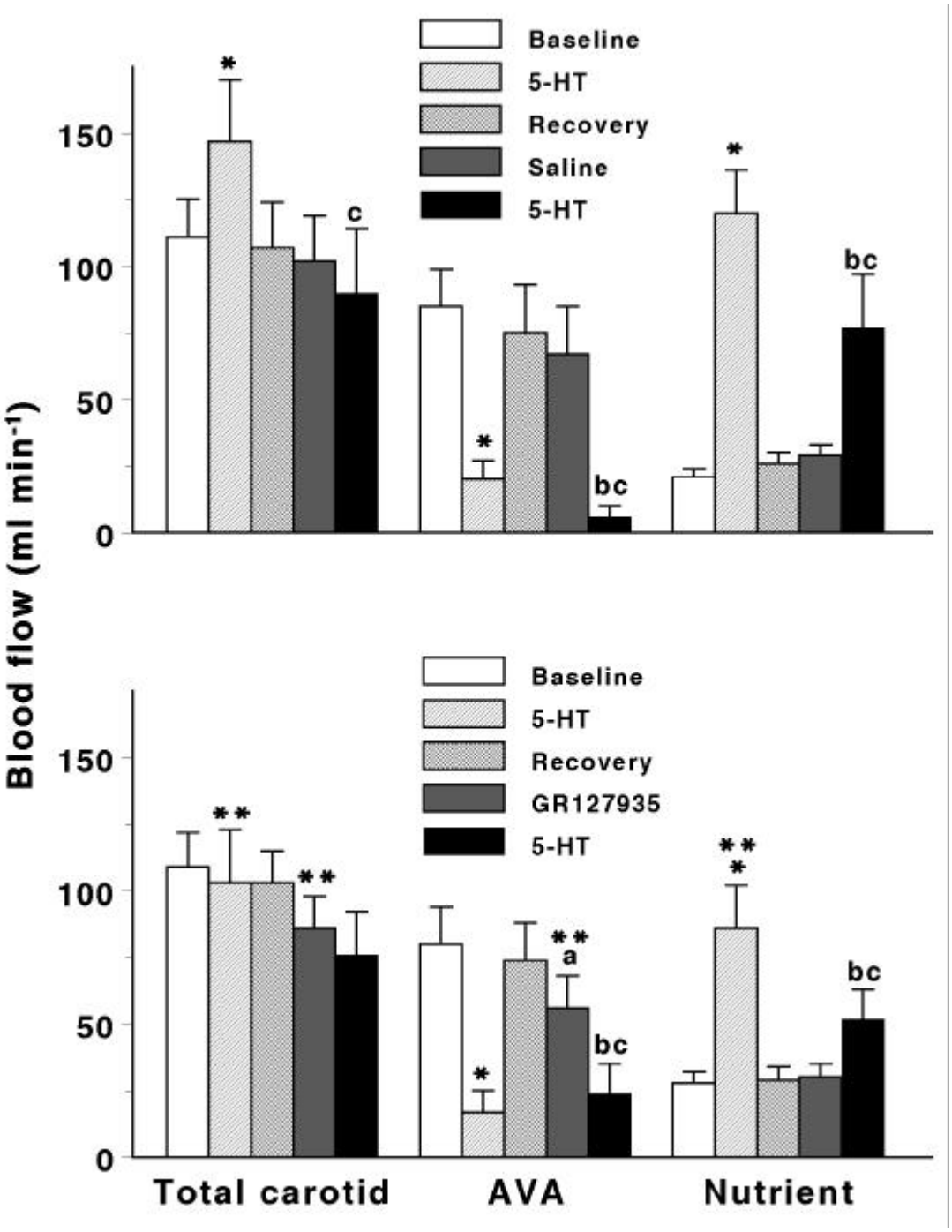

Figure 5.1 Distribution of total carotid blood flow into its arteriovenous anastomotic (AVA) and nutrient fractions in ketanserin (0.5 mg kg^{-1}, i.v.)-pretreated pigs. Effects of 10-min intracarotid infusions of 5-HT (2 µg kg^{-1} min^{-1}) before and after saline (upper panel; n=5) or 0.5 mg kg^{-1} (i.v.) of GR127935 (lower panel; n=7). *****, P<0.05 vs baseline; **a**, P<0.05 vs recovery value; **b**, P<0.05 vs values after saline or GR127935; **c**, P<0.05 vs first response to 5-HT; ******, P<0.05 vs response in control animals.

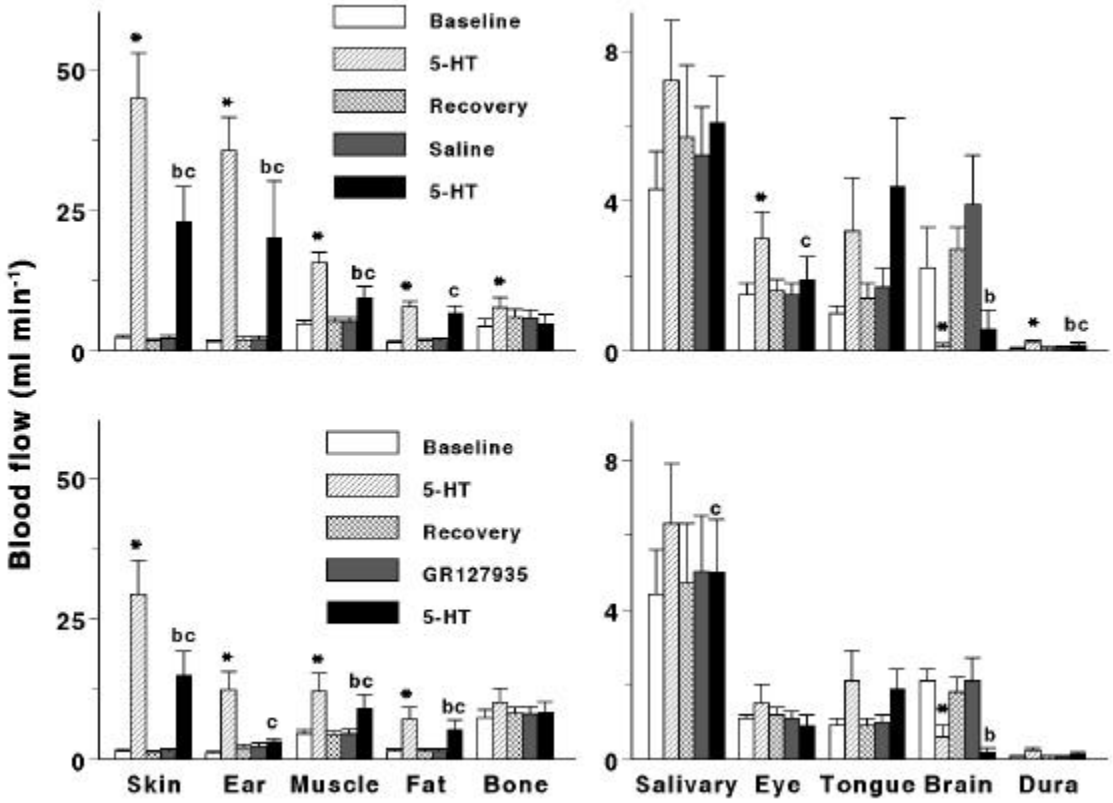

Figure 5.2 Distribution of total carotid blood flow to the different cranial tissues in ketanserin (0.5 mg kg^{-1}, i.v.)-pretreated pigs. Effects of 10-min intracarotid infusions of 5-HT (2 µg kg^{-1} min^{-1}) before and after saline (upper panel; n=5) or 0.5 mg kg^{-1} (i.v.) of GR127935 (lower panel; n=7). *****, P<0.05 vs baseline; **a**, P<0.05 vs recovery value; **b**, P<0.05 vs values after saline or GR127935; **c**, P<0.05 vs first response to 5-HT; ******, P<0.05 vs response in control animals.

The distribution of carotid blood flow to the different head tissues is depicted in Figure 5.2. In both groups of animals, 5-HT increased blood flow significantly towards the skin, ears, fat, and muscles and decreased blood flow to the brain; exclusively in the control group (Figure 5.2, upper panels) significant increases were observed in bones, eye and dural blood flow. In keeping with the above, a marked, sharply defined, redness of the skin and ear, confined to the ipsilateral side of the head, was observed in all animals during the infusion with 5-HT. These variables, including the redness of the skin, returned to baseline values after a 30 min recovery period and remained unaltered after the subsequent administration of saline or GR127935. Overall, the 5-HT-induced increases in blood flow towards the different

head tissues were slightly, but significantly, attenuated to the same extent by treatment with saline, as well as by GR127935; however, the increase in ear blood flow was abolished by GR127935, while it was only partly attenuated by saline. The decrease in blood flow to the brain was not affected by either saline or GR127935. The degree of redness caused by 5-HT was not visibly different before and after GR127935 or saline.

5.3.3 Effects of ergot alkaloids in animals pretreated with physiological saline or GR127935 on systemic haemodynamics

As shown in Table 5.2, ergotamine (2.5-20 μg kg^{-1}) and dihydroergotamine (3-100 μg kg^{-1}) did not cause major changes in heart rate or mean arterial blood pressure; only the highest dose of dihydroergotamine produced small, but significant, tachycardiac and hypertensive effects in control and GR127935-pretreated animals, respectively.

In saline-treated animals, both ergot compounds produced dose-dependent increases in the A-V SO_2. In animals pretreated with GR127935, the ergotamine-induced increases in the A-V SO_2 seemed to be attenuated, although only at 5 μg kg^{-1} significance was reached. The dihydroergotamine-induced increases in the A-V SO_2 were abolished after pretreatment with GR127935, although, compared to saline treated animals, this was only significant at a dose of 10 μg kg^{-1}.

Table 5.2 Values of heart rate, mean arterial blood pressure and difference in arterial and jugular venous oxygen saturation at baseline and after cumulative doses of ergotamine and dihydroergotamine in animals pretreated with either saline (Control) or GR127935 (0.5 mg kg^{-1}).

		Ergotamine (µg kg^{-1}, i.v.)			
	Baseline	*2.5*	*5*	*10*	*20*
Heart rate (beats min^{-1})					
Control	97±4	96±4	96±4	95±4	95±4
GR127935	93±4	90±4	88±4	88±4	88±4
Mean arterial blood pressure (mmHg)					
Control	95±2	104±4	101±6	98±7	101±6
GR127935	93±3	97±6	98±7	97±8	102±7
Arterial-jugular venous oxygen saturation difference (%)					
Control	4.1±0.7	10.0±2.9*	15.5±3.3*	17.6±2.5*	21.2±2.6*
GR127935	11.9±5.6	14.6±16.5	16.7±6.9*[a]	19.9±7.0*	22.6±6.2*

		Dihydroergotamine (µg kg^{-1}, i.v.)			
	Baseline	*3*	*10*	*30*	*100*
Heart rate (beats min^{-1})					
Control	91±3	91±3	90±3	91±2	94±3*
GR127935	94±4	94±4	93±3	94±3	98±3
Mean arterial blood pressure (mmHg)					
Control	106±4	118±5	116±6	116±7	117±7
GR127935	100±2	99±1[a]	98±1	103±2	107±2*
Arterial-jugular venous oxygen saturation difference (%)					
Control	6.2±1.5	9.4±1.6*	12.5±1.6*	14.7±1.7	16.6±2.1*
GR127935	15.5±4.9	14.8±4.3	15.4±4.0[a]	18.5±3.8	20.4±3.0

All values have been presented as the mean±s.e.mean. *, $P<0.05$ vs baseline; [a], $P<0.05$ vs response by corresponding dose in saline-pretreated animals.

Table 5.3 Values of total carotid, arteriovenous anastomotic (AVA) and nutrient vascular conductance at baseline and after cumulative doses of ergotamine and dihydroergotamine in animals pretreated with either saline (control; n=6 each) or GR127935 (0.5 mg kg^{-1}; n=6 each).

		Ergotamine (µg kg^{-1}, i.v.)			
	Baseline	*2.5*	*5*	*10*	*20*
Total carotid vascular conductance					
Control	136±13	92±10*	85±9*	82±10*	71±8*
GR127935	126±8	116±9*[a]	103±8*[a]	94±9*	81±10*
AVA vascular conductance					
Control	108±14	50±11*	32±8*	24±7*	13±5*
GR127935	89±6	74±9*[a]	61±9*[a]	43±7*[a]	25±6*[a]
Nutrient vascular conductance					
Control	24±3	36±6*	46±6*	50±6*	49±5*
GR127935	32±3	37±4	36±3[a]	45±6*[a]	49±5*

		Dihydroergotamine (µg kg^{-1}, i.v.)			
	Baseline	*3*	*10*	*30*	*100*
Total carotid vascular conductance					
Control	113±10	86±6*	81±7*	78±8*	72±7*
GR127935	136±10	137±11[a]	131±10[a]	109±6*[a]	89±6*
AVA vascular conductance					
Control	84±10	45±10*	30±7*	23±6*	12±3*
GR127935	102±11	94±12[a]	79±12*[a]	59±8*[a]	33±6*[a]
Nutrient vascular conductance					
Control	25±3	36±7*	45±6*	50±6*	54±6*
GR127935	28±3	36±4*	43±4*	43±5*	47±3*

All values have been presented as mean±s.e.mean and expressed in 10^{-2} ml min^{-1} $mmHg^{-1}$. *, P<0.05 vs baseline; [a], P<0.05 vs response by corresponding dose in control animals.

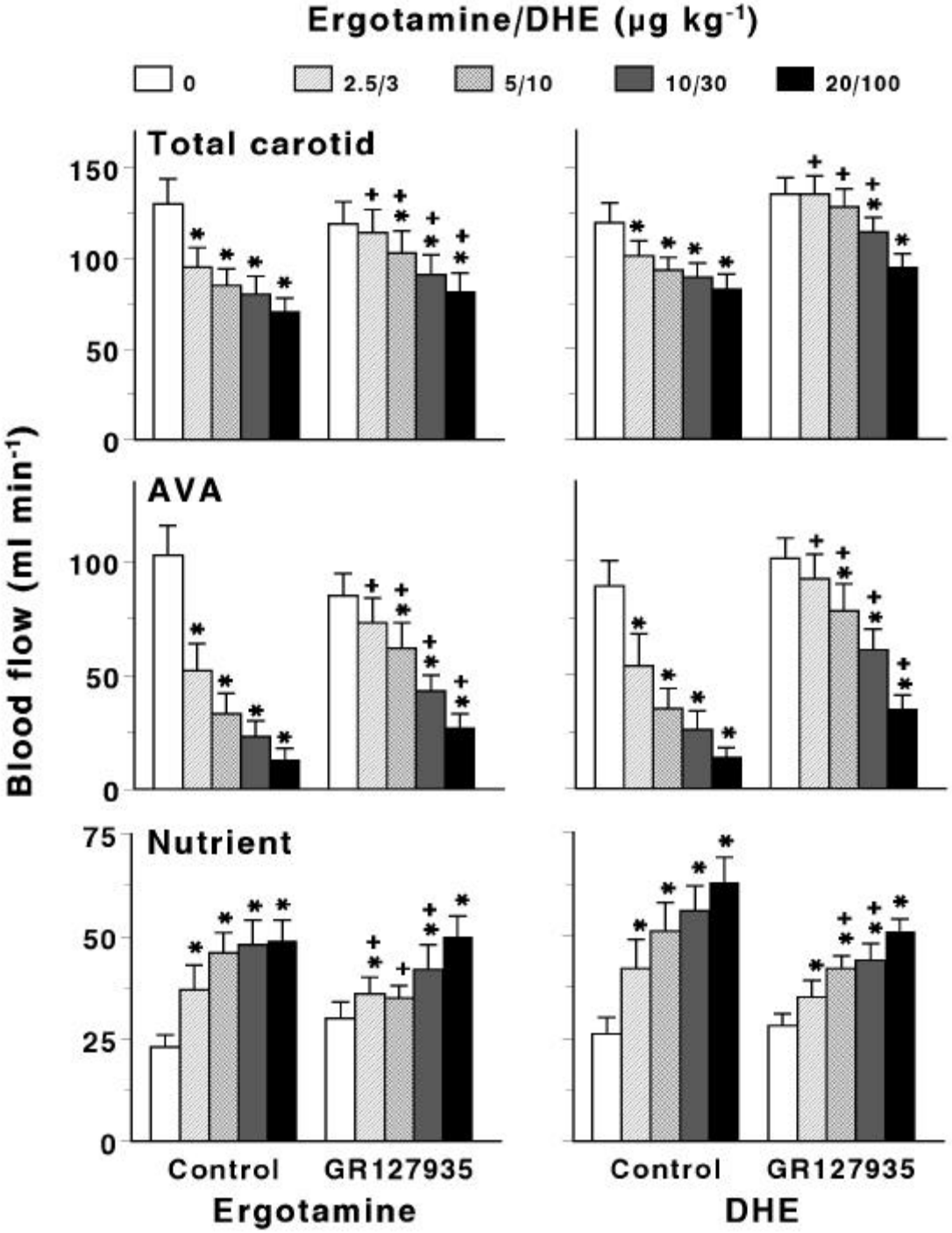

Figure 5.3 Effects of ergotamine (*left panels*) and dihydroergotamine (DHE; *right panels*) on the distribution of total carotid blood flow into its arteriovenous anastomotic (AVA) and nutrient fractions in pigs pretreated with either saline (Control; n=6 each) or GR127935 (0.5 mg kg^{-1}; n=6 each). All values are presented as the mean±s.e.mean. *, P<0.05 vs baseline; +, P<0.05 vs response by corresponding dose in control animals.

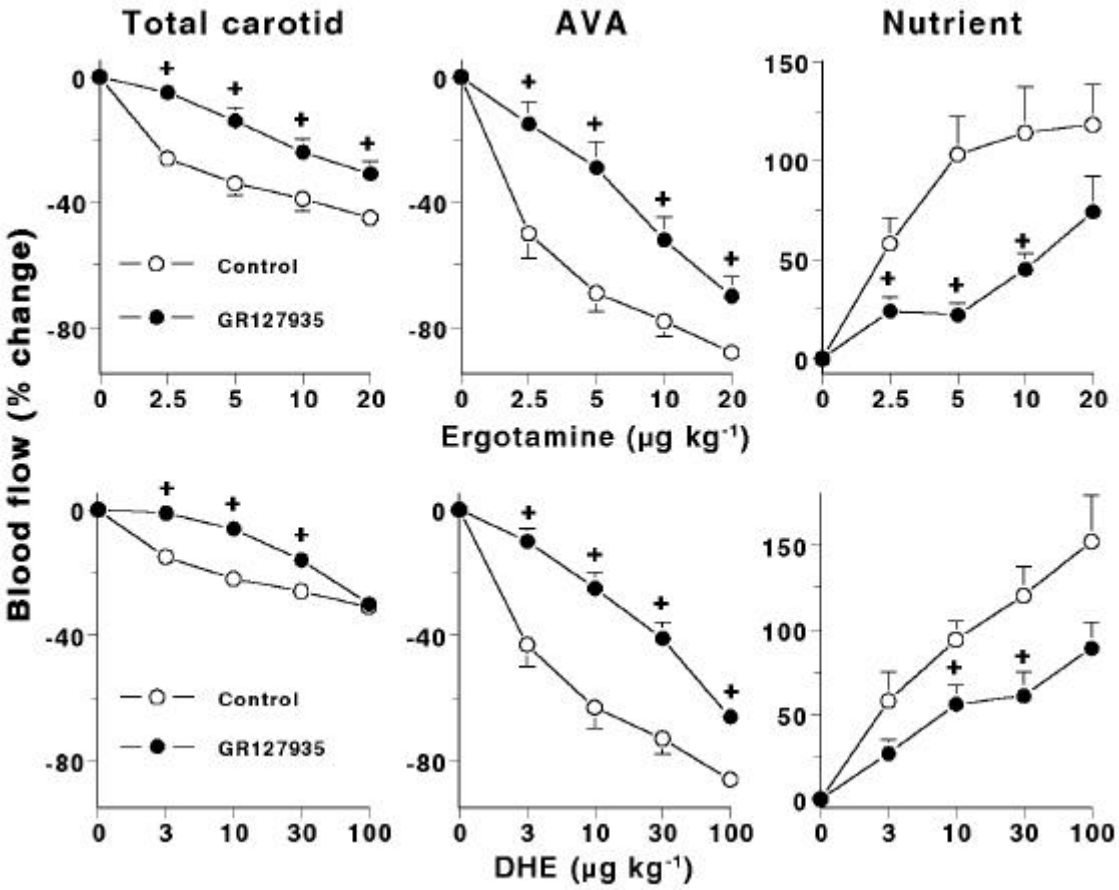

Figure 5.4 Percent changes from baseline values (n=6 each) of total carotid, arteriovenous anastomotic (AVA) and nutrient blood flow by ergotamine (upper panels) and dihydroergotamine (lower panels) in pigs pretreated with either saline (Control) or GR127935 (0.5 mg kg^{-1}). All values are presented as the mean±s.e.mean. +, $P<0.05$ vs control.

5.3.4 Carotid haemodynamic effects of ergot alkaloids in animals pretreated with physiological saline or GR127935

As shown in Figures 5.3 (absolute values) and 5.4 (percent changes from baseline), ergotamine (2.5-20 µg kg^{-1}), as well as dihydroergotamine (3-100 µg kg^{-1}) elicited dose-dependent decreases in the total carotid blood flow (maximal decreases: 45±3% and 31±2%, respectively) and in its conductance (maximal decreases: 47±4% and 37±2%, respectively; Table 5.3). These decreases in the total carotid blood flow were exclusively attributable to marked decreases in its arteriovenous anastomotic fraction; the highest dose of ergotamine (20 µg kg^{-1}) decreased arteriovenous anastomotic

blood flow and its conductance by 88±3% and 88±4%, respectively, whereas dihydroergotamine (100 µg kg^{-1}) similarly decreased these variables by 86±3% and 87±2%, respectively. Additionally, ergotamine and dihydroergotamine increased nutrient blood flow by up to 118±17% and 152±27%, respectively, accompanied by increases in nutrient vascular conductance (Table 5.3). Treatment with GR127935 (0.5 mg kg^{-1}) significantly attenuated the above ergot-induced responses. Maximal changes in the total carotid, arteriovenous anastomotic and nutrient blood flow induced by ergotamine were -31±4%, -70±6% and +74±18%, respectively, while dihydroergotamine produced changes of -30±2%, -66±3% and +89±15%, respectively. GR127935 (0.5 mg kg^{-1}) produced a 5.5-fold (from 0.8±0.2 to 4.4±0.8 µg kg^{-1}) and 12.2-fold (from 1.1±0.5 to 13.4±2.3 µg kg^{-1}) increases in the ED_{30} (dose eliciting a 30% decrease in arteriovenous anastomotic conductance, calculated using linear regression analysis) for ergotamine and dihydroergotamine, respectively.

The ergotamine- and dihydroergotamine-induced changes in the distribution of carotid blood flow to the different head tissues are depicted in Figure 5.5. Both ergot derivatives produced significant, dose-dependent increases in blood flow to skin, ear, fat, bone, brain and dura mater, of which only bone and brain blood flow values were slightly, though significantly, attenuated by GR127935; exclusively in muscle and salivary gland ergotamine-induced blood flow increases were observed, which were slightly less in animals pretreated with GR127935.

5.4 Discussion

5.4.1 General

We have previously shown that sumatriptan-induced carotid haemodynamic responses in pigs (see Chapter 4), as well as rabbits (Chapter 3) are mediated by 5-$HT_{1B/1D}$ receptors as these responses are completely antagonised by a single i.v. dose of 0.3-0.5 mg kg^{-1} of GR127935. The main aim of the present investigation in the anaesthetised pig was to establish whether the carotid vascular responses to the endogenous ligand (5-HT) and the antimigraine drugs, ergotamine and dihydroergotamine, are also mediated by 5-$HT_{1B/1D}$ receptors. Due to the different pharmacological properties of 5-HT and the ergot compounds, separate protocols were used.

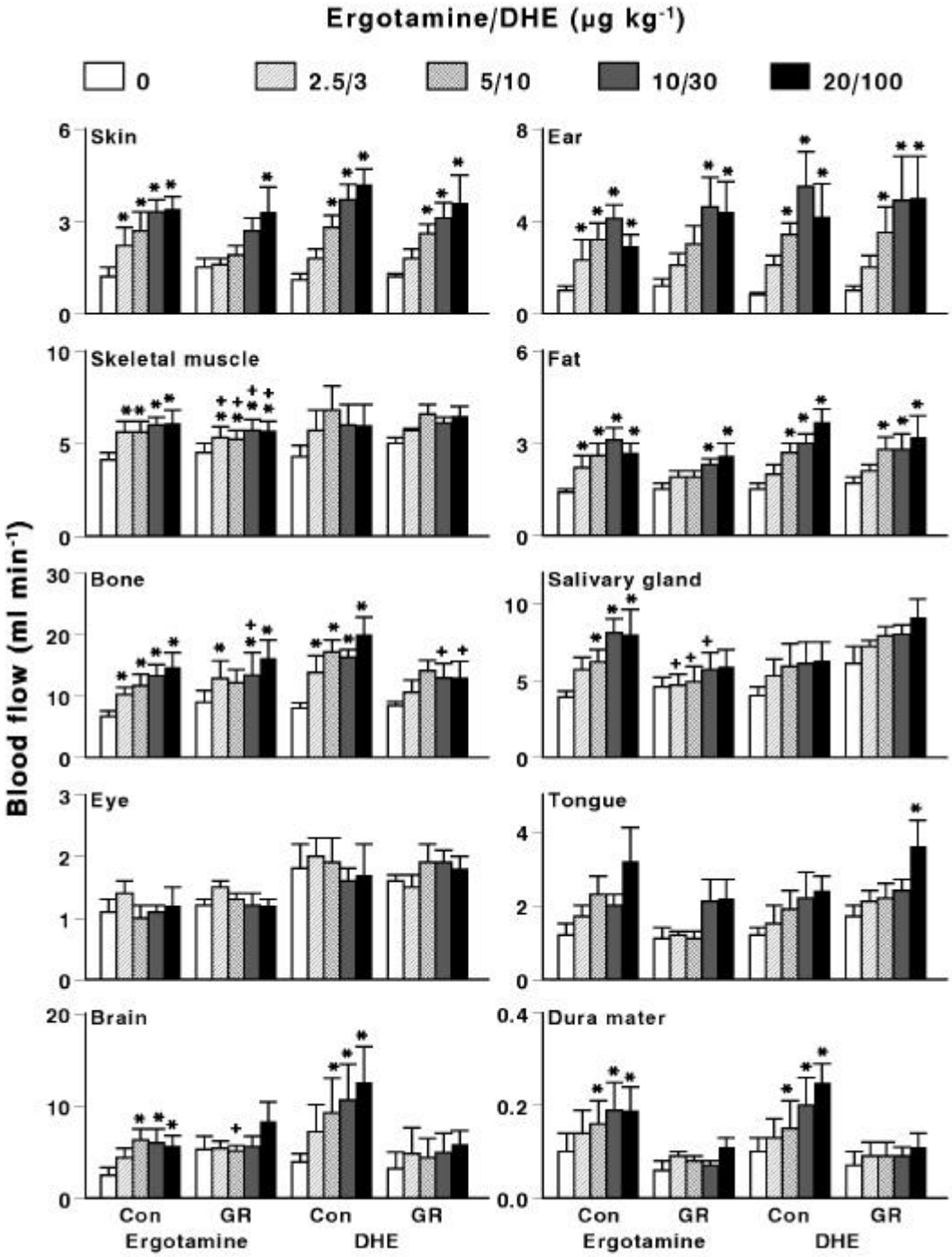

Figure 5.5 Effects of ergotamine and dihydroergotamine (DHE) on the distribution of total carotid blood flow to the different cranial tissues in pigs pretreated with either saline (Con; n=6 each) or GR127935 (0.5 mg kg^{-1}; n=6 each). All values are presented as the mean±s.e.mean. *, P<0.05 vs baseline; +, P<0.05 vs response by corresponding dose in control animals.

Firstly, as 5-HT_2 receptors play a role in carotid vascular effects by 5-HT (Saxena & Verdouw, 1982; Verdouw *et al*., 1984b), but not by the ergot alkaloids (Saxena *et al*., 1983; Bom *et al*., 1989a), the animals receiving 5-HT were pretreated with ketanserin. Ketanserin is a potent 5-HT_{2A} receptor antagonist and has also been shown to possess a moderate blocking property at the h5-HT_{1D} receptor (Table 1.2). It should be kept in mind, however, that in the present experiments we cannot provide evidence that the employed dosage also blocked the porcine 5-HT_{1D} receptor. Secondly, due to the short duration of action of 5-HT, the compound was infused directly into the carotid artery, whereas the long-acting compounds, ergotamine and dihydroergotamine, were administered intravenously. Moreover, the effect of GR127935 (0.5 mg kg^{-1}) on 5-HT-induced changes was studied within animals, while the ergot-induced changes were studied in different groups pretreated with either saline or GR127935. It should be noted that the intrinsic activity of GR127935 at receptors mediating arteriovenous anastomotic constriction (Chapter 3 and 4), also observed in the present experiments (see Figure 5.1, *lower panel*), does not allow us to use higher doses of the compound.

5.4.2 Systemic haemodynamics

As observed previously (Saxena & Verdouw, 1982), intracarotid infusion of 5-HT (2 µg kg^{-1} min^{-1}) produced a slight tachycardia and hypotension. The tachycardia could be due to an action at cardiac 5-HT_4 receptors (Villalón *et al*., 1990b), but it should be noted that heart rate did not increase during the second 5-HT infusion after treatment with physiological saline. Therefore, the apparent attenuation of the 5-HT-induced tachycardic effect by GR127935 seems to be a non-specific (tachyphylactic) effect. The small hypotensive response caused by 5-HT may be due to stimulation of 5-HT_7 receptors (see Chapter 10).

5.4.3 Arterio-jugular venous oxygen saturation difference (A-V SO_2)

5-HT did not increase the A-V SO_2. Considering the potent vasoconstriction of cephalic arteriovenous anastomoses by 5-HT (see below), one may expect an increase in the A-V SO_2 as observed with the ergot alkaloids (present results; Den Boer *et al*., 1991a) and sumatriptan (see Chapter 4; Den Boer *et al*., 1991b). However, in contrast to the latter compounds, 5-HT produces a very *pronounced* arteriolar dilatation, leading to a *huge* increase in nutrient (capillary) blood flow. As a consequence,

arterial blood with high O_2 saturation mixes with jugular venous blood ("physiological shunting"), thereby decreasing the A-V SO_2.

The effects of the ergot alkaloids on A-V SO_2 were not potently blocked, whereas those of sumatriptan were completely antagonised by GR127935 (see Chapter 4). This is in keeping with the extent of blockade of arteriovenous anastomotic constriction by GR127935, which abolished the response to sumatriptan, but only partly affected that to the ergot alkaloids (see below).

5.4.4 Effects of GR127935 on carotid vascular responses to 5-HT and ergot alkaloids

In control animals, intracarotid infusions of 5-HT decreased arteriovenous anastomotic blood flow, but caused a marked arteriolar vasodilatation; as a result the total carotid blood flow increased. This is in keeping with our previous reports, where treatment with 5-HT_2 receptor antagonists, such as ketanserin, cyproheptadine and WAL1307, partly blocked 5-HT-induced arteriovenous anastomotic constriction and enhanced arteriolar dilatation (Saxena & Verdouw, 1982; Verdouw *et al*., 1984b). Surprisingly, the second 5-HT infusion, as well as the initial 5-HT infusion in the other subgroup, did not produce significant changes in the total carotid blood flow. We have also previously observed that, whereas the magnitude of 5-HT-induced decreases in arteriovenous anastomotic blood flow appears to be constant, the magnitude of increases in nutrient blood flow is subject to variation (see Saxena & Verdouw, 1982; Saxena & Verdouw, 1984; Verdouw *et al*., 1984b; Saxena *et al*., 1986; Den Boer *et al*., 1992b). Although we have no direct explanation for this variable response in the arteriolar vascular bed, it is worth noticing that differences in the degree of pre-existing sympathetic vascular tone, probably produced by the degree of initial anaesthesia (Den Boer *et al*., 1993), might play a role.

As observed previously in several species (Saxena, 1974a; Mylecharane *et al*., 1978; Spierings & Saxena, 1980; Den Boer *et al*., 1991a), both ergotamine and dihydroergotamine dose-dependently decreased the total carotid blood flow, and this response was exclusively caused by a selective constriction of carotid arteriovenous anastomoses with concomitant conductance changes.

Our findings show that the arteriovenous anastomotic constriction by 5-HT was partly attenuated after GR127935. However, it should be noted that the observed attenuation might be, at least in part, a result of the second 5-HT infusion starting at a

lower level of blood flow, being related to the intrinsic activity of GR127935 itself (see above). Accordingly, the same minimum level of arteriovenous anastomotic blood flow was obtained before and after GR127935 and, when compared to the corresponding response by 5-HT in control animals, no significance was reached. These results lead us to conclude that the 5-HT-induced carotid arteriovenous anastomotic constriction in ketanserin-pretreated pigs is primarily mediated by receptors *not* identical to the 5-$HT_{1B/1D}$ receptor subtypes. As the arteriovenous anastomotic constriction by sumatriptan was abolished by a 0.5 mg kg^{-1} dose of GR127935 (Chapter 4), these findings imply that 5-HT and sumatriptan constrict porcine arteriovenous anastomoses predominantly via distinct receptors. In keeping with the above, Yu *et al.* (1997) have recently reported that, although GR127935 was capable of antagonising sumatriptan-induced inhibition of neurogenic plasma extravasation in the guinea-pig, 5-CT-induced effects remained unaffected. On the other hand, the decrease in arteriovenous anastomotic conductance by both ergotamine and dihydroergotamine was attenuated to a considerable degree (5.5- and 12.2-fold shifts, respectively, in ED_{30} values) by GR127935 (0.5 mg kg^{-1}). Den Boer *et al.* (1991a) have previously shown that methiothepin (3 mg kg^{-1}) caused 3.1- and 5.2-fold increases in ED_{30} values of ergotamine and dihydroergotamine, respectively. The higher shifts in ED_{30} values by GR127935 are in keeping with the higher affinities displayed by GR127935 at h5-$HT_{1B/1D}$ receptors compared to those exhibited by methiothepin (see Table 4.1). In view of the high affinities displayed at 5-HT_{1B} and 5-HT_{1D} receptors by ergotamine (Table 7.1), methiothepin, as well as by GR127935, it is suggested that both ergot compounds partly constrict porcine arteriovenous anastomoses via 5-$HT_{1B/1D}$ receptors.

5.4.5 Do the GR127935-resistant receptors resemble any of the other 5-HT receptors?

Based on the above, the greater part of the 5-HT-induced, as well as a considerable part of the ergot-induced arteriovenous anastomotic constriction does not seem to be mediated by 5-$HT_{1B/1D}$ receptors. Furthermore, as the arteriovenous anastomotic constriction by 5-HT was completely blocked (Saxena *et al.*, 1986) and that by the ergots only partly affected (Den Boer *et al.*, 1991a) methiothepin, it is likely that 5-HT and the ergot compounds act via different (non-5-$HT_{1B/1D}$) receptors. In this context, several subtypes of the 5-HT_1 receptor family (5-HT_{1A}, 5-ht_{1E} and 5-HT_{1F}) may be

considered as possible candidates. Whereas the involvement of 5-HT_{1A} receptors has already been excluded (Saxena & Villalón, 1990; Den Boer *et al.*, 1992b), the participation of 5-ht_{1E} and 5-HT_{1F} receptors is also unlikely because: (i) 5-CT, a compound with little affinity at 5-ht_{1E} and 5-HT_{1F} receptors (Table 1.2), is a potent agonist (Saxena & Verdouw, 1985a); (ii) the 5-HT-induced arteriovenous anastomotic constriction was abolished by methiothepin (Saxena *et al.*, 1986), but not by GR127935 (present results), despite their similar affinities at the h5-ht_{1F} receptor (Table 4.1); and (iii) ergotamine and dihydroergotamine display low affinities (see Table 7.1).

We have previously shown that the 5-HT- and ergot-induced arteriovenous anastomotic constriction is not mediated by 5-HT_2 or 5-HT_3 receptors (Saxena *et al.*, 1983; Saxena *et al.*, 1986; Bom *et al.*, 1989a). The pharmacological profile of the receptors mediating ergot- or 5-HT-induced carotid vascular effects also seems to be inconsistent with 5-HT_4, 5-ht_5 or 5-ht_6 classification on the basis of: (i) the high potency of 5-CT relative to 5-HT (Saxena & Verdouw, 1985a), an order which is reversed for the 5-HT_4 and 5-ht_6 receptors (Hoyer *et al.*, 1994); (ii) the potent blockade of 5-HT-induced arteriovenous anastomotic constriction by methiothepin (Saxena *et al.*, 1986), which is inactive at 5-HT_4 receptors (Hoyer *et al.*, 1994); (iii) dihydroergotamine displaying low affinity at gp5-HT_4 receptors (Table 7.1) and; (iv) the absence of *m*RNA for 5-ht_5 receptors on blood vessels (Ullmer *et al.*, 1995). Similarly, 5-HT_7 receptors are not likely to mediate constrictor responses on the basis of positive coupling to adenylyl cyclase and, therefore, leading to an increase in cAMP, which is associated with vasorelaxation (Rand *et al.*, 1987; Hoyer *et al.*, 1994). Additionally, sumatriptan, which potently constricts porcine arteriovenous anastomoses, is inactive at 5-HT_7 receptors (Hoyer *et al.*, 1994).

Since in the dog the carotid vasoconstrictor effects by ergot-derivatives is abolished by a combination of GR127935 and yohimbine (Chapter 7), α_2-adrenoceptors may have mediated the GR127935-resistant part of the ergot-induced arteriovenous anastomotic constriction in the present experiments. The latter requires further investigation.

5.4.6 5-HT-induced arteriolar dilatation in cranial tissues

In accordance with earlier observations (Saxena & Verdouw, 1982; Verdouw *et al.*, 1984b; Saxena *et al.*, 1986; Den Boer *et al.*, 1992b), 5-HT produced arteriolar

dilatation, which was confined to the head skin and ears. As demonstrated in other vascular preparations (see Chapters 10 and 12; Eglen *et al.*, 1997; Saxena *et al.*, 1998b), it is possible that this carotid arteriolar dilatation may also be mediated by the 5-HT_7 receptor. This contention is supported by the fact that (i) the vasodilator response is potently mimicked by 5-CT (Saxena & Verdouw, 1985a), but only weakly by sumatriptan (see Chapter 4; Den Boer *et al.*, 1991b) and (ii) methiothepin, a compound with high affinity at cloned 5-HT_7 receptors (see Table 10.2), acts as a potent antagonist (Saxena *et al.*, 1986). Furthermore, in contrast to the vasodilator effect of 5-HT (Figure 5.2), the sumatriptan-induced vasodilatation is abolished by GR127935 (Chapter 4), implying the involvement of 5-$HT_{1B/1D}$ receptors, as also shown in the porcine coronary artery (Schoeffter & Hoyer, 1990). In view of the attenuation of the ergot-induced (weak) arteriolar dilatation by GR127935 (Figure 5.5), their vasodilator effect also seems to be mediated, at least in part, via 5-$HT_{1B/1D}$ receptors.

In conclusion, our results show that in pigs pretreated with ketanserin, 5-HT constricts arteriovenous anastomoses primarily via a receptor type that cannot be classified using guidelines for the current nomenclature. Similarly, although the present results demonstrate a substantial role for 5-$HT_{1B/1D}$ receptors, but yet to be identified receptors mediate the GR127935-resistant part of the ergot-induced arteriovenous anastomotic constriction. Since previous reports have shown that methiothepin completely blocks 5-HT-induced, but only partly attenuates the ergot-induced arteriovenous anastomotic constriction, multiple receptors seem to be involved. In view of the putative role of cranial arteriovenous anastomotic dilatation during migraine headache (Heyck, 1969; Saxena, 1995), as well as the high predictive value of constriction of these structures in antimigraine therapy (Saxena, 1995), these novel receptors could be a target for further antimigraine drug development (Villalón *et al.*, 1997b).

Chapter 6

The porcine carotid and systemic haemodynamic effects of the 5-$HT_{1B/1D}$ receptor agonists, alniditan, BMS181885, avitriptan, eletriptan and GMC2021; comparison with sumatriptan

Summary In previous studies, we have shown that sumatriptan potently constricts porcine carotid arteriovenous anastomoses. This effect seems to be of high predictive value for antimigraine activity. In the present experiments, we studied the effects of several new tryptamine (BMS181885, avitriptan, eletriptan and GMC2021) as well as non-tryptamine derivatives (alniditan) with high affinity at 5-$HT_{1B/1D}$ receptors on systemic and carotid haemodynamics in anaesthetised pigs. Intravenous administrations of the above drugs decreased total carotid blood flow by a selective vasoconstriction of the carotid arteriovenous anastomoses. The apparent rank order of potency was: alniditan > sumatriptan > avitriptan > eletriptan > GMC2021, whereas BMS181885 seemed to have a lower intrinsic activity. The arteriovenous anastomotic constriction by alniditan, eletriptan and GMC2021 was potently, but not completely, antagonised by 0.5 mg kg^{-1} of GR127935. After similar reductions in carotid blood flow following a single 100 µg kg^{-1} intravenous dose at 30 min, the effect of BMS181885 lasted longer than that of sumatriptan. These results suggest that the new triptans selectively constrict porcine carotid arteriovenous anastomoses mainly via 5-$HT_{1B/1D}$ receptors and should be able to abort migraine headaches. The latter has indeed been confirmed in initial clinical studies in man. Lastly, the long-lasting effect of BMS181885 may reduce the recurrence rate in migraine.

Based on:

De Vries, P., Willems, E.W., Heiligers, J.P.C., Villalón, C.M. & Saxena, P.R. (1998). The antimigraine agent alniditan selectively constricts porcine carotid arteriovenous anastomoses via 5-$HT_{1B/1D}$ receptors. *Eur. J. Pharmacol.*, **351**, 193-201.

Saxena, P.R., De Vries, P., Heiligers, J.P.C., Bax, W.A., MaassenVanDenBrink, A. & Yocca, F.D. (1998). BMS-181885, a highly potent 5-$HT_{1B/1D}$ receptor ligand: effects in experimental models predictive of antimigraine activity and coronary side-effect potential. *Eur. J. Pharmacol.*, **351**, 329-339.

Saxena, P.R., De Vries, P., Wang, W., Heiligers, J.P.C., MaassenVanDenBrink, A., Bax, W.A. & Yocca, F.D. (1997). Effects of avitriptan, a new 5-$HT_{1B/D}$ receptor agonist, in experimental models predictive of antimigraine activity and coronary side-effect potential. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **355**, 295-302.

Saxena, P.R., De Vries, P., Heiligers, J.P.C., MaassenVanDenBrink, A., Bax, W.A., Barf, T. & Wikstrom, H. (1996). Investigations with GMC2021 in experimental models predictive of antimigraine activity and coronary side-effect potential. *Eur. J. Pharmacol.*, **312**, 53-62.

Willems, E.W., De Vries, P., Heiligers, J.P.C. & Saxena, P.R. (1998). Porcine carotid vascular effects of eletriptan (UK-116,044): a new 5-$HT_{1B/1D}$ receptor agonist with anti-migraine activity. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **358**, 212-219.

6.1 Introduction

The indole derivative sumatriptan was introduced several years ago (Humphrey *et al*., 1988) and since then many studies have established the effectiveness of the drug in the acute treatment of migraine (see Chapter 2). Sumatriptan constricts several large cranial and extracranial blood vessels, including porcine carotid arteriovenous anastomoses, via 5-HT_1-like receptors (Den Boer *et al*., 1991b). Using the selective 5-$HT_{1B/1D}$ receptor antagonist GR127935 (see Chapter 3; Clitherow *et al*., 1994; Pauwels, 1996; Skingle *et al*., 1996), it has been shown that the sumatriptan-sensitive 5-HT_1-like receptors in several tissues (Pauwels, 1996; Skingle *et al*., 1996; Villalón *et al*., 1996), including porcine carotid arteriovenous anastomoses (see Chapter 4), are identical to 5-$HT_{1B/1D}$ receptors.

The success of selective 5-$HT_{1B/1D}$ receptor agonists in the treatment of migraine, combined with some shortcomings of sumatriptan (e.g. headache recurrence, coronary artery constriction, low oral bioavailability) has prompted the development of several new compounds acting specifically at this receptor class (see Chapter 2). Alniditan, BMS181885, avitriptan, eletriptan and GMC2021 are such compounds. Like sumatriptan, these compounds are substituted indoles, although alniditan is a benzopyran derivative (see Figure 6.1). As shown in Table 6.1, all compounds display high affinities at the 5-$HT_{1B/1D}$ receptors, alniditan and BMS181885 being the most potent. Moreover, alniditan displays virtually no affinity at the 5-HT_{1F} receptor, but the highest at 5-HT_{1A} receptors. The 5-ht_{1E} and 5-HT_{1F} receptor binding data have not been reported for avitriptan, BMS181885 and GMC2021.

Isolated cerebral arteries potently and efficaciously constrict in response to alniditan (Janssens *et al*., 1997), avitriptan (Yocca *et al*., 1995; Goggins *et al*., 1996) and eletriptan (Gupta *et al*., 1999), whereas alniditan (Limmroth *et al*., 1997) and eletriptan (Gupta *et al*., 1996) were also shown to block plasma extravasation evoked by trigeminal ganglion stimulation in the rat dura mater. BMS181885, however, displayed weak activity (1,000-fold less potent than sumatriptan) in reducing electrically-evoked plasma extravasation in guinea-pig dura-mater, yet the drug potently constricted cranial blood vessels, although lower maximal contractions compared to sumatriptan were reached (Yocca *et al*., 1997). Additionally, alniditan (Van de Water *et al*., 1996), BMS181885 (Yocca *et al*., 1997), avitriptan (Yocca *et al*., 1995) and eletriptan (Gupta *et al*., 1996) have shown to reduce carotid

blood flow in the dog. Interestingly, BMS181885 does not constrict the canine saphenous vein, but even behaved as a competitive antagonist (Yocca *et al*., 1997), whereas eletriptan behaves as a partial agonist in this blood vessel (Gupta *et al*., 1999).

Table 6.1 Binding values of several antimigraine agents at human 5-HT_1 receptor subtypes.

	pK_i				
	5-HT_{1A}	**5-HT_{1B}**	**5-HT_{1D}**	**5-ht_{1E}**	**5-HT_{1F}**
Sumatriptan[a]	6.4	7.8	8.5	5.8	7.9
Alniditan[a]	8.4	9.0	9.4	6.6	6.4
BMS181885[b]	6.4	9.0	8.7	ND	ND
Avitriptan[c]	7.2	7.7	8.4	ND	ND
Eletriptan[d]	7.4	8.0	8.9	7.3	8.0
GMC2021[e]	7.4	7.5	8.5	ND	ND

pK_i values are taken from: [a], Leysen *et al.* (1996); [b], Yocca *et al.* (1997); [c], Yocca *et al.* (1995); [d], Napier *et al.* (1999); [e], Saxena *et al.* (1996). ND, not determined.

In the present study, we analysed the effects of alniditan, BMS181885, avitriptan, eletriptan and GMC2021 in the porcine carotid vascular bed, with particular emphasis on the arteriovenous anastomotic fraction, since constriction of cranial arteriovenous anastomoses has previously been shown to be of high predictive value for antimigraine activity (e.g. Saxena, 1995; Saxena *et al*., 1997). Moreover, in the case of alniditan, eletriptan and GMC2021 we established the contribution of 5-$HT_{1B/1D}$ receptors using GR127935, at a dose capable of abolishing sumatriptan-induced effects in the same experimental model (see Chapter 4). The above effects were compared to previous results obtained with sumatriptan, described in Chapter 4. Lastly, in view of the slow dissociation rate of BMS181885 from 5-$HT_{1B/1D}$ receptors (Yocca *et al*., 1997), we studied the duration of action of this drug, compared to that of sumatriptan.

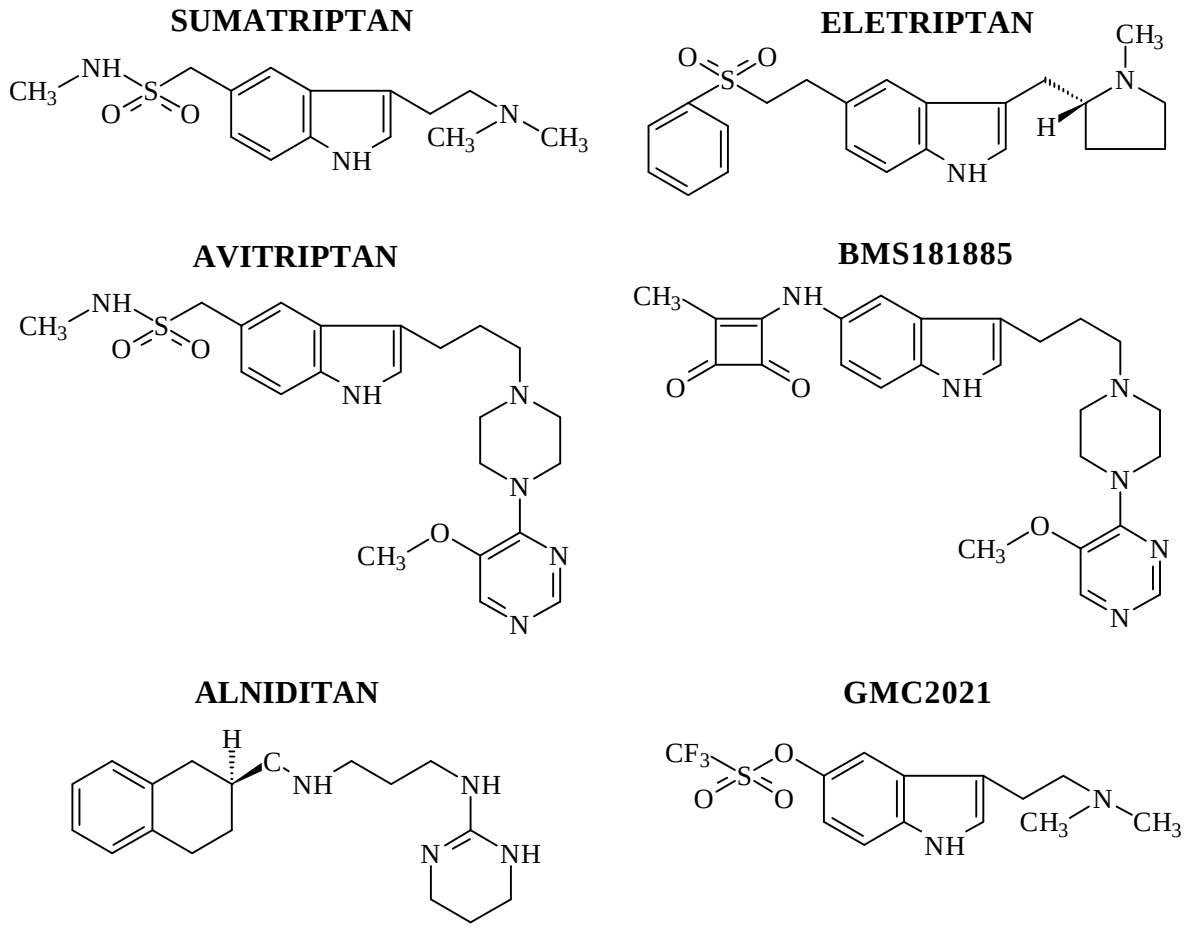

Figure 6.1 Chemical structures of several compounds with antimigraine activity

6.2 Methods

6.2.1 General

The methods used for anaesthesia, surgical preparations and determination of systemic and carotid haemodynamics are described in detail in Chapter 4.

6.2.2 Experimental protocol

After a stabilisation period of about 1 h, the animals were divided into three groups. The first (n=42) and second group (n=19) were pretreated with saline or GR127935 (0.5 mg kg^{-1}), respectively, given over a period of 5 min at a rate of 1 ml min^{-1} (i.v.). After a waiting period of 15 min, baseline values of heart rate, mean arterial blood pressure, carotid blood flow and its distribution, as well as arterial and jugular venous

blood gases were measured. Subsequently, the first group was subdivided into 6 subgroups, receiving sequential i.v. doses of either alniditan (3, 10, 30 and 100 μg kg^{-1}; n=6), BMS181885 (10, 30, 100 and 300 μg kg^{-1}; n=7), avitriptan (10, 30, 100 and 300 μg kg^{-1}; n=7), eletriptan (10, 30, 100, 300 and 1000 μg kg^{-1}; n=6, 9, 9, 8 and 3, respectively), GMC2021 (30, 100, 300 and 1000 μg kg^{-1}; n=6) or saline (four consecutive injections of 0.5 ml; n=7). The second group was subdivided into 3 subgroups receiving alniditan (n=6), eletriptan (n=7) or GMC2021 (n=6), at the above doses. Each dose of the above agonists was given every 20 min. Fifteen min after each dose, all haemodynamic variables were assessed again.

After measuring the systemic and carotid haemodynamic variables at baseline, the third group (n=12) was divided into two subgroups (n=6 each), receiving a single i.v. dose (100 μg kg^{-1}) of either sumatriptan or BMS181885. All haemodynamic parameters were reassessed 30, 60, 90 and 120 min after the drug administration.

6.2.3 Data presentation and statistical analysis

All data have been expressed as the mean±s.e.mean. The significance of the difference between the variables within one group was evaluated with Duncan's new multiple range test, once an analysis of variance (randomised block design) had revealed that the samples represented different populations (Steel & Torrie, 1980). The changes caused by the agonists in the GR127935-pretreated groups were compared to the corresponding responses in the saline-pretreated groups by using Student's unpaired *t*-test. Statistical significance was accepted at $P<0.05$ (two-tailed). In the saline-pretreated groups, the agonist dose eliciting a 50% decrease (ED_{50}) in arteriovenous anastomotic vascular conductance was calculated using linear regression analysis.

6.2.4 Drugs

Apart from the anaesthetics (see Chapter 4), the compounds used in this study were: alniditan (Janssen Research Foundation, Belgium), BMS181885 and avitriptan (both from Bristol-Myers Squibb, Wallingford, CT, USA), eletriptan (Pfizer Limited, UK), GMC2021 (Department of Medicinal Chemistry, University of Groningen, The Netherlands), sumatriptan succinate and GR127935 (Glaxo Group Research, Ware, UK). Heparin sodium (Leo Pharmaceutical Products, Weesp, The Netherlands) was used to prevent clotting of the catheters. All agonists were dissolved in physiological

saline, whereas GR127935 was dissolved by heating the dispersion in distilled water to about 70°C and then allowing to cool down to room temperature. All doses refer to the respective salts.

6.3 Results

6.3.1 Systemic haemodynamics

As shown in Table 6.2, bradycardia, hypotension and increases in the arteriovenous oxygen saturation difference (A-V SO_2) were observed (with maximum changes in heart rate, blood pressure and A-V SO_2, respectively) with alniditan (4±1, 23±5 and 252±78%), BMS181885 (6±2, 7±4 and 126±59%), avitriptan (17±4, 13±6 and 182±70%), eletriptan (11±3, 32±1 and 163±101%) and GMC2021 (5±1, 30±1 and 102±36%). GR127935 attenuated the hypotension by eletriptan and GMC2021 (maximum decreases: 19±3 and 11±3%, respectively), as well as the decreases in A-V SO_2 by alniditan, eletriptan and GMC2021 (maximum changes: 57±14, 137±72 and 25±14%, respectively). The infusions of saline were devoid of systemic effects.

As depicted in Table 6.3, a single dose (100 μg kg^{-1}) of sumatriptan or BMS181885 moderately decreased heart rate, which remained lower throughout the observation period of 2 h; blood pressure was not affected by the drugs. Whereas sumatriptan did not change A-V SO_2, BMS181885 increased this parameter from the first dose onwards and this effect was evident even after 120 min.

Table 6.2 Systemic haemodynamic effects after i.v. infusions of several 5-$HT_{1B/1D}$ receptor agonists (μg kg^{-1}) or corresponding volumes of saline (ml).

Alniditan	*Baseline*	*3*	*10*	*30*	*100*
HR (beats min^{-1})	95±4	93±3*	92±4*	91±4*	91±4*
MAP (mmHg)	95±3	95±4	90±4*	80±3*	73±3*
A-V SO_2 (%)	4.6±1.3	7.8±2.3	9.7±3.2*	13.1±3.4*	14.6±3.8*
BMS181885	*Baseline*	*10*	*30*	*100*	*300*
HR (beats min^{-1})	113±4	111±4	109±4*	108±4*	106±4*
MAP (mmHg)	95±4	97±5	97±4	94±5	88±6*
A-V SO_2 (%)	14.6±3.2	19.1±4.2*	22.2±4.0*	24.2±3.8*	25.5±3.8*
Avitriptan	*Baseline*	*10*	*30*	*100*	*300*
HR (beats min^{-1})	90±4	85±5	79±5*	77±5*	75±5*
MAP (mmHg)	93±5	91±4	90±5	88±5	80±5*
A-V SO_2 (%)	4.4±0.5	5.1±1.2	6.9±1.1	9.5±1.9*	12.5±2.5*
Eletriptan	*Baseline*	*30*	*100*	*300*	*1000*
HR (beats min^{-1})	101±3	97±3*	95±2*	92±3*	90±3*
MAP (mmHg)	96±3	89±2*	85±2*	80±3*	69±1*
A-V SO_2 (%)	13.8±5.2	16.0±5.0	19.6±4.5	22.1±4.5*	32.8±4.5
GMC2021	*Baseline*	*30*	*100*	*300*	*1000*
HR (beats min^{-1})	94±2	93±2*	91±2*	90±2*	89±2*
MAP (mmHg)	98±3	94±4*	88±4*	79±3*	69±2*
A-V SO_2 (%)	10.1±1.5	11.6±2.7	11.2±1.9	14.6±2.1	18.9±2.9*
Saline	*Baseline*	*0.5*	*0.5*	*0.5*	*0.5*
HR (beats min^{-1})	98±2	97±2	96±2	96±2	95±2
MAP (mmHg)	100±3	95±3	96±3	96±2	97±3
A-V SO_2 (%)	6.6±2.2	6.6±1.6	6.8±2.2	6.7±1.3	7.5±2.0

HR, heart rate; MAP, mean arterial blood pressure; A-V SO_2, oxygen saturation difference between arterial and jugular venous blood. *, $P<0.05$ vs baseline. The effects of 10 μg kg^{-1} of eletriptan are not shown.

Table 6.3 Systemic haemodynamic effects at different time-points after a single i.v. infusion of sumatriptan or BMS181885 in anaesthetised pigs.

		Time (min) after 100 µg kg^{-1}			
	Baseline	*30*	*60*	*90*	*120*
Sumatriptan					
HR (beats min^{-1})	108±2	102±3*	99±3*	97±3*	95±2*
MAP (mmHg)	86±3	86±4	83±5	83±5	83±4
A-V SO_2 (%)	17.7±4.5	20.9±6.3	19.1±5.9	18.6±5.9	18.2±5.4
BMS181885					
HR (beats min^{-1})	111±4	100±4*	97±3*	95±4*	93±4*
MAP (mmHg)	83±1	85±4	84±5	84±6	83±7
A-V SO_2 (%)	13.6±3.7	23.8±5.4*	23.1±6.5*	21.4±5.6*	23.6±7.5*

HR, heart rate; MAP, mean arterial blood pressure; A-V SO_2, oxygen saturation difference between arterial and jugular venous blood. *, $P<0.05$ vs baseline.

6.3.2 Carotid haemodynamics

Alniditan, BMS181885, avitriptan, eletriptan and GMC2021, decreased total carotid blood flow, which was in all cases entirely due to a decrease in its arteriovenous anastomotic, non-nutrient fraction, as also observed with sumatriptan (Figure 6.2). Nutrient blood flow increased in response to the agonists. The decreases in arteriovenous anastomotic blood flow were accompanied by reductions in carotid arteriovenous anastomotic conductance (where the changes in blood flow are corrected for changes in blood pressure), indicative of a vasoconstrictor action on these blood vessels (Figure 6.3).

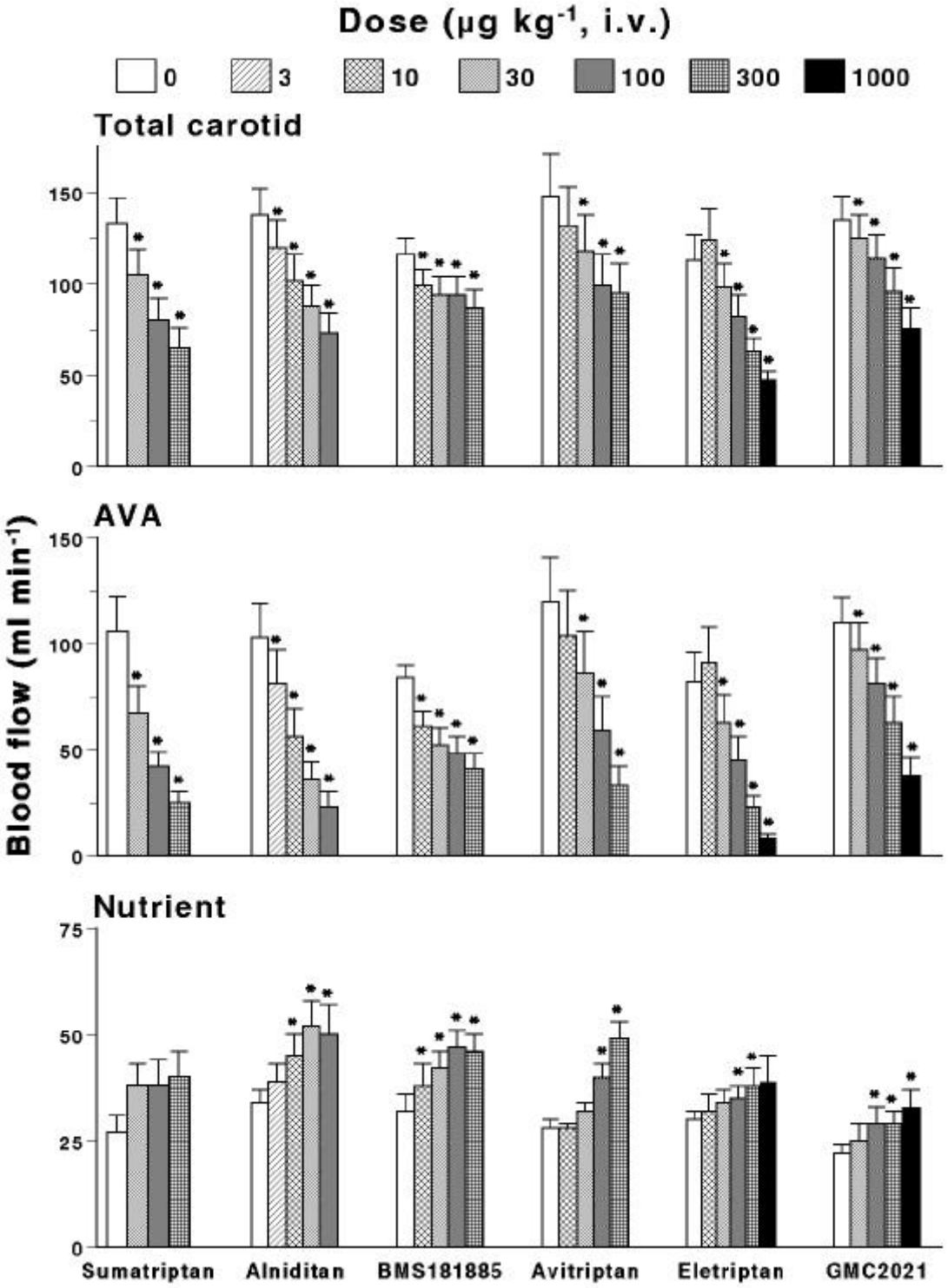

Figure 6.2 The effect of several 5-$HT_{1B/1D}$ receptor agonists on porcine total carotid, arteriovenous anastomotic (AVA) and nutrient blood flow. *, $P<0.05$ vs baseline. For comparison, the effects of sumatriptan are given (data from Chapter 4).

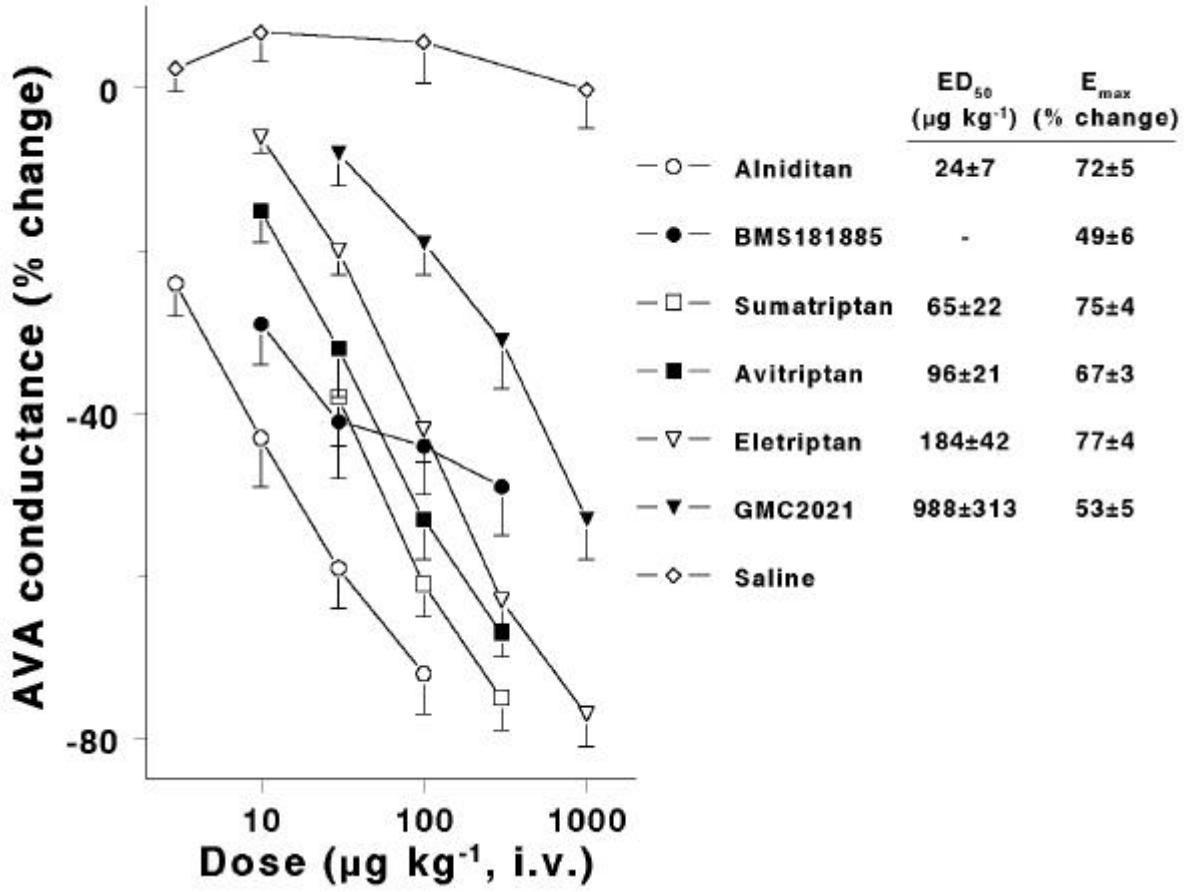

Figure 6.3 Percentage changes in arteriovenous anastomotic (AVA) conductance by saline (4 consecutive infusions of 5 ml) and several 5-$HT_{1B/1D}$ receptor agonists in anaesthetised pigs. The dose producing a 50% decrease (ED_{50}) and the maximum percent changes (E_{max}) in AVA conductance are shown in the right panel. All values are presented as the mean±s.e.mean. For comparison the effects of sumatriptan are given (data from Chapter 4).

The rank order of agonist potency was: alniditan > sumatriptan > avitriptan > eletriptan > GMC2021, whereas BMS181885 seemed to have a lower intrinsic activity (see Figure 6.3). As shown in Figure 6.4, the alniditan-, eletriptan- and GMC2021-induced decreases in arteriovenous anastomotic conductances were potently, but not completely, antagonised by 0.5 mg kg^{-1} of GR127935.

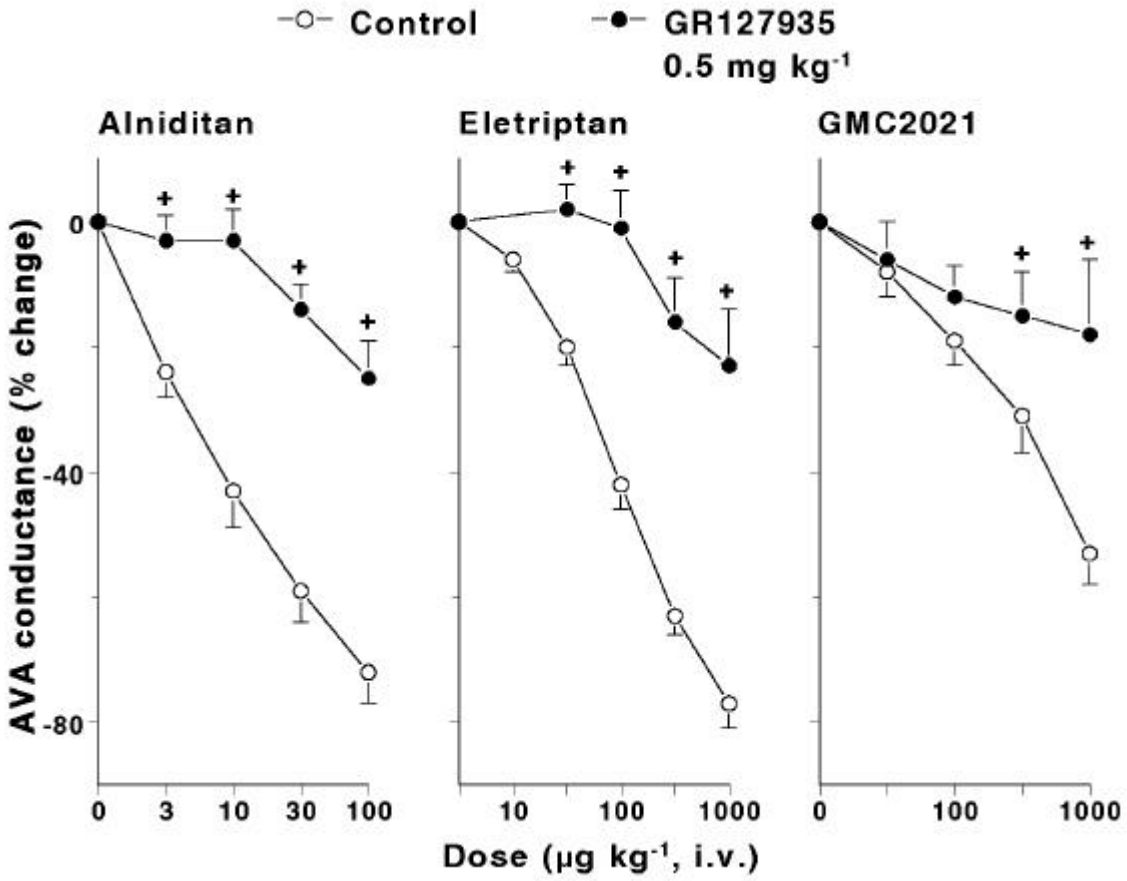

Figure 6.4 Percent changes from baseline values caused by alniditan, eletriptan and GMC2021 in carotid arteriovenous anastomotic conductance in pigs pretreated with either saline (Control) or 0.5 mg kg^{-1} of GR127935. +, $P<0.05$ vs control.

As depicted in Figure 6.5, the single dose of sumatriptan and BMS181885 decreased total carotid and arteriovenous anastomotic blood flows. BMS181885, but not sumatriptan, also increased nutrient blood flow. The effects of BMS181885 persisted for the 120-min observation period. In contrast, the effects of sumatriptan disappeared after 30-60 min.

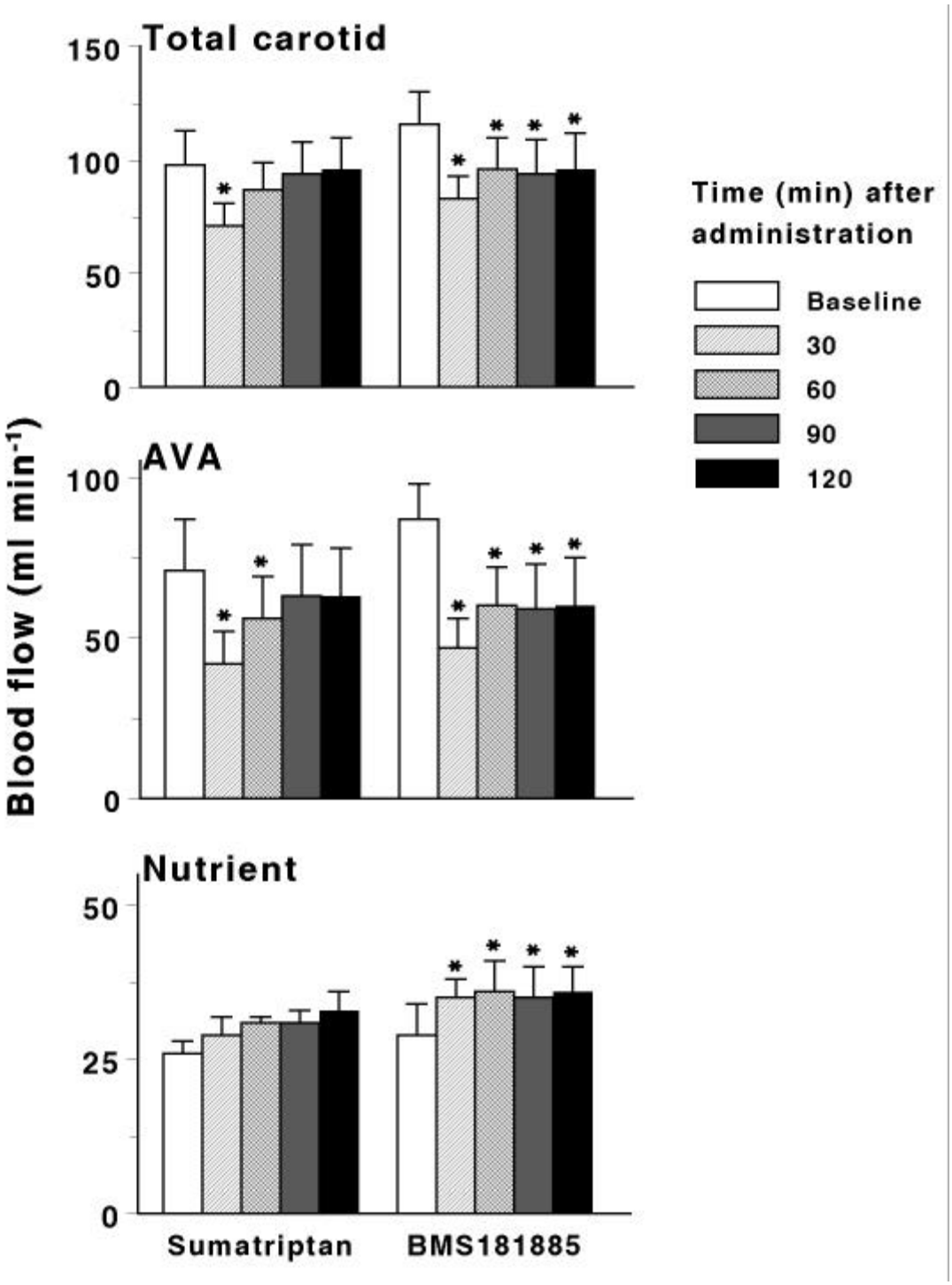

Figure 6.5 The effects of a single infusion of sumatriptan or BMS181885 (both 100 µg kg^{-1}, i.v.) at different time-points on total carotid, arteriovenous anastomotic (AVA) and nutrient blood flow. *, P<0.05 vs baseline.

6.4 Discussion

6.4.1 Systemic haemodynamics

All 5-$HT_{1B/1D}$ receptor agonists produced a small bradycardia and a hypotension, of which the decrease in blood pressure was especially pronounced with alniditan, eletriptan and GMC2021. This has also been observed with sumatriptan, although the magnitude was rather small (see Chapter 4). The decreases in blood pressure may be related to an action at central or peripheral receptors mediating decreases in sympathetic outflow (Saxena & Villalón, 1990). It should be noted, however, that alniditan, BMS181885 and sumatriptan are not able to cross the blood brain barrier easily, due to their low lipophilicity (Saxena & Tfelt-Hansen, 1993; Bonaventura *et al*., 1997); avitriptan, eletriptan and GMC2021 are more lipophilic compounds (Rance *et al*., 1997, Svensson, K., personal communication). In any case, the hypotension may be related to an action at 5-HT_{1A} receptors, in view of the lack (alniditan, data not shown) or weak (eletriptan and GMC021, data not shown) blockade by GR127935. Indeed, these drugs display moderate affinities for the 5-HT_{1A} receptor (see Table 6.1). In any case, no major systemic haemodynamic changes were observed with alniditan (Goldstein *et al*., 1996), avitriptan (Couch *et al*., 1996), eletriptan (Milton *et al*., 1998) and sumatriptan (Saxena & Tfelt-Hansen, 1993) in clinical studies.

6.4.2 Carotid haemodynamics

The success of the 5-$HT_{1B/1D}$ receptor agonist sumatriptan in the treatment of migraine has prompted the development of several new compounds acting specifically at this receptor class (The Subcutaneous Sumatriptan International Study Group, 1991; Visser *et al*., 1996a), in order to overcome the shortcomings of sumatriptan, such as the low oral bioavailability (Saxena & Tfelt-Hansen, 1993), headache recurrence (Visser *et al*., 1996a) and coronary artery constriction (MaassenVanDenBrink *et al*., 1998).

As depicted in Figure 6.2, all second-generation triptans (admittedly, alniditan is a non-tryptamine derivative) potently and selectively decrease arteriovenous anastomotic blood flow. The latter is solely responsible for the decrease in total carotid blood flow; in fact, all triptans slightly increase blood flow in the tissue nutrient fraction (Figure 6.2), being most pronounced in the skin and ears (see Chapter 4). Since arteriovenous anastomotic conductance (where blood flow values

are corrected for blood pressure changes) also decreased (Figure 6.3) the blood flow changes were caused by a vasoconstriction of these vessels. In keeping with a vasoconstriction of the cranial arteriovenous anastomoses, all drugs increased A-V SO_2.

When using the dose of agonist that produced a 50 % decrease from baseline values in arteriovenous anastomotic conductance (ED_{50}) as a measure of potency, the following rank order of potency was obtained (see Figure 6.3): alniditan > sumatriptan ≥ avitriptan ≥ eletriptan > GMC2021. BMS181885 also seems to be a potent carotid vasoconstrictor compound, but no ED_{50} value could be determined in view of its low efficacy (Figure 6.3). The above rank order of agonist potency closely correlates with their reported binding values at 5-HT_{1B} receptors (Table 6.1), although based on these data a 5-HT_{1D} receptor involvement cannot be excluded. In keeping with the above, the arteriovenous anastomotic constriction induced by sumatriptan (see Chapter 4), alniditan, eletriptan and GMC2021 is strongly antagonised by the potent and selective 5-$HT_{1B/1D}$ receptor antagonist GR127935 (see Figure 6.4). The low expression of 5-HT_{1D} receptor *m*RNA (Bouchelet *et al*., 1996) or even absence of the corresponding protein (Longmore *et al*., 1997) in cephalic blood vessels, indicates that the 5-HT_{1B} receptor, which is abundantly expressed in cranial vasculature (Bouchelet *et al*., 1996; Longmore *et al*., 1997), is probably responsible for the constriction of arteriovenous anastomoses. More definitive evidence for the involvement of the 5-HT_{1B}, but not 5-HT_{1D} receptor, using subtype selective antagonists, is discussed in Chapters 8 and 9. Although Bouchelet *et al.* (1996) also reported some 5-HT_{1F} receptor transcript expression in cranial blood vessels, this receptor is unlikely to be involved, in view of the low affinity displayed by alniditan at this receptor (Table 6.1). Moreover, LY344864, a selective 5-HT_{1F} receptor agonist (Table 1.2; Phebus *et al*., 1997), did not produce canine external carotid vasoconstriction (Villalón, C.M., personal communication) and SB224289, a selective 5-HT_{1B} ligand with very low affinity for 5-HT_{1F} receptors (Table 1.2), completely blocked sumatriptan-induced porcine and canine carotid vasoconstriction (Chapter 8-9).

As 0.5 mg kg^{-1} of GR127935, a dose that abolished the sumatriptan-induced arteriovenous anastomotic constriction (Chapter 4), did not completely block the carotid arteriovenous anastomotic constriction by alniditan, eletriptan and GMC2021, it may be that receptors other than 5-$HT_{1B/1D}$ subtypes are involved. Indeed, this also seems to be the case for the ergotamine-, dihydroergotamine- and 5-HT-induced

arteriovenous anastomotic constriction (Chapter 5). On the other hand, it is possible that higher doses of GR127935 may be needed for a complete blockade of the alniditan-induced changes. Unfortunately, the partial agonist property of GR127935 (Chapter 3) precludes the use of higher doses of this antagonist. The mechanism of action involved in the carotid vasoconstriction by BMS181885 and avitriptan was not studied, but based on similar pharmacological profiles it is reasonable to assume that mainly 5-$HT_{1B/1D}$ (most likely 5-HT_{1B}) receptors were involved.

Similar to sumatriptan (see Figure 4.4), all triptans produced a dilatation in carotid arterioles (nutrient vascular bed; Figure 6.2), which was observed in many cranial tissues, being most pronounced in skin and ear. This dilatation seems to be due to activation of 5-$HT_{1B/1D}$ receptors, in view of the antagonism by GR127935. It is noteworthy that Schoeffter and Hoyer (1990) have reported of 5-HT receptors similar to 5-$HT_{1B/1D}$ subtypes mediating endothelium-dependent relaxations in porcine isolated coronary artery, although it may also be argued that the nutrient dilatation is an indirect consequence of the closure of arteriovenous anastomoses.

Interestingly, despite the observation that eletriptan behaved as partial, but sumatriptan as a full agonist in the dog saphenous vein (Gupta *et al*., 1999), eletriptan was apparently as efficacious as sumatriptan in contracting the carotid arteriovenous anastomoses. Additionally, Gupta *et al.* (1999) showed that the maximum vasoconstrictor effect of eletriptan was less in peripheral (dog saphenous vein) than cranial (dog basilar artery) blood vessels, when compared to sumatriptan (see also Table 2.5). BMS181885 seems to display an even lower intrinsic efficacy than eletriptan, since the drug is a competitive antagonist, devoid of intrinsic efficacy, in dog saphenous vein, yet it behaves as a potent (partial) agonist in isolated cephalic blood vessels (Yocca *et al*., 1997) and the porcine (see Figure 6.3), feline and canine (Yocca *et al*., 1997) carotid vasculature. This could be explained in terms of a larger receptor reserve in the cranial circulation compared to that in the isolated saphenous vein (Kenakin, 1984). If other systemic vascular beds (e.g. coronary vasculature) also exhibit less receptor expression, an antimigraine drug with partial agonist properties may be able to exert a tissue selective pharmacological effect and, consequently, may show less side-effects on peripheral vasculature. The latter needs clinical confirmation, but it has already been demonstrated that BMS181885 (Saxena *et al*., 1998a), but not eletriptan (MaassenVanDenBrink *et al*., 1999a), is a partial agonist in the human isolated coronary artery.

The vasoconstrictor effects of BMS181885 on porcine carotid arteriovenous anastomoses was qualitatively similar to that of sumatriptan, but the drug appears not to reach full efficacy. However, after similar reductions in carotid blood flow following a single 100 μg kg^{-1} intravenous dose at 30 min the effect of BMS181885 lasted longer than that of sumatriptan (Figure 6.5). Although differences in the plasma half-life values can explain the longer duration, an equally likely factor may be the slow dissociation rate of BMS181885 from 5-$HT_{1B/1D}$ receptors (Yocca *et al*., 1997).

6.4.3 Conclusions

In conclusion, our results show that in the anaesthetised pig, sumatriptan and the new generation antimigraine agents, alniditan, GMC2021, avitriptan, BMS181885 as well as eletriptan, potently and selectively constrict carotid arteriovenous anastomoses via 5-$HT_{1B/1D}$ receptors, of which the 5-HT_{1B} receptor is the most likely candidate. Since constriction of porcine carotid arteriovenous anastomoses has over the years been shown to be of high predictive value for antimigraine activity, all drugs with this activity should be able to abort migraine headaches. Indeed, this has been shown for sumatriptan (The Subcutaneous Sumatriptan International Study Group, 1991; Visser *et al*., 1996a), avitriptan (Ryan *et al*., 1997; Schoenen, 1997), BMS181885 (Yocca *et al*., 1997), alniditan (Goldstein *et al*., 1996; Diener & De Beukelaar, 1997) and eletriptan (Jackson, 1996). Interestingly, BMS181885 was shown to only weakly inhibit the trigeminally-evoked plasma protein extravasation in the guinea-pig (Yocca *et al*., 1997). The fact that the compound did produce porcine arteriovenous anastomotic constriction and showed clinical efficacy, reinforces the idea that carotid arteriovenous anastomotic constriction, but not inhibition of neurogenic inflammation, is predictive of antimigraine potential (see also Chapter 2). Moreover, the clinical effectiveness of alniditan, which is, in contrast to sumatriptan, virtually devoid of affinity at the h5-HT_{1F} receptor, implies that an action at the 5-HT_{1F} receptor is not essential for antimigraine activity. The longer duration of action of BMS181885 as compared to sumatriptan in the present experiments suggests that potentially BMS181885 may have less headache recurrence rates than sumatriptan.

Chapter 7

Canine external carotid vasoconstriction to methysergide, ergotamine and dihydroergotamine involves 5-$HT_{1B/1D}$ receptors and α_2-adrenoceptors, but not α_1-adrenoceptors

Summary The antimigraine drugs methysergide, ergotamine and dihydroergotamine (DHE) produce selective vasoconstriction in the external carotid bed of vagosympathectomised dogs anaesthetised with pentobarbital and artificially respired, but the receptors involved have not yet been completely characterised. Since the above drugs display affinity for several binding sites, including α-adrenoceptors and several 5-HT_1 and 5-HT_2 receptor subtypes, this study has analysed the mechanisms involved in the above responses. Intracarotid (i.c.) infusions during 1-min of methysergide (31-310 μg min^{-1}), ergotamine (0.56-5.6 μg min^{-1}) or DHE (5.6-31 μg min^{-1}) dose-dependently reduced external carotid blood flow (ECBF) by up to 46±4, 37±4 and 49±5%, respectively. Blood pressure and heart rate remained unchanged. The reductions in ECBF by methysergide were abolished and even reversed to increases in animals pretreated with GR127935 (10 μg kg^{-1}, i.v.). The reductions in ECBF by ergotamine and DHE remained unchanged in animals pretreated (i.v.) with prazosin (300 μg kg^{-1}), but were partly antagonised in animals pretreated with either GR127935 (10 or 30 μg kg^{-1}) or yohimbine (1000 μg kg^{-1}). Pretreatment with a combination of GR127935 (30 μg kg^{-1}) and yohimbine (1000 μg kg^{-1}) abolished the responses to both ergotamine and DHE. The above doses of antagonists were shown to produce selective antagonism at their respective receptors. These results suggest that the external carotid vasoconstrictor responses to methysergide primarily involve 5-$HT_{1B/1D}$ receptors, whereas those to ergotamine and DHE are mediated by 5-$HT_{1B/1D}$ receptors as well as α_2-adrenoceptors.

7.1 Introduction

Several lines of pharmacological evidence show that 5-HT and the antimigraine drug sumatriptan constrict the carotid vascular bed in vagosympathectomised animals via 5-HT_1-like receptors (Den Boer *et al*., 1991b; Villalón *et al*., 1995c). Using the potent and selective 5-$HT_{1B/1D}$ receptor antagonist, GR127935 (see Chapter 3; Clitherow *et al*., 1994; Pauwels, 1996; Skingle *et al*., 1996), it was subsequently demonstrated that the carotid vasoconstrictor 5-HT_1-like receptors correspond to

Based on: Villalón, C.M., De Vries, P., Rabelo, G., Centurión, D., Sánchez-López, A. & Saxena, P.R. (1999). Canine external carotid vasoconstriction to methysergide, ergotamine and dihydroergotamine: role of 5-$HT_{1B/1D}$ receptors and α_2-adrenoceptors. *Br. J. Pharmacol.*, **126**, 585-594.

5-$HT_{1B/1D}$ subtypes in the pig (Chapter 4) rabbit (Chapter 3) and dog (Villalón *et al*., 1996). Additionally, it has been previously shown that the classical antimigraine agents methysergide, ergotamine and dihydroergotamine (DHE) exert a selective vasoconstrictor action in the carotid vascular bed of several species, including dogs (Saxena, 1974b; Saxena *et al*., 1983), pigs (see Chapter 5; Saxena & Verdouw, 1984; Den Boer *et al*., 1991a), cats (Spierings & Saxena, 1980) and monkeys (Mylecharane *et al*., 1978).

Surprisingly, very little is known about the receptor mechanisms involved in the external carotid vasoconstriction induced by methysergide, ergotamine and DHE in the dog. In this respect, we have shown that vasoconstriction in the canine external carotid bed may be mediated by activation of 5-$HT_{1B/1D}$ receptors (Villalón *et al*., 1996), α_1- and α_2-adrenoceptors (Villalón & Terrón, 1994b; Terrón *et al*., 1996), but not by 5-HT_2 (Villalón *et al*., 1995c) or D_2 receptors (Villalón & Terrón, 1994b). Indeed, as shown in Table 7.1, ergotamine and DHE are able to interact with a wide variety of receptors mediating vasoconstriction, including 5-$HT_{1/2}$ receptors and $\alpha_{1/2}$-adrenoceptors, whereas methysergide seems to interact mainly with 5-$HT_{1/2}$ receptors. In the light of these findings, the present study set out to characterise the pharmacological profile of the receptors involved in the vasoconstriction of the canine external carotid vascular bed in response to methysergide, ergotamine and DHE, with particular emphasis on verifying the possible involvement of the 5-$HT_{1B/1D}$ receptor subtypes and α_1- and α_2-adrenoceptors. For this purpose, we made use of antagonists at 5-$HT_{1B/1D}$ receptor subtypes (GR127935; Skingle *et al*., 1996), α_1- (prazosin) and α_2- (yohimbine) adrenoceptors (Hoffman & Lefkowitz, 1996), after determining the doses of these antagonists that produce selective blockade at their respective receptors.

Table 7.1 Pharmacological profile (pK_i values, except when stated otherwise) of agonists and antagonists used in the present study.

Receptor	*Methysergide*	*Ergotamine*	*DHE*	*GR127935*	*Prazosin*	*Yohimbine*
5-HT_{1A}	7.6[a]	8.4[a]	9.3[b]	7.2[c]	5.0[a]	6.9[a]
r5-HT_{1B}	5.8[a]	8.7[a]	7.9[b]	8.5[d]	5.1[a]	5.5[a]
h5-HT_{1B}	ND	7.9*	9.2[b]	9.0[c]/9.9[d]	ND	ND
h5-HT_{1D}	ND	8.4*	8.6[b]	8.6[c]/8.9[d]	ND	ND
5-$HT_{1B/1D}$	8.4[a]	7.6[a]	7.7[b]	ND	7.1[a]	7.1[a]
5-ht_{1E}	6.6[e]	6.2[e]	6.2[b]	5.4[c]/6.2[d]	ND	5.9[e]
5-HT_{1F}	7.5[e]	6.8[e]	7.0[b]	6.4[c]/7.3[d]	ND	7.0[e]
5-HT_{2A}	8.6[a]	7.7[a]	8.5[b]	7.8[c]/7.2[d]	5.0[a]	6.0[a]
5-HT_{2B}	UA[f]	8.2[g]	7.7[g]	<6.0[f]	ND	7.9[f]
5-HT_{2C}	8.9[h]	7.3	7.5[a]/7.4[b]	7.0[c]/6.2[d]	4.7[a]	4.4[a]
5-ht_{5A}	7.2[h]	7.3*	7.3*	5.2[d]	ND	6.0[h]
5-ht_{5B}	6.9[h]	8.5[h]	ND	ND	ND	6.0[h]
5-ht_{6}	6.4[h]	ND	8.0[b]/6.8*	5.8[c]	ND	ND
5-HT_{7}	7.9[h]	7.5[h]	7.8[b]/7.2*	6.2[c]/5.5[d]	ND	5.6[i]
α_1	5.6[j]	8.0[k]	8.0[b]	<6.0[d]	9.2[j]	6.4[j]
α_2	5.6[j]	8.2[k]	8.0[b]	<6.0[d]	5.3[j]	6.8[j]
D_2	6.7[j]	8.5[k]	7.0[k]	<5.0[d]	<6.0[j]	6.2[j]

[a], Hoyer (1988), pK_D value; [b], Leysen *et al.* (1996); [c], Price *et al.* (1997); [d], Pauwels (1996); [e], Adham *et al.* (1993); [f], Baxter *et al.* (1994), pA_2 value; [g], Glusa & Roos (1996), pEC_{50} value; [h], Hoyer *et al.* (1994), pK_D value; [i], Bard *et al.* (1993); [j], Leysen (1985); [k], Leysen & Gommeren (1984). DHE, dihydroergotamine; UA, Unsurmountable Antagonist; ND, Not Determined, r5-HT_{1B}, rodent 5-HT_{1B}; h5-HT_{1B} and h5-HT_{1D} human receptors, respectively (Hartig *et al.*, 1996); 5-$HT_{1B/1D}$ refers to the, then called, 5-HT_{1D} receptor binding site in calf caudate membrane, at which time the experimental conditions allowed the inclusion of the 5-HT_{1B} and 5-HT_{1D} receptor sites.

7.2 Methods

7.2.1 General

Experiments were carried out in a total of 72 dogs (15-31 kg) not selected for breed or sex. The animals were anaesthetised with an intravenous (i.v.) bolus injection of sodium pentobarbitone (30 mg kg^{-1}) and additional amounts (1 mg kg^{-1}, i.v.) were provided when required. All dogs were intubated with an endotracheal tube and artificially respired with room air; for this purpose, a Palmer ventilation pump was used at a rate of 20 strokes min^{-1} and a stroke volume of 13-16 ml kg^{-1}, as previously established by Kleinman & Radford (1964). Catheters were placed in the inferior vena cava via a femoral vein for the administration of antagonist drugs and in the aortic arch via a femoral artery, connected to a Statham pressure transducer (P23 ID), for the measurement of blood pressure. After drug administration, the venous catheter was flushed with 3 ml of saline. Heart rate was measured with a tachograph (7P4F, Grass Instrument Co., Quincy, MA, U.S.A.) triggered from the blood pressure signal. The right common carotid artery was dissected free and the corresponding internal carotid and occipital arteries were ligated. Thereafter, an ultrasonic flow probe (4 mm R-Series) connected to an ultrasonic T201D flowmeter (Transonic Systems Inc., Ithaca, N.Y., U.S.A.) was placed around the right common carotid artery, and the flow through this artery was considered as the external carotid blood flow (for further details see Villalón *et al*., 1993b). Bilateral cervical vagosympathectomy was systematically performed in order to produce one of the main features of migraine, i.e. external carotid vasodilatation (Saxena & De Vlaam-Schluter, 1974). The agonists were administered into the carotid artery by a Harvard model 901 pump (Harvard Apparatus Co. Inc., Millis, MA, U.S.A.) with a catheter inserted into the right cranial thyroid artery. Blood pressure, heart rate and external carotid blood flow were recorded simultaneously by a model 7D polygraph (Grass Instrument Co., Quincy, MA, U.S.A.). The body temperature of the animals was maintained between 37-38°C.

7.2.2 Experimental protocol

After a stable haemodynamic condition for at least 30 min, baseline values of blood pressure, heart rate and external carotid blood flow were determined. At this point, the dogs were divided into two groups (n=56 and 16, respectively). The first group (n=56) was subdivided into three subgroups. In the first subgroup (n=8), the effects of sequential 1-min intracarotid (i.c.) infusions of methysergide (31, 100 and

310 µg min^{-1}) were analysed in animals pretreated i.v. with either saline (0.1 ml kg^{-1}; n=4) or GR127935 (10 µg kg^{-1}; n=4). In the second and third subgroups (n=24 each), the effects of cumulative 1-min i.c. infusions of ergotamine (0.56, 1, 1.8, 3.1 and 5.6 µg min^{-1}) or DHE (5.6, 10, 18 and 31 µg min^{-1}), respectively, were analysed in animals pretreated with i.v. infusions of either (n=4 each) saline (0.1 ml kg^{-1}), GR127935 (10 µg kg^{-1}), GR127935 (30 µg kg^{-1}), prazosin (300 µg kg^{-1}), yohimbine (1000 µg kg^{-1}) or the combination of GR127935 (30 µg kg^{-1}) and yohimbine (1000 µg kg^{-1}). In the second group (n=16), all animals received subsequent 1-min i.c. infusions of clonidine (1 µg min^{-1}), phenylephrine (10 µg min^{-1}) and sumatriptan (30 µg min^{-1}). Then, this group of animals was subdivided into four subgroups (n=4 each). In these subgroups, the responses to clonidine, phenylephrine and sumatriptan were reanalysed after i.v. administration of either prazosin (300 µg kg^{-1}), yohimbine (1000 µg kg^{-1}), GR127935 (30 µg kg^{-1}) or the combination of GR127935 (30 µg kg^{-1}) and yohimbine (1000 µg kg^{-1}). The dose-intervals between the different doses of methysergide ranged between 5 and 10 min, as in each case we waited until the blood flow had returned completely to baseline values. Moreover, after the administration of an antagonist or saline a period of about 10 min was allowed to elapse before the responses to the respective agonists were elicited.

7.2.3 Data presentation and statistical evaluation

All data have been expressed as the mean±s.e.mean. The peak changes in external carotid blood flow (calculated as percent change from baseline) by methysergide, ergotamine and DHE after pretreatment with a dose of a particular antagonist were compared to the respective responses by these agonists in saline-pretreated animals by Student's unpaired *t*-test. Furthermore, the peak percent changes in external carotid blood flow by clonidine, phenylephrine and sumatriptan before and after antagonist treatment were compared by using Student-Newman-Keuls test, once an analysis of variance (randomised block design) had revealed that the samples represented different populations (Steel & Torrie, 1980). Statistical significance was accepted at $P<0.05$ (two-tailed).

7.2.4 Drugs

Apart from the anaesthetic (sodium pentobarbitone), the drugs used in the present study (obtained from the sources indicated) were the following: phenylephrine

hydrochloride, clonidine hydrochloride and prazosin hydrochloride (Sigma Chemical Company, St. Louis, MO, U.S.A.); GR127935 and sumatriptan succinate (gifts from Dr. M. Skingle, Glaxo Group Research, Ware, Herts, UK and Dr. H.E. Connor, Glaxo Group Research, Stevenage, Hertfordshire, UK, respectively); methysergide maleate, DHE mesylate and ergotamine tartrate (gift: Sandoz A.G., Basel, Switzerland). All compounds were dissolved in physiological saline. When needed, 5% (v v^{-1}) propylene glycol (prazosin) was added; this vehicle had no effect on the haemodynamic variables. GR127935 was solubilised according to the instructions of the supplier by heating the dispersion in bidistilled water to about 70°C for 10 s and then allowing to cool down to room temperature. All doses of antagonists refer to the respective salts, whereas those of the agonists refer to the free base.

7.3 Results

7.3.1 Systemic haemodynamic variables

Baseline values of mean arterial blood pressure, heart rate and external carotid blood flow in the 72 vagosympathectomised dogs were, respectively, 142±5 mmHg, 178±8 beats min^{-1} and 163±21 ml min^{-1}. No significant differences were observed in the initial values between the 18 subgroups. As depicted in Table 7.2, these haemodynamic variables remained essentially unchanged after administrations of saline, GR127935, yohimbine, or the combination of GR127935 and yohimbine, at all doses tested. Only prazosin produced a significant decrease in both mean blood pressure and external carotid blood flow by 29±15% and 29±16%, respectively, without affecting the corresponding carotid vascular conductance (not shown). This prazosin-induced hypotension and the resulting decrease in external carotid blood flow has previously been reported to be attributable to its α_1-adrenoceptor blocking properties (Terrón *et al*., 1996). In any case, these effects were not accompanied by changes in heart rate (Table 7.2), as previously shown by Massingham and Hayden (1975).

Table 7.2 Mean arterial blood pressure (MAP; mmHg), heart rate (HR; beats min^{-1}) and external carotid blood flow (ECBF; ml min^{-1}) before and after i.v. administration of saline, GR127935, prazosin, yohimbine or the combination of GR127935 and yohimbine.

	Dose		*MAP*		*HR*		*ECBF*	
Treatment	*(µg kg^{-1})*	*n*	*Before*	*After*	*Before*	*After*	*Before*	*After*
Saline	0.1[1]	12	152±11	152±12	176±12	173±11	151±8	153±6
GR127935	10	12	141±6	148±5	183±7	182±8	163±13	155±13
GR127935	30	8	137±5	145±6	181±5	181±7	134±12	127±9
Prazosin	300	8	143±8	101±14*	184±8	188±10	171±21	122±15*
Yohimbine	1000	8	143±6	138±10	171±8	186±14	177±32	139±22
GR127935	30	8	138±5	128±7	176±9	199±18	170±19	143±18
+	+							
Yohimbine	1000							

All values have been presented as the mean±s.e.mean. [1], ml kg^{-1}; *, $P<0.05$ vs before values.

7.3.2 Initial effects of methysergide, ergotamine and DHE on external carotid blood flow

As shown in Figure 7.1, i.c. infusions (during 1 min) of methysergide (31, 100 and 310 µg min^{-1}), ergotamine (0.56, 1, 1.8, 3.1 and 5.6 µg min^{-1}) and DHE (5.6, 10, 18 and 31 µg min^{-1}) produced dose-dependent decreases in external carotid blood flow, without changes in mean blood pressure or heart rate (not shown). The above responses, which were immediate in onset, are drug-induced as 1-min i.c. infusions of the corresponding volumes of saline did not affect any haemodynamic parameter for the duration of the experiments (data not shown). The duration of action of ergotamine and DHE could not be established, as external carotid blood flow did not return to baseline values, as previously reported (Saxena, 1974a; Villalón *et al*., 1992). In contrast, the duration of action of methysergide was 2.0±0.7, 7.0±1.2 and 9.0±1.9 min after 31, 100 and 310 µg min^{-1}, respectively. The apparent rank order of potency obtained in the present experiments was ergotamine > DHE > methysergide (Figure 7.1).

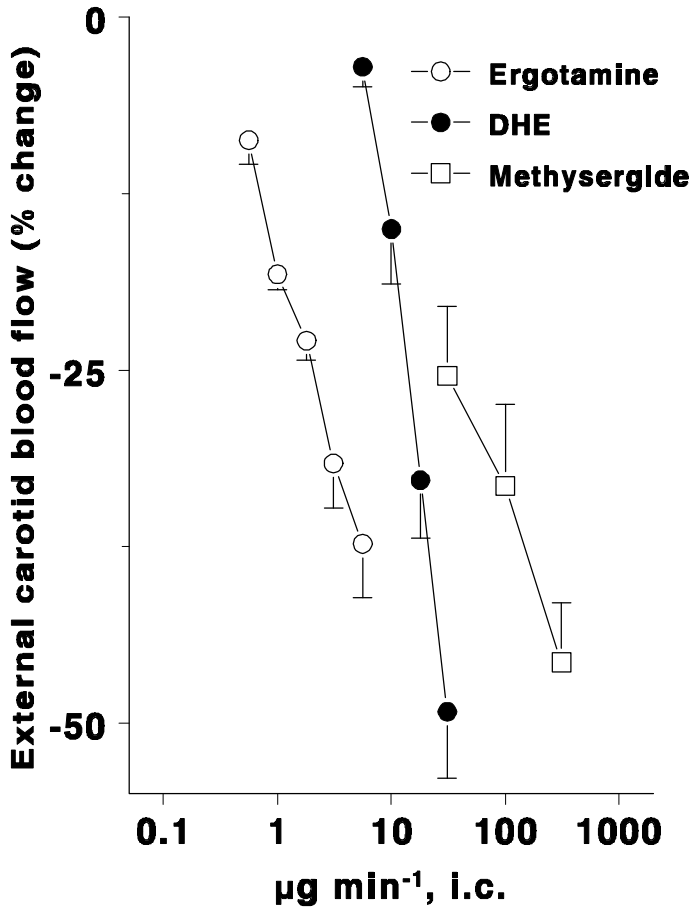

Figure 7.1 Effects of 1-min i.c. infusions of ergotamine, dihydroergotamine (DHE) and methysergide (n=4 each) on external carotid blood flow in vagosympathectomised dogs. All values have been presented as the mean±s.e.mean.

7.3.3 Effects of GR127935 on the methysergide-induced external carotid vasoconstriction

As depicted in Figure 7.2, in animals pretreated with 10 µg kg^{-1} of GR127935, the decreases in external carotid blood flow by methysergide were abolished and even reversed to an external carotid vasodilatation.

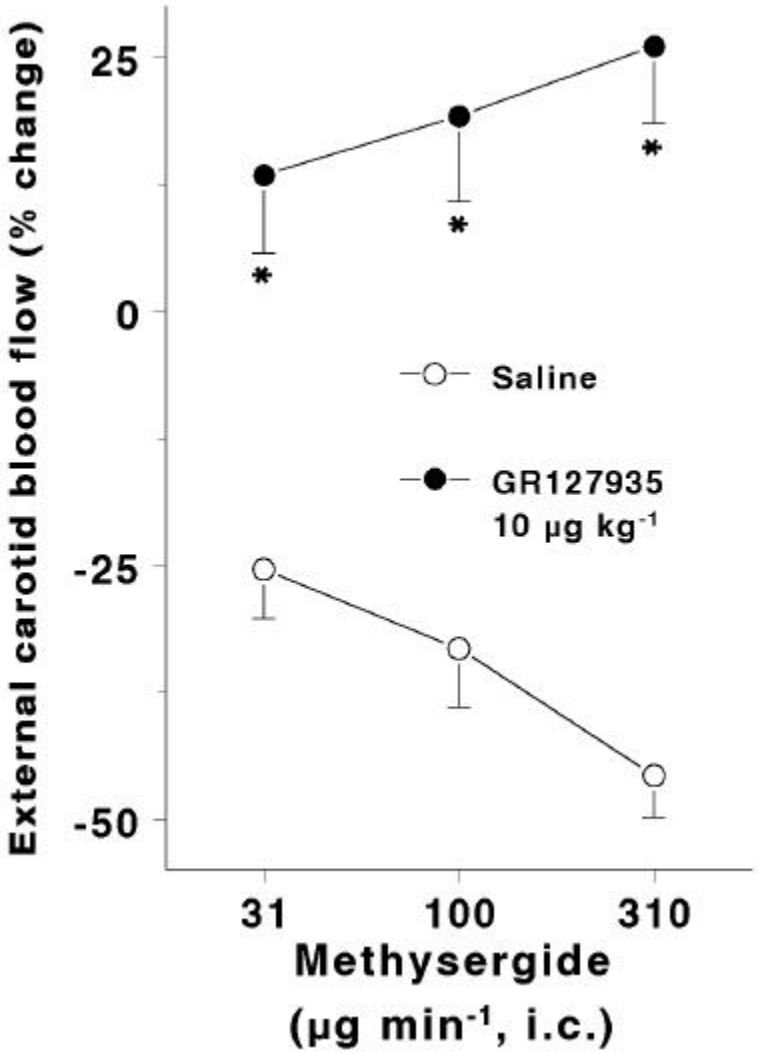

Figure 7.2 Effects of 1-min i.c. infusions of methysergide on external carotid blood flow in dogs pretreated (i.v.; n=4 each) with either saline or GR127935. All values have been presented as the mean±s.e.mean. *, P<0.05 vs response in saline-pretreated animals.

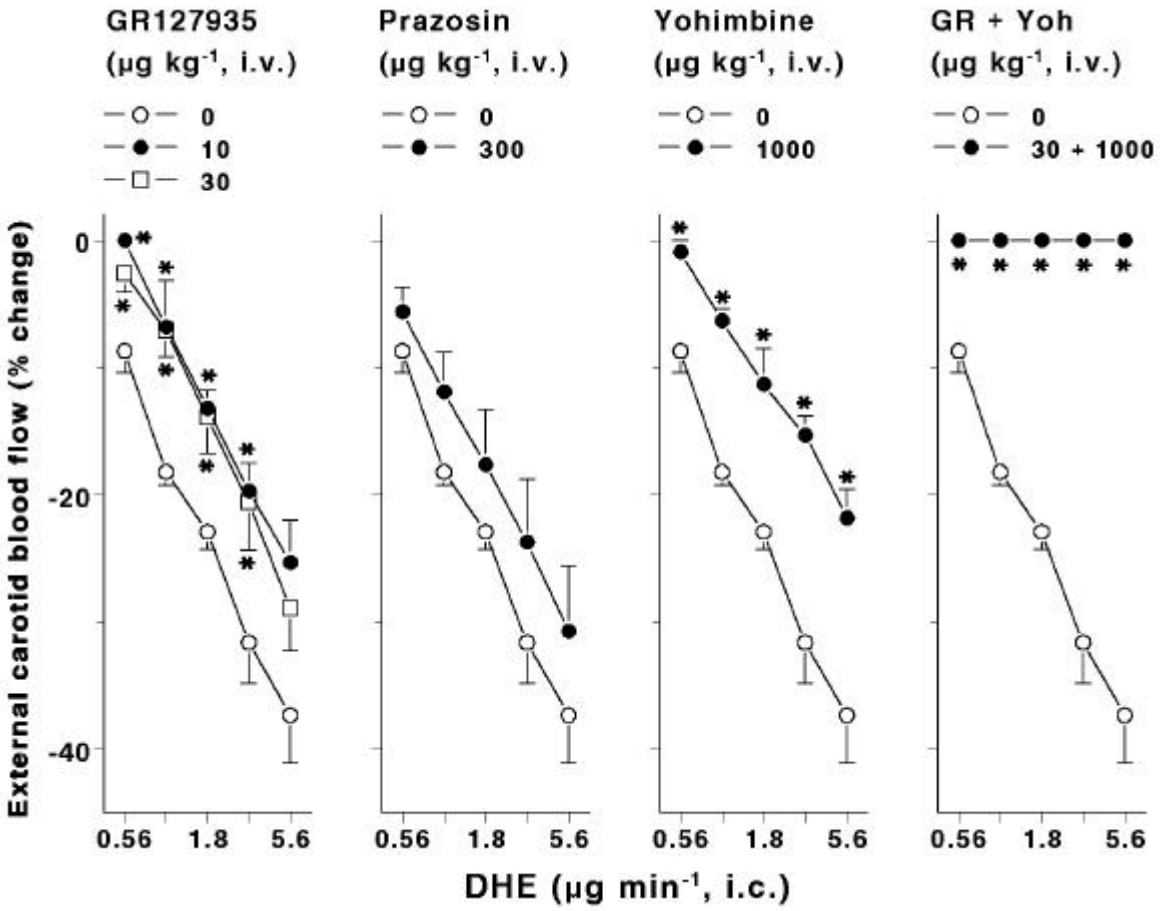

Figure 7.3 Effects of 1-min i.c. infusions of ergotamine on external carotid blood flow in dogs pretreated (n=4 each) with either saline (control), GR127935, prazosin, yohimbine or the combination of GR127935 and yohimbine (GR+Yoh). All values have been presented as the mean±s.e.mean. For the sake of clarity, the responses to ergotamine in control animals are shown in all panels. *, P<0.05 vs control.

7.3.4 Effects of GR127935, prazosin, yohimbine or the combination of GR127935 and yohimbine on the ergotamine- and DHE-induced external carotid vasoconstriction

As shown in Figures 7.3 (ergotamine) and 7.4 (DHE), 10 µg kg^{-1} of GR127935 only partly blocked the external carotid vasoconstrictor responses to ergotamine and DHE. Interestingly, a higher dose of GR127935 (30 µg kg^{-1}) did not produce a further blockade. Moreover, whereas prazosin (300 µg kg^{-1}) did not affect the ergotamine- and DHE-induced decreases in external carotid blood flow, yohimbine (1000 µg kg^{-1})

brought about a partial blockade. Significantly, the combination of GR127935 (30 µg kg^{-1}) and yohimbine (1000 µg kg^{-1}) completely blocked the external carotid vasoconstriction induced by ergotamine (Figure 7.3) and DHE (Figure 7.4). The above doses of antagonists, as explained below, were shown to produce selective antagonism at their respective receptors.

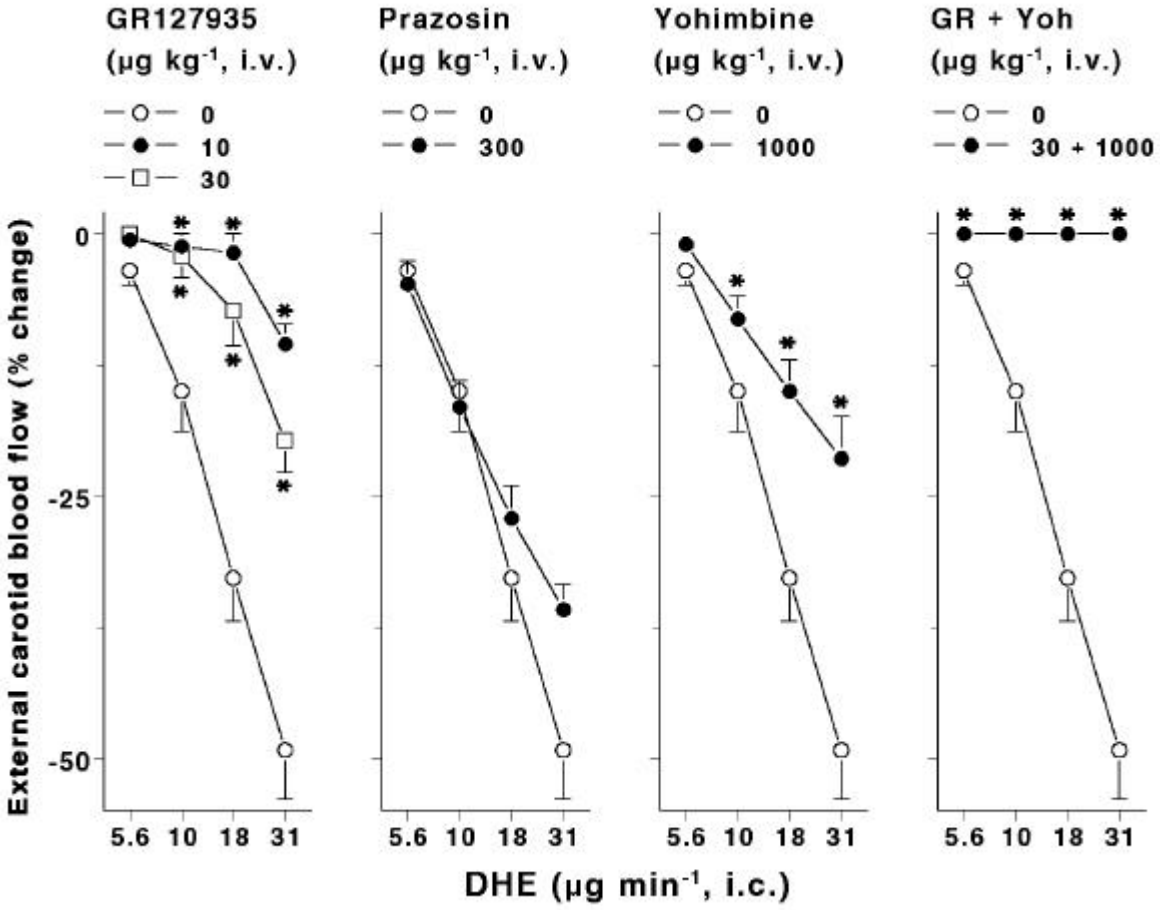

Figure 7.4 Effects of 1-min i.c. infusions of dihydroergotamine on external carotid blood flow in dogs pretreated (n=4 each) with either saline (control), GR127935, prazosin, yohimbine or the combination of GR127935 and yohimbine (GR+Yoh). All values have been presented as the mean±s.e.mean. For the sake of clarity, the responses to dihydroergotamine in control animals are shown in all panels. *, $P<0.05$ vs control.

7.3.5 Effects of GR127935, prazosin, yohimbine and the combination of GR127935 and yohimbine on the external carotid vasoconstriction produced by clonidine, phenylephrine and sumatriptan

As depicted in Figure 7.5, GR127935 (30 µg kg^{-1}) selectively antagonised the external carotid vasoconstriction to sumatriptan, as the responses to clonidine and phenylephrine remained unaffected. Similarly, prazosin (300 µg kg^{-1}) exclusively abolished the phenylephrine-induced decreases in external carotid blood flow, leaving the clonidine- and sumatriptan-induced changes intact. Lastly, while yohimbine (1000 µg kg^{-1}) abolished only the clonidine-induced carotid vascular effects, the combination of GR127935 (30 µg kg^{-1}) and yohimbine (1000 µg kg^{-1}) completely blocked the clonidine-, as well as the sumatriptan-induced constriction of the canine carotid vascular bed. It should be pointed out that the external carotid vasoconstrictor responses to phenylephrine, clonidine and sumatriptan are highly reproducible, as they remained essentially unchanged in control animals receiving two subsequent bolus injections of physiological saline (Villalón & Terrón, 1994b; Villalón *et al*., 1996).

7.4 Discussion

7.4.1 General

The major findings of the present study in vagosympathectomised dogs were that: (i) the methysergide-induced external carotid vasoconstriction was abolished, and even reversed to carotid vasodilatation, by the selective 5-$HT_{1B/1D}$ receptor antagonist, GR127935 and (ii) the external carotid vasoconstrictor responses by ergotamine and DHE were only partly antagonised by GR127935 or yohimbine, but were completely blocked by the combination of GR127935 and yohimbine. Apart from the implications discussed below, these data indicate that the vasoconstrictor responses to ergotamine and DHE in the external carotid vascular bed involve both 5-$HT_{1B/1D}$ receptors and α_2-adrenoceptors, whereas methysergide seems to exert external carotid vasoconstriction primarily via 5-$HT_{1B/1D}$ receptors.

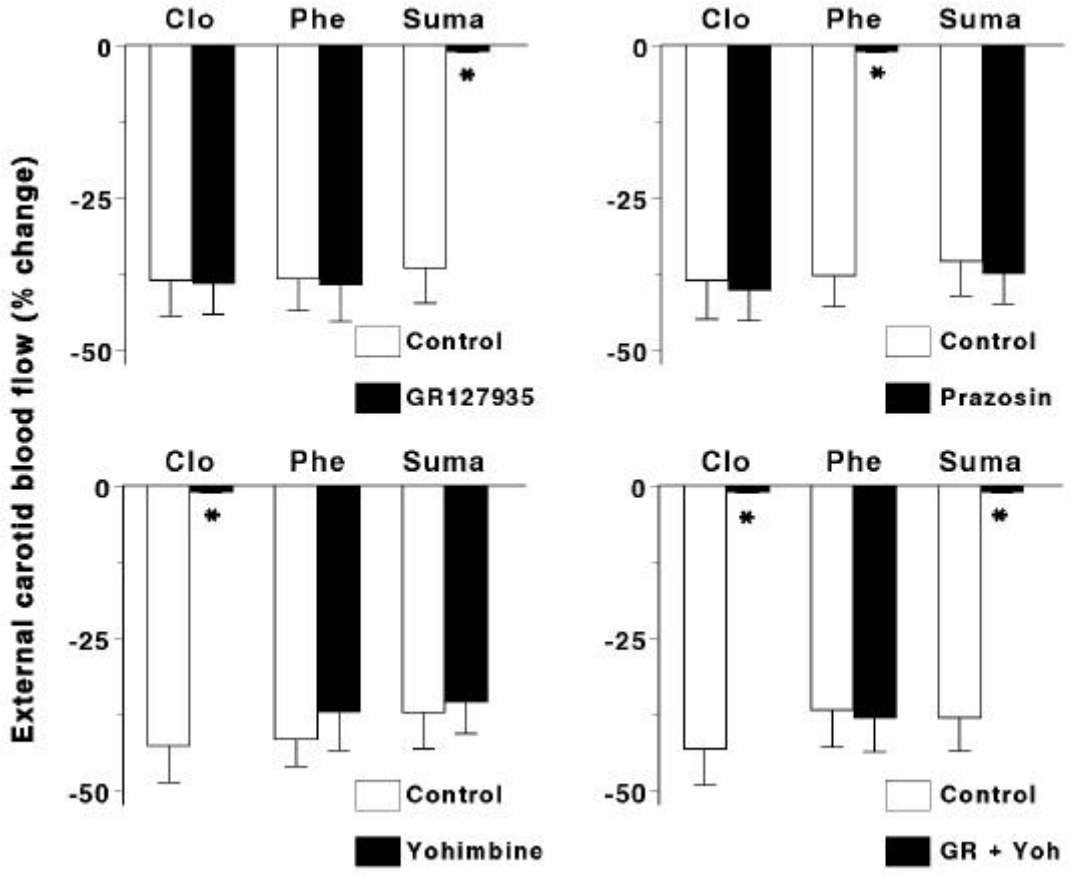

Figure 7.5 Effects of 1-min i.c. infusions of clonidine (Clo; 1 µg min^{-1}), phenylephrine (Phe; 10 µg min^{-1}) or sumatriptan (Suma; 30 µg min^{-1}) on canine external carotid blood flow before and after i.v. administrations (n=4 each) of either GR127935 (30 µg kg^{-1}), prazosin (300 µg kg^{-1}), yohimbine (1000 µg kg^{-1}) or the combination of GR127935 and yohimbine (GR+Yoh; 30+1000 µg kg^{-1}). All values have been presented as the mean±s.e.mean. *, $P<0.05$ after vs before.

7.4.2 Receptors involved in the methysergide-induced carotid vasoconstrictor responses

As previously observed using i.v. administrations (Villalón *et al*., 1996), 1 min i.c. infusions of methysergide dose-dependently decreased external carotid blood flow, an effect which has been suggested to be due to an agonist action of the ergot derivative at smooth muscle 5-HT_1-like receptors (Saxena & Verdouw, 1984; Martin, 1994). As methysergide did not affect heart rate or blood pressure, these data suggest that under our experimental conditions methysergide induced a selective vasoconstriction in the

external carotid circulation. A complete blockade of the methysergide-induced carotid vasoconstriction was observed after treatment with 10 μg kg^{-1} of GR127935; this dose of GR127935 has previously been shown to abolish sumatriptan-induced external carotid vasoconstriction without affecting that to noradrenaline or oxymetazoline (Villalón *et al*., 1996). In the present study, our results further demonstrate that doses of GR127935 up to 30 μg kg^{-1} selectively abolish sumatriptan-induced canine external carotid vasoconstriction, since the corresponding responses to phenylephrine and clonidine remained unaltered (Figure 7.5), as expected from its binding profile (see Table 7.1). Thus, these data show that methysergide constricts the external carotid vasculature predominantly via the 5-$HT_{1B/1D}$ receptor subtypes, at which methysergide displays high affinities (see Table 7.1). In view of the complete blockade by GR127935, as well as the low affinities exhibited by methysergide at α_1- and α_2-adrenoceptors (see Table 7.1), we decided not to further analyse the effects of prazosin or yohimbine on the methysergide-induced external carotid vasoconstriction.

7.4.3 Contribution of 5-$HT_{1B/1D}$ receptors in the carotid vasoconstrictor responses to ergotamine and DHE

1 Min i.c. infusions of ergotamine and DHE induced dose-dependent decreases in the external carotid blood flow, without affecting heart rate or mean arterial blood pressure. Thus, these findings suggest that such responses were caused by a selective constriction in the external carotid vascular bed. Notwithstanding, unlike methysergide, the responses to ergotamine and DHE were of long duration, as previously observed *in vivo* (Saxena, 1974a; Den Boer *et al*., 1991a; Villalón *et al*., 1992; De Vries *et al*., 1998) and *in vitro* (Müller-Schweinitzer & Weidmann, 1978; MaassenVanDenBrink *et al*., 1998). Although there is no clear-cut explanation for this difference, it has been suggested, albeit not categorically proven, that it may be due to a slow dissociation from its receptors or a sequestration and subsequent diffusion out of a local nonsaturable compartment (Müller-Schweinitzer & Weidmann, 1978; Martin *et al*., 1995).

Regarding the possible receptor mechanisms involved in the vasoconstrictor responses to ergotamine and DHE, previous results in anaesthetised dogs have shown that the external carotid vasoconstrictor responses to ergotamine and DHE are not mediated by 5-HT_2 receptors, whereas only part of the effects seem to be mediated by phentolamine-sensitive α-adrenoceptors (Saxena *et al*., 1983). Moreover, 5-HT_1-like

receptors are only partly involved in the ergotamine- and DHE-induced constriction of the carotid vasculature in pigs (Den Boer *et al.*, 1991a). Since 5-HT_1-like receptors mediating porcine as well as canine carotid vasoconstriction are similar to the 5-$HT_{1B/1D}$ receptor subtypes (Chapter 4; Villalón *et al.*, 1996), we decided to explore the possible involvement of 5-$HT_{1B/1D}$ receptors in the present study. Indeed, the responses to ergotamine and DHE in dogs were, in contrast to the methysergide- and sumatriptan-induced effects, only partly antagonised by 10 μg kg^{-1} of GR127935; the fact that pretreatment with a higher dose of GR127935 did not produce any further blockade, implies that the ergotamine- and DHE-induced external carotid vasoconstriction involves a mixed population of receptors. Thus, we suggest that the responses to ergotamine and DHE are partly mediated via 5-$HT_{1B/1D}$ receptors, but a considerable part is mediated via GR127935-resistant receptors. Consistent with these findings, we have recently shown that ergotamine and DHE act, at least partly, via GR127935-sensitive 5-$HT_{1B/1D}$ receptors in the porcine carotid vascular bed, but additional receptors/mechanisms seem to be involved (Chapter 5).

7.4.4 Contribution of a_1- and/or a_2-adrenoceptors in the carotid vasoconstrictor responses to ergotamine and DHE

As discussed above, a considerable part of the external carotid vasoconstriction by both ergotamine and DHE is mediated by non-5-$HT_{1B/1D}$ receptors. These GR127935-resistant receptors may be of the α-adrenoceptor class, as (i) agonists at α_1- and α_2-adrenoceptors can produce potent vasoconstrictor responses in the carotid circulation of dogs (Villalón & Terrón, 1994b; Terrón *et al.*, 1996) and pigs (Willems *et al.*, 1999); and (ii) ergotamine and DHE display high affinities at α_1- and α_2-adrenoceptors (Table 7.1).

Nevertheless, 300 μg kg^{-1} of prazosin, a dose which is three-fold higher than that required to abolish methoxamine-induced canine external carotid vasoconstriction (Terrón *et al.*, 1996), did not affect the vasoconstrictor responses to either ergotamine (Figure 7.3) or DHE (Figure 7.4). These results suggest that ergotamine and DHE do not display α_1-adrenoceptor agonist properties under our experimental conditions. Indeed, this suggestion is strengthened by our results showing that the above dose of prazosin produced a selective and complete blockade of the α_1-adrenoceptor-mediated external carotid vasoconstriction induced by phenylephrine (Figure 7.5). It might still

be argued that ergotamine and DHE could have behaved as α-adrenoceptor antagonists. Nonetheless, at such low total doses (5.6 and 31 μg for ergotamine and DHE, respectively) these ergot derivatives did not modify the external carotid vasoconstrictor responses to phenylephrine and clonidine (data not shown).

Contrasting with prazosin, yohimbine was capable of producing a partial blockade of the vasoconstrictor responses to ergotamine and DHE (see Figure 7.3 and 7.4) at a dose (1000 μg kg^{-1}) that selectively abolished clonidine-induced canine external carotid vasoconstriction (Figure 7.5). Taken together, the above results imply that α_2-, but not α_1-adrenoceptors, are involved, as previously suggested by other studies (Müller-Schweinitzer & Weidmann, 1978; Kalkman, 1983; Müller-Schweinitzer, 1984).

Nevertheless, it must be recognised that yohimbine also displays moderate affinity for other receptors, including α_1-adrenoceptors and 5-$HT_{1B/1D}$ receptors (see Table 7.1); thus, it is reasonable to assume that the above dose of the drug could have been high enough to block these, and other, receptors. Yet, lower doses of yohimbine (100 and 300 μg kg^{-1}, i.v.) did not completely block clonidine-induced external carotid vasoconstriction and higher doses (3000 μg kg^{-1}, i.v.) were not selective in our model as they also blocked phenylephrine-induced external carotid vasoconstriction (data not shown). Indeed, other *in vivo* studies have shown that yohimbine displays moderate selectivity for α_2- over the α_1-adrenoceptor in anaesthetised cats (Ramage & Tomlinson, 1985) and dogs (Shepperson *et al*., 1981).

However, as shown in Figure 7.5, the antagonism produced by 1000 μg kg^{-1} of yohimbine at external carotid α_2-receptors was selective, since this dose completely blocked the clonidine-induced carotid vascular effects without affecting those to sumatriptan, which involve 5-$HT_{1B/1D}$ receptors (Villalón *et al*., 1996) and phenylephrine, which involve α_1-adrenoceptors (Villalón & Terrón, 1994b). In any case, the latter suggestion is validated by the fact that prazosin, at doses producing selective α_1-adrenoceptor antagonism (see above) did not affect the responses to ergotamine and DHE. Although beyond the scope of the present investigation, further studies using highly selective agonists and antagonists will be required to ascertain which specific subtype(s) of the α_2-adrenoceptor is (are) producing vasoconstriction.

7.4.5 Possible involvement of other receptors in the carotid vasoconstrictor responses to ergotamine and DHE

As discussed above, the constriction of the canine carotid vascular bed by ergotamine and DHE is likely to be mediated by 5-$HT_{1B/1D}$ receptors, as well as α_2-adrenoceptors. The question remains open, however, whether additional receptors and/or mechanisms play a role in their effects. For this reason we studied the effects of a combination of GR127935 and yohimbine on the carotid vascular responses by the ergot alkaloids. Indeed, after simultaneous, selective blockade of both 5-$HT_{1B/1D}$ receptors and α_2-adrenoceptors, the carotid vascular effects of ergotamine and DHE were abolished. Therefore, the above results, taken collectively, suggest that the vasoconstriction within the carotid vascular bed induced by ergotamine and DHE in vagosympathectomised dogs is primarily mediated by both 5-$HT_{1B/1D}$ receptors and α_2-adrenoceptors.

On the other hand, since methysergide, ergotamine and DHE (as well as GR127935 and yohimbine) display also moderate affinity for 5-HT_{1F} receptors (see Table 7.1), its potential role cannot be categorically excluded in the present study, particularly when considering that *m*RNA for 5-HT_{1F} receptors has been shown in cranial blood vessels (Bouchelet *et al*., 1996). Indeed, sumatriptan, which displays reasonable affinity for the recombinant 5-HT_{1F} receptor (Table 1.2), also produced a GR127935-sensitive vasoconstriction in the canine external carotid bed (Villalón *et al*., 1996). Notwithstanding, two recent findings from our laboratory apparently argue against the role of 5-ht_{1F} receptors (and simultaneously reinforce the role of 5-HT_{1B} receptors): (i) 1 min i.c. infusions of LY344864 (1-3100 µg min^{-1}), a selective 5-HT_{1F} receptor agonist (Table 1.2; Phebus *et al*., 1997), did not produce canine external carotid vasoconstriction (unpublished); and (ii) SB224289 (300 µg kg^{-1}), a selective 5-HT_{1B} ligand with very low affinity (pK_i: <5.0) for 5-HT_{1F} receptors (Table 1.2), completely blocked 5-HT- as well as sumatriptan-induced canine external carotid vasoconstriction (Chapter 8).

7.4.6 Possible involvement of external carotid vasodilator mechanisms by ergot derivatives

Interestingly, after treatment with GR127935, not only was the methysergide-induced carotid constriction abolished, but a clear vasodilator response was unmasked. We

have previously demonstrated that the 5-HT-induced external carotid vasodilatation in GR127935-pretreated vagosympathectomised dogs is mediated via 5-HT_7 receptors (Villalón *et al.*, 1997a), as shown in other vascular preparations (Saxena *et al.*, 1998b). Therefore, it is tempting to suggest that methysergide could have acted via smooth muscle relaxant 5-HT_7 receptors, for which the compound displays high affinity (see Table 7.1). Nevertheless, this seems less likely, as methysergide behaves as a silent antagonist at other cardiovascular 5-HT_7 receptors, including those mediating feline tachycardia (Chapter 12) and rat hypotension (Saxena & Lawang, 1985). Alternatively, methysergide may have stimulated an endothelial receptor resulting in the release of NO, possibly of the 5-HT_{2B} receptor subtype, as shown previously for other ergot derivatives in the porcine pulmonary circulation (Glusa & Roos, 1996). In support of this contention, the methysergide-induced carotid dilatory response was inhibited in animals treated with the NO synthetase inhibitor L-NAME (unpublished observations).

As opposed to methysergide, ergotamine and DHE did not induce any vasodilator effects in the external carotid circulation, even after complete blockade of the vasoconstrictor effects by GR127935 and yohimbine. This seems to argue against the possible involvement of endothelial dilatory (5-HT) receptors (see above), as these ergot derivatives have been shown to be potent agonists at the (5-HT_{2B}) receptors mediating endothelium-dependent vasorelaxation of porcine isolated pulmonary arteries (Glusa & Roos, 1996). It should be noted, however, that yohimbine has been shown to possess high antagonist affinity at 5-HT_{2B} receptors (Table 7.1) and, therefore, may have inhibited the potential ergot-induced vasorelaxant effects at these receptors. In this context, in view of the low affinity displayed by yohimbine at 5-HT_7 receptors (Table 7.1), it is highly unlikely that 5-HT_7 receptors mediate the potential ergotamine- and DHE-induced external carotid vasodilatation.

In conclusion, our results show that in vagosympathectomised, anaesthetised dogs, methysergide constricts the external carotid vascular bed predominantly via GR127935-sensitive 5-$HT_{1B/1D}$ receptors. After administration of GR127935, an external carotid vasodilator (possibly endothelial) component was unmasked. Additionally, the present results show the predominant involvement of both 5-$HT_{1B/1D}$ receptors and α_2-adrenoceptors, but not α_1-adrenoceptors, in the external carotid constriction by ergotamine and DHE. The therapeutic efficacy of these classical

antimigraine drugs is likely to be mediated by these receptors, as constriction of the carotid vascular bed is highly predictive of antimigraine potential (Saxena *et al*., 1997). In this context, we have clearly demonstrated that the decrease in carotid blood flow by antimigraine agents is exclusively due to vasoconstriction of carotid arteriovenous anastomoses (for references see Saxena, 1995; Saxena *et al*., 1997), which have been suggested to be in a dilated state during migraine headaches (e.g. Heyck, 1969; Saxena, 1995). Lastly, although we cannot categorically exclude other, possibly novel receptors/mechanisms, as shown recently in the porcine carotid vasculature (Chapter 5), it seems that, if present at all, these are of minor importance in the dog external carotid vascular bed.

Chapter 8

The canine external carotid vasoconstrictor 5-HT_1 receptor: blockade by 5-HT_{1B} (SB224289), but not by 5-HT_{1D} (BRL15572) receptor antagonists

Summary In vagosympathectomised dogs pretreated intravenously (i.v.) with mesulergine (300 μg kg^{-1}), 1-minute (1-min) intracarotid (i.c.) infusions of 5-hydroxytryptamine (5-HT; 0.3-30 μg min^{-1}) and sumatriptan (1-30 μg min^{-1}) dose-dependently decreased external carotid blood flow, without affecting mean blood pressure or heart rate. Treatment with the selective 5-HT_{1B} receptor antagonist SB224289 (30-300 μg kg^{-1}, i.v.) produced a potent, specific and dose-dependent blockade of this response, whereas the selective 5-HT_{1D} receptor antagonist BRL15572 (30-300 μg kg^{-1}, i.v.) was ineffective. It is concluded that mainly 5-HT_{1B}, but not 5-HT_{1D} receptors mediate the canine external carotid vasoconstriction by 5-HT and sumatriptan.

8.1 Introduction

We have previously reported that 1-minute (1-min) intracarotid (i.c.) infusions of 5-HT or sumatriptan produce selective vasoconstriction in the external carotid vascular bed of vagosympathectomised dogs via 5-HT_1-like receptors (Villalón *et al*., 1995c). Subsequently, it was shown that these 5-HT_1-like receptors closely resemble the 5-$HT_{1B/1D}$ receptor subtypes (Villalón *et al*., 1996), as they are highly sensitive to the antagonist action of GR127935, a potent and selective 5-$HT_{1B/1D}$ receptor ligand (Skingle *et al*., 1996). Based on the presence of 5-HT_{1B}, but not of 5-HT_{1D} receptor *m*RNA in vascular smooth muscle (Ullmer *et al*., 1995; Bouchelet *et al*., 1996), it was suggested that the receptor mediating this response appears to be of the 5-HT_{1B} subtype (Villalón *et al*., 1996). Notwithstanding, it remained virtually impossible to pharmacologically attribute the external carotid vasoconstrictor responses to either the 5-HT_{1B} or 5-HT_{1D} receptor due to the lack of potent and selective ligands at these subtypes. In this context it should be emphasised that, in contrast to other species, ketanserin cannot discriminate between the canine 5-HT_{1B} and 5-HT_{1D} receptor subtypes (Branchek *et al*., 1995). Considering the recent availability of potent and

Based on: De Vries, P., Sánchez-López, A., Centurión, D., Heiligers, J.P.C., Saxena, P.R. & Villalón, C.M. (1998). The canine external carotid vasoconstrictor 5-HT_1 receptor: blockade by 5-HT_{1B} (SB224289), but not by 5-HT_{1D} (BRL15572) receptor antagonists. *Eur. J. Pharmacol.*, **362**, 69-72 (Short communication).

selective antagonists at either 5-HT$_{1B}$ (SB224289) or 5-HT$_{1D}$ (BRL15572) receptors (Hagan *et al*., 1997), in the present study we decided to further analyse the 5-HT$_{1B/1D}$ receptors involved in canine external carotid vasoconstrictor responses to 5-HT and sumatriptan.

8.2 Methods

8.2.1 General

Experiments were carried out in a total of 12 dogs (16-26 kg) not selected for breed or sex. The animals were anaesthetised with intravenously (i.v.) administered sodium pentobarbitone (30 mg kg^{-1}) and additional amounts (1 mg kg^{-1}) were provided when required. All dogs were intubated with an endotracheal tube and artificially respired with room air using a Palmer ventilation pump at a rate of 20 strokes min^{-1} and a stroke volume of 13-16 ml kg^{-1}. Bilateral cervical vagosympathectomy was systematically performed and mean arterial blood pressure and heart rate were recorded. The right femoral vein was cannulated for drug injection. The right common carotid artery was dissected free and the corresponding internal carotid and occipital arteries were ligated. The blood flow through the right common carotid artery, measured with ultrasonic flowmetry, was considered as the external carotid blood flow (for further details see Villalón *et al*., 1993b). The agonists were administered into the right common carotid artery with a cannula inserted into the right cranial thyroid artery. Body temperature was maintained between 37-38°C.

After a stable haemodynamic condition for at least 30 min, baseline values of blood pressure, heart rate and external carotid blood flow were determined. After i.v. pretreatment with 300 µg kg^{-1} of mesulergine, in order to block 5-HT$_7$ receptor-mediated external carotid vasodilatation (see Villalón *et al*., 1997a), sequential 1-min intracarotid (i.c.) infusions of 5-HT (0.3, 1, 3, 10 and 30 µg min^{-1}), sumatriptan (1, 3, 10 and 30 µg min^{-1}) and noradrenaline (0.1, 0.3, 1 and 3 µg min^{-1}) were given. The animals were then divided into 2 groups (n=6 each), where the responses to the above agonists were reanalysed after i.v. treatment with 30, 100 and 300 µg kg^{-1} of either SB224289 or BRL15572. The dose-intervals between the different doses of agonists ranged between 5 and 20 min, as in each case we waited until the blood flow had returned completely to baseline values. After the administration of an antagonist or saline a period of about 10 min was allowed to elapse before the responses to the respective agonists were elicited.

The drugs used in this study were: 5-HT creatinine sulphate and noradrenaline bitatrate (Sigma Chemical Co., St. Louis, Mo., USA), sumatriptan succinate (gift: Dr. M. Skingle, Glaxo Group Research, Ware, UK), mesulergine hydrochloride (gift: Sandoz A.G., Basel, Switzerland) and SB224289 and BRL15572 (SmithKline Beecham Pharmaceuticals, Harlow, Essex, UK). All compounds were dissolved in distilled water; when needed 20% (v v^{-1}) propylene glycol (SB224289 and BRL15572) was added, which did not affect any haemodynamic variable. The peak changes in external carotid blood flow by the agonists, expressed as percent changes from baseline, before and after the different doses of antagonists were compared by Student's paired *t*-test. A P-value of 0.05 or less (two-tailed) was considered statistically significant. All data are reported as the mean±s.e.mean.

8.3 Results

8.3.1 Initial effects of 5-HT, sumatriptan and noradrenaline on external carotid blood flow

Baseline values (n=12) of mean arterial blood pressure, heart rate and external carotid blood flow were 125±2 mmHg, 175±3 beats min^{-1} and 184±5 ml min^{-1}, respectively. 5-HT (0.3, 1, 3, 10 and 30 µg min^{-1}), sumatriptan (1, 3, 10 and 30 µg min^{-1}) and noradrenaline (0.1, 0.3, 1 and 3 µg min^{-1}) produced dose-dependent decreases in external carotid blood flow in mesulergine-pretreated vagosympathectomised dogs (Figure 8.1). The apparent rank order of agonist potency was noradrenaline> 5-HT≥sumatriptan. 1-min i.c. infusions of corresponding volumes of saline did not affect any haemodynamic parameter for the duration of the experiments (data not shown). Since the agonists were devoid of effects on mean arterial blood pressure and heart rate (data not shown), a local vasoconstrictor effect in the external carotid vasculature is implied, as previously discussed (Villalón *et al*., 1993b). The 5-HT-induced decreases in external carotid blood flow were preceded by small, non-dose-dependent increases in external carotid blood flow (see below).

8.3.2 Effects of SB224289 and BRL15572 on the external carotid vascular responses to 5-HT, sumatriptan and noradrenaline

As shown in the *upper panels* of Figure 8.1, SB224289 dose-dependently antagonised the external carotid vasoconstriction induced by 5-HT and sumatriptan, without

affecting that to noradrenaline. Additionally, after SB224289 the non-dose-dependent 5-HT-induced increases in external carotid blood flow were potentiated and a dose-dependency was unmasked. The increases after 0.3, 1, 3, 10 and 30 µg min^{-1} of 5-HT were 6.6±2.7, 15.5±6.5, 17.2±10.3, 14.0±6.6 and 13.1±9.9 %, respectively before SB224289 and 0.5±0.1, 7.2±3.5, 21.8±9.9, 43.6±14.8 and 63.2±17.4 %, respectively after the highest dose of SB224289 (300 µg kg^{-1}). In contrast, BRL15572 did not inhibit the decreases in external carotid blood flow by either 5-HT, sumatriptan or noradrenaline (Figure 8.1, *lower panels*); at 300 µg kg^{-1}, BRL15572 even slightly enhanced the decrease in external carotid blood flow by 0.3 µg min^{-1} of 5-HT. The small increases in the external carotid blood flow by 5-HT remained unchanged after BRL15572 (data not given). The doses of the antagonists used in this study were devoid of any haemodynamic effects *per se*.

8.4 Discussion

We have previously shown that 5-HT and sumatriptan constrict the canine external carotid vasculature via GR127935-sensitive 5-HT$_{1B/1D}$ receptors (Villalón *et al*., 1996). The recent availability of silent and selective antagonists for the 5-HT$_{1B}$ and 5-HT$_{1D}$ receptor subtypes led us to further analyse the receptors mediating these responses.

5-HT, sumatriptan and noradrenaline produced external carotid vasoconstriction with an apparent rank order of agonist potency (noradrenaline>5-HT≥sumatriptan), similar to that reported earlier (Villalón *et al*., 1993b; 1995c; 1997a). These effects are highly reproducible as they remain essentially unchanged after 3 subsequent infusions of saline (Villalón *et al*., 1995c; 1996). The 5-HT$_{1B}$ receptor ligand SB224289 (Table 9.1) dose-dependently antagonised these responses and the highest dose of SB224289 virtually abolished both the 5-HT- as well as sumatriptan-induced carotid vasoconstriction. This antagonism by SB224289 was specific, as the noradrenaline-induced effects remained unaffected. In contrast, the 5-HT$_{1D}$ receptor ligand BRL15572 (Table 9.1) did not affect the carotid vascular effects of 5-HT, sumatriptan or noradrenaline in any way. As SB224289 and BRL15572 display similar affinities at their respective receptors (Table 9.1), the lack of inhibitory effects by BRL15572, combined with the potent blockade by SB224289 at similar doses, clearly indicates that 5-HT$_{1B}$, but not 5-HT$_{1D}$

receptors, are involved in the canine external carotid vasoconstriction by 5-HT and sumatriptan.

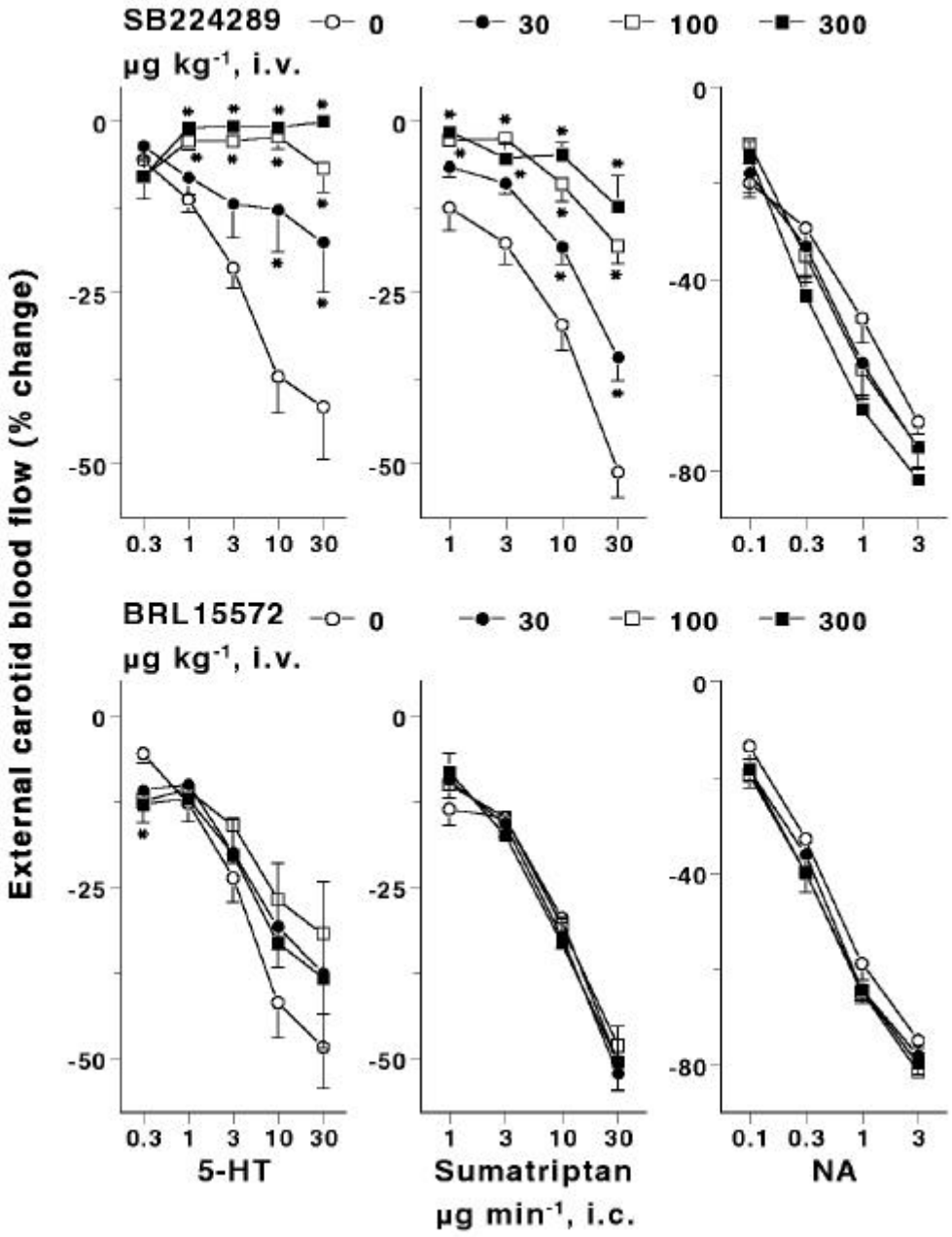

Figure 8.1 The effects of SB224289 (*upper panels*) and BRL15572 (*lower panels*) on the decreases in canine external carotid blood flow observed with 1-min intracarotid (i.c.) infusions of 5-HT, sumatriptan or noradrenaline (NA). All values are presented as the mean±s.e.mean. *, $P<0.05$ vs control.

Admittedly, this conclusion is based on the assumption that species differences between the binding of SB224289 and BRL15572 to canine and human 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors do not play a major role. In any case, the involvement of 5-HT$_{1B}$, but not 5-HT$_{1D}$ receptors is supported by the following observations: (i) the 5-HT-induced contraction of the human isolated temporal artery is similarly antagonised by SB224289 with a potency of 1 nM, but not by BRL15572 in doses up to 500 nM (Verheggen *et al*., 1998); (ii) *m*RNA (Bouchelet *et al*., 1996; Verheggen *et al*., 1998) and even the corresponding receptor protein (Longmore *et al*., 1997) of the 5-HT$_{1B}$, but not of the 5-HT$_{1D}$ receptor, has been detected in cranial blood vessels; and (iii) high doses of ketanserin or ritanserin (potential 5-HT$_{1D}$ receptor antagonists) do not display antagonism against sumatriptan-induced vasoconstriction (for reviews see Kaumann *et al*., 1993; Saxena *et al*., 1998b). Additionally, in view that SB224289 produced a complete blockade of the 5-HT- and sumatriptan-induced carotid vascular effects, it seems highly unlikely that additional receptors/mechanisms play a role, if any.

The 5-HT-induced decreases in external carotid blood flow, but not those to sumatriptan and noradrenaline, were preceded by a small vasodilator response, which was enhanced after blockade of the carotid vasoconstriction by SB224289, as also previously observed with GR127935 (Villalón *et al*., 1996). This carotid vasodilator response was recently shown to be mediated by the 5-HT$_7$ receptor (Villalón *et al*., 1997a). Indeed, due to the systematic pretreatment in the present experiments with the 5-HT$_7$ receptor ligand, mesulergine, the magnitude of the external carotid dilatory responses was much less marked than that observed previously (Villalón *et al*., 1997a).

In conclusion, using selective antagonists the present results obtained in the canine external carotid vasculature represent the first *in vivo* evidence that vascular constriction induced by 5-HT and sumatriptan is mediated primarily via 5-HT$_{1B}$, but not 5-HT$_{1D}$ receptors. Thus, SB224289 and BRL15572 seem to be excellent tools for further investigating the pharmacology of the 5-HT$_{1B/1D}$ receptors.

Chapter 9

Sumatriptan constricts porcine carotid arteriovenous anastomoses via 5-HT_{1B} receptors

Summary It has previously been shown that the antimigraine drug sumatriptan constricts porcine carotid arteriovenous anastomoses via 5-HT_1-like receptors, identical to 5-$HT_{1B/1D}$ receptors. The recent availability of silent antagonists selective for the 5-HT_{1B} (SB224289) and 5-HT_{1D} (BRL15572) receptor led us to further analyse the nature of receptors involved. In pentobarbital-anaesthetised, bilaterally vagosympathectomised pigs, sumatriptan (30, 100 and 300 µg kg^{-1}, i.v.) dose-dependently decreased carotid arteriovenous anastomotic conductance by up to 70±5%. The dose-related decreases in carotid arteriovenous anastomotic conductance by sumatriptan remained unchanged in animals treated (i.v.) with 1 mg kg^{-1} of BRL15572 (maximum decrease: 72±3%), but were significantly attenuated by 1 mg kg^{-1} (maximum decrease: 30±11%) and abolished by 3 mg kg^{-1} (maximum decrease: 3±7%) of SB224289. The highest dose of SB224289 did not attenuate the hypertension, tachycardia or increases in carotid blood flow induced by bolus injections of noradrenaline (0.1-3 µg kg^{-1}, i.v.). The results indicate that sumatriptan constricts porcine carotid arteriovenous anastomoses primarily via 5-HT_{1B}, but not via 5-HT_{1D} receptors.

9.1 Introduction

It has previously been shown in several species that 5-HT causes constriction within the carotid vascular bed, predominantly via 5-HT_1-like receptors and this effect is potently mimicked by the antimigraine drug, sumatriptan (Den Boer *et al*., 1991b; Villalón *et al*., 1995c). The carotid vasoconstrictor effect of sumatriptan and other acutely acting antimigraine agents is exclusively due to vasoconstriction of carotid arteriovenous anastomoses (see Saxena *et al*., 1997; De Vries *et al*., 1999b), which may open up during migraine headaches (Heyck, 1969; Saxena, 1995). Since sumatriptan displayed high affinity at the 5-HT_{1D} receptor binding sites identified in calf and human caudate membranes (Hoyer *et al*., 1994; Martin, 1994), it was suggested that "5-HT_{1D}" receptors were responsible for the sumatriptan-induced carotid vasoconstriction. Presently, we know that the experimental conditions in the above experiments allowed the inclusion of 5-HT_{1B}, 5-HT_{1D} as well as 5-HT_{1F} receptors (see Saxena *et al*., 1998b). GR127935, the first potent and selective antagonist at the "5-HT_{1D}" receptor (see

Based on: De Vries, P., Willems, E.W., Heiligers, J.P.C., Villalón, C.M. & Saxena, P.R. (1999). Investigation of the role of 5-HT_{1B} and 5-HT_{1D} receptors in the sumatriptan-induced constriction of porcine carotid arteriovenous anastomoses. *Br. J. Pharmacol.*, **127**, 405-412.

Chapter 3; Clitherow *et al*., 1994; Pauwels, 1996; Skingle *et al*., 1996), was shown to inhibit the sumatriptan-induced carotid vasoconstriction in pigs (see Chapter 4) and dogs (Villalón *et al*., 1996). In the mean time, it was demonstrated that the human "5-HT$_{1D}$" receptor was encoded by two structurally distinct genes, named 5-HT$_{1D\alpha}$ and 5-HT$_{1D\beta}$ (Weinshank *et al*., 1992). The 5-HT$_{1D\beta}$ receptor was shown to be the human homologue of the rat 5-HT$_{1B}$ receptor and, consequently, renamed 5-HT$_{1B}$, while the 5-HT$_{1D\alpha}$ receptor was renamed 5-HT$_{1D}$ receptor (see Figure 1.1). Unfortunately, GR127935 does not distinguish between these 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors (Table 9.1). Recently, however, two new potent compounds were developed, SB224289 (Hagan *et al*., 1997; Gaster *et al*., 1998) and BRL15572 (Price *et al*., 1997), which show a high degree of selectivity for the 5-HT$_{1B}$ and 5-HT$_{1D}$ receptor, respectively (Table 9.1). Using these compounds, it was shown that sumatriptan-induced canine external carotid vasoconstriction (Chapter 8) and human isolated temporal artery contraction (Verheggen *et al*., 1998) as well as hypothermia induced by SKF-99101H in the guinea-pig (Hagan *et al*., 1997) are mediated by SB224289-sensitive 5-HT$_{1B}$ receptors, whereas the human atrium heteroreceptor resembles BRL15572-sensitive 5-HT$_{1D}$ receptors (Schlicker *et al*., 1997). In the light of the availability of the above selective ligands, we decided to verify whether 5-HT$_{1B}$ or 5-HT$_{1D}$ receptors are involved in the constriction of carotid arteriovenous anastomoses in anaesthetised pigs by the antimigraine drug, sumatriptan.

9.2 Methods

9.2.1 General

The methods used for anaesthesia, surgical preparations and determination of systemic and carotid haemodynamics are described in detail in Chapter 4.

Table 9.1 pK_i values of sumatriptan, GR127935, SB224289 and BRL15572 at human cloned 5-HT_1 receptor subtypes.

	5-HT_{1A}	**5-HT_{1B}**	**5-HT_{1D}**	**5-ht_{1E}**	**5-HT_{1F}**
Sumatriptan[a]	6.4	7.8	8.5	5.8	7.9
GR127935[b]	7.2	9.0	8.6	5.4	6.4
SB224289[c]	5.5	8.0	6.2	<5.0	<5.0
BRL15572[b]	7.7	6.1	7.9	5.2	6.0

[a], Leysen *et al.* (1996); [b], Price *et al.* (1997); [c], Hagan *et al.* (1997).

9.2.2 Experimental protocol

After a stabilisation period of about 1 h, baseline values of heart rate, mean arterial blood pressure, carotid blood flow and its distribution, as well as arterial and jugular venous blood gases were determined. At this point the animals (n=21) were divided into 4 groups, receiving an i.v. infusion (1 ml min^{-1} over a period of 5 min) of either vehicle (distilled water, 20% propylene glycol, v v^{-1}; n=6), SB224289 (1 mg kg^{-1}; n=6), SB224289 (3 mg kg^{-1}; n=3) or BRL15572 (1 mg kg^{-1}; n=6). After a waiting period of 15 min, all parameters were reassessed. Subsequently, sequential i.v. doses of sumatriptan (30, 100 and 300 µg kg^{-1}) were given to all animals every 20 min. 15 Min after each dose of sumatriptan, all haemodynamic variables were assessed again. In the group treated with 3 mg kg^{-1} of SB224289, i.v. bolus injections of noradrenaline (0.1, 0.3, 1 and 3 µg kg^{-1}) were given at the start of the experiment (before SB224289) and at the end of the experiment (after SB224289 and the 3 doses of sumatriptan).

9.2.3 Data presentation and statistical analysis

All data have been expressed as the mean±s.e.mean. The significance of the difference between the variables within one group was evaluated with Duncan's new multiple range test, once an analysis of variance (randomised block design) had revealed that the samples represented different populations (Steel & Torrie, 1980). The percent changes caused by sumatriptan in animals treated with SB224289 or BRL15572 were compared to the corresponding responses in animals treated with vehicle by using Student's unpaired *t*-test. The peak changes induced by

noradrenaline before and after 3 mg kg^{-1} of SB224289 were compared by Student's paired *t*-test. Statistical significance was accepted at $P<0.05$ (two-tailed).

9.2.4 Drugs

Apart from the anaesthetics, azaperone (Janssen Pharmaceuticals, Beerse, Belgium), midazolan hydrochloride (Hoffmann La Roche b.v., Mijdrecht, The Netherlands) and pentobarbitone sodium (Apharmo, Arnhem, The Netherlands), the compounds used in this study were: sumatriptan succinate (gift from Dr. H.E. Connor, Glaxo Group Research, Stevenage, Hertfordshire, UK), SB224289 and BRL15572 (both gifts from Dr. A.A. Parsons, SmithKline Beecham Pharmaceuticals, Harlow, Essex, UK) and noradrenaline bitatrate (Sigma Chemical Co., St. Louis, Mo., USA). All compounds were dissolved in distilled water; when needed 20% (v v^{-1}) propylene glycol (SB224289 and BRL15572) was added. Heparin sodium (Leo Pharmaceutical Products, Weesp, The Netherlands) was used to prevent clotting of the catheters. All doses refer to the respective salts.

9.3 Results

9.3.1 Systemic and carotid haemodynamic effects by vehicle, SB224289 or BRL15572

The effects of the two antagonists and vehicle on systemic (mean arterial blood pressure and heart rate) and carotid (total, arteriovenous anastomotic and nutrient vascular conductances and the difference between oxygen saturation of arterial and jugular venous blood; A-V SO_2) are shown in Table 9.2. Except for a moderate, but significant increase in mean arterial blood pressure (12±2%) and decrease in carotid arteriovenous anastomotic conductance (21±5%) after the 1 mg kg^{-1}, i.v. dose of SB224289, there were no changes in these variables.

Table 9.2 The effects of (i.v.) vehicle, SB224289 and BRL15572 on heart rate (HR; beats min^{-1}), mean arterial blood pressure (MAP; mmHg), difference in arterial and jugular venous oxygen saturation (A-V SO_2; %), total carotid (TCa), arteriovenous anastomotic (AVA) and nutrient (Nut) vascular conductances (VC; 10^{-2} ml min^{-1} $mmHg^{-1}$).

	HR	MAP	A-V SO_2	TCa VC	AVA VC	Nut VC
Vehicle						
before	93±3	105±1	5.6±0.8	116±6	92±7	24±4
after	93±2	103±3	5.1±0.7	118±6	89±7	29±3
SB224289 (1 mg kg^{-1})						
before	100±2	94±2	8.5±1.8	138±10	109±10	29±3
after	101±2	105±4*	6.9±1.4	122±13	88±13*	35±3
SB224289 (3 mg kg^{-1})						
before	101±6	89±2	10.8±3.8	201±44	149±35	51±10
after	103±7	96±6	10.0±4.7	174±29	119±20	55±10
BRL15572 (1 mg kg^{-1})						
before	100±3	90±2	9.1±3.5	124±7	87±8	37±5
after	99±3	92±3	9.4±3.7	123±8	86±8	37±4

*, $P<0.05$ after vs before.

9.3.2 Systemic haemodynamic effects of sumatriptan in pigs treated with vehicle, SB224289 or BRL15572

Systemic haemodynamic changes induced by sumatriptan in the 4 different treatment groups are depicted in Table 9.3. In vehicle-treated animals, sumatriptan slightly decreased heart rate (maximum decrease: 4±1%) and blood pressure (maximum decrease: 7±3%). The sumatriptan-induced bradycardia was not affected by treatment with either SB224289 (maximum decreases after 1 or 3 mg kg^{-1}: 5±1% or 4±1%, respectively) or BRL15572 (maximum decrease: 5±2%). On the other hand, after treatment with 1 mg kg^{-1} of SB224289, sumatriptan produced a significantly more pronounced hypotension (maximum decrease: 19±3%). Similarly, 3 mg kg^{-1} significantly potentiated the hypotension of SB224289 induced by 30 and 100 µg kg^{-1}

of sumatriptan. Treatment with BRL15572 (1 mg kg^{-1}) did not affect the sumatriptan-induced decrease in blood pressure (maximum decrease: 7±5%).

Table 9.3 Systemic haemodynamic effects of sequential doses of sumatriptan in pigs treated with vehicle (n=6), SB224289 (1 or 3 mg kg^{-1}; n=6 or 3, respectively) or BRL15572 (n=6).

		Sumatriptan (µg kg^{-1}, i.v.)		
Treatment	*Baseline*	*30*	*100*	*300*
Heart rate (beats min^{-1})				
Vehicle	93±2	91±2*	90±2*	89±2*
SB224289 (1 mg kg^{-1})	101±2	100±2	98±2*	96±2*
SB224289 (3 mg kg^{-1})	103±7	102±6	100±5*	99±5*
BRL15572 (1 mg kg^{-1})	99±3	98±3	96±3*	94±4*
Mean arterial blood pressure (mmHg)				
Vehicle	103±3	103±4	103±4	97±5*
SB224289 (1 mg kg^{-1})	105±4	94±6*,$^{+}$	90±5*,$^{+}$	86±5*,$^{+}$
SB224289 (3 mg kg^{-1})	96±6	88±7^{+}	81±3*,$^{+}$	81±2*
BRL15572 (1 mg kg^{-1})	92±3	92±2	90±3	85±4*
Arterial-jugular venous oxygen saturation difference (%)				
Vehicle	5.1±0.8	7.0±0.8	11.4±2.6*	17.7±2.8*
SB224289 (1 mg kg^{-1})	6.9±1.4	12.1±2.8*	12.1±3.3*	14.9±2.2*
SB224289 (3 mg kg^{-1})	10.0±4.7	10.3±2.7	8.4±3.7	10.0±4.2^{+}
BRL15572 (1 mg kg^{-1})	9.4±3.7	10.8±3.4	14.8±5.0*	18.6±4.8*

*, P<0.05 vs baseline; $^{+}$, P<0.05 vs response by corresponding dose of sumatriptan in animals treated with vehicle.

Sumatriptan dose-dependently increased A-V SO_2 by up to 270±71%. The increases in A-V SO_2 by sumatriptan were not significantly modified by treatment with 1 mg kg^{-1} of SB224289 or BRL15572 (maximum increases: 161±55 and 151±38%, respectively), whereas after 3 mg kg^{-1} of SB224289 this effect was absent.

9.3.3 Carotid haemodynamic effects of sumatriptan in pigs treated with vehicle, SB224289 or BRL15572

Changes in carotid haemodynamics by sumatriptan in the 4 different groups are depicted in Figures 9.1 (absolute values) and 9.2 (percent changes). In vehicle-treated animals, sumatriptan dose-dependently decreased total carotid blood flow and conductance by up to 44±7% and 40±7%, respectively, accompanied by decreases in arteriovenous anastomotic blood flow and conductance by up to 72±4% and 70±5%, respectively. Nutrient blood flow and conductance increased after sumatriptan by up to 55±25% and 65±26%, respectively. These effects of sumatriptan were dose-dependently reduced by SB224289. Thus, in animals treated with 1 mg kg^{-1} of SB224289 the sumatriptan-induced decreases in total carotid blood flow and conductance amounted to only 32±6% and 17±7%, respectively, while carotid arteriovenous anastomotic blood flow and conductance decreased by only up to 43±9% and 30±11%, respectively. After the higher dose (3 mg kg^{-1}) of SB224289, the sumatriptan-induced maximal decreases in total carotid and arteriovenous anastomotic blood flows (12±7% and 18±3%, respectively) and conductances (4±12% and 3±7%, respectively) were completely blocked. Treatment with BRL15572 (1 mg kg^{-1}) did not affect the sumatriptan-induced decreases in total carotid and arteriovenous anastomotic blood flows (maximum decreases: 37±3% and 74±3%, respectively) and conductances (maximum decreases: 32±5% and 72±3%, respectively). Neither SB224289 nor BRL15572 significantly affected the increases in nutrient blood flow and conductance.

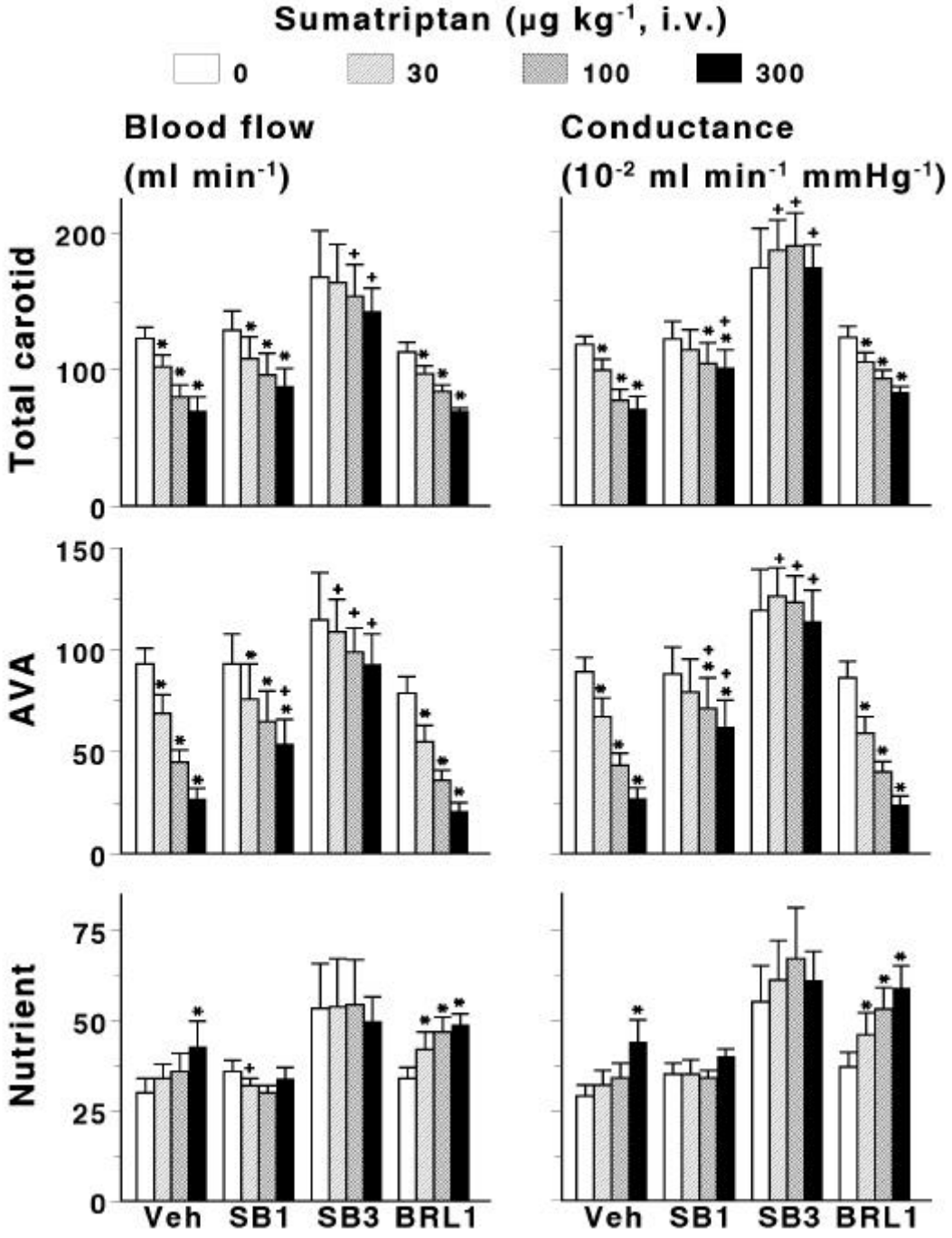

Figure 9.1 Values of total carotid, arteriovenous anastomotic (AVA) and nutrient blood flows (*left panels*) and conductances (*right panels*) at baseline and after sumatriptan in animals treated i.v. with vehicle (Veh; n=6), 1 mg kg^{-1} of SB224289 (SB 1; n=6), 3 mg kg^{-1} of SB224289 (SB 3; n=3) or 1 mg kg^{-1} of BRL15572 (BRL1; n=6). All values are presented as the mean±s.e.mean. *, P<0.05 vs baseline. +, P<0.05 vs response by corresponding dose in vehicle-treated animals.

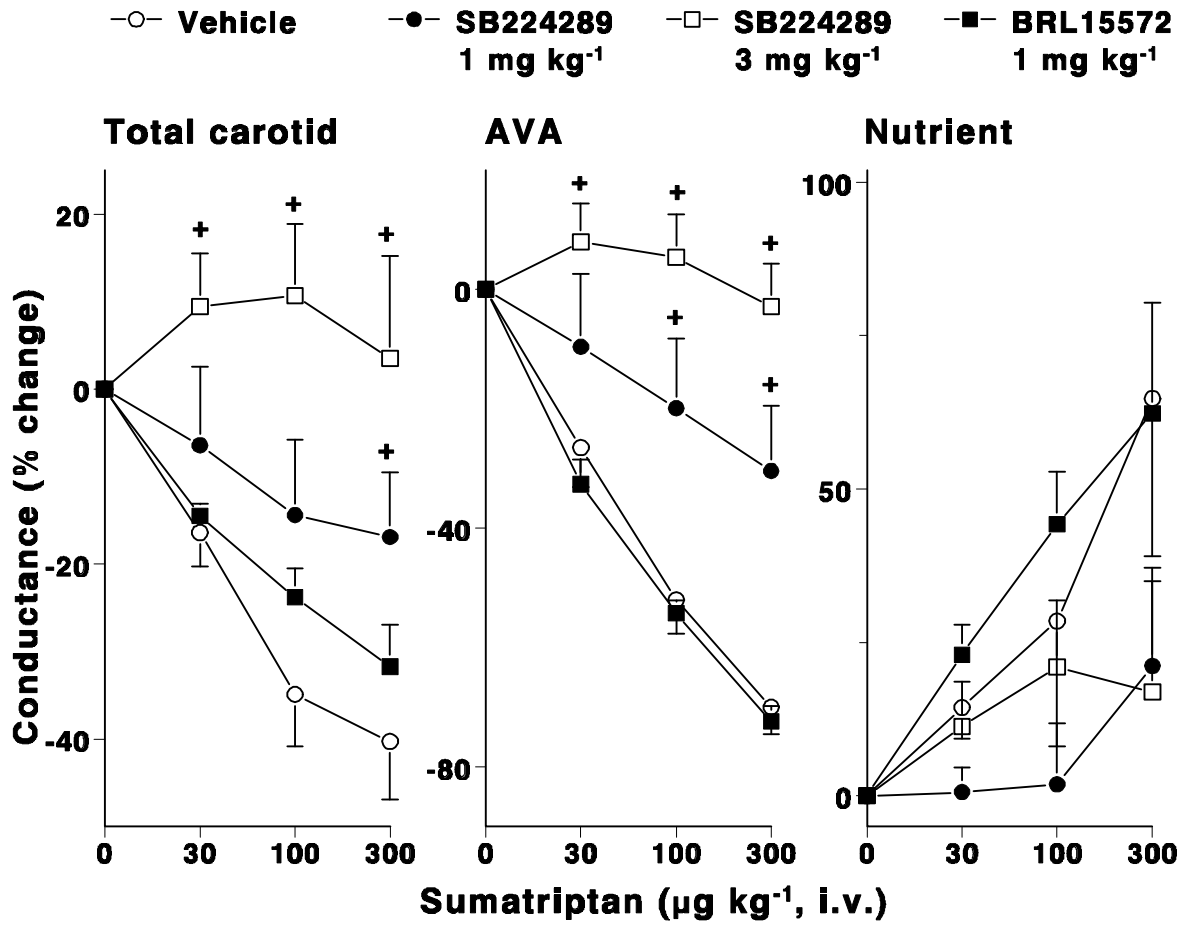

Figure 9.2 Effect of sumatriptan on total carotid, arteriovenous anastomotic (AVA) and nutrient conductances in animals treated with vehicle (n=6), 1 mg kg^{-1} of SB224289 (n=6), 3 mg kg^{-1} of SB224289 (n=3) or BRL15572 (1 mg kg^{-1}; n=6). All values are presented as mean±s.e.mean. +, P<0.05 vs response by corresponding dose in vehicle-treated animals.

9.3.4 Effect of SB224289 on noradrenaline-induced changes in heart rate, mean arterial blood pressure and total carotid blood flow and conductance

As shown in Figure 9.3, bolus injections of noradrenaline (0.1-3 µg kg^{-1}, i.v.) produced dose-dependent increases (with maximum changes) in heart rate (+82±7%), mean arterial blood pressure (+31±2%) and total carotid blood flow (+26±5%) without affecting total carotid vascular conductance (maximum change: +4±1%). The maximum changes by noradrenaline remained either unchanged (heart rate, 76±3%;

mean arterial blood pressure, 43±4%; total carotid vascular conductance, 5±4%) or were even enhanced (total carotid blood flow, 47±8%) after SB224289 (3 mg kg^{-1}, i.v.).

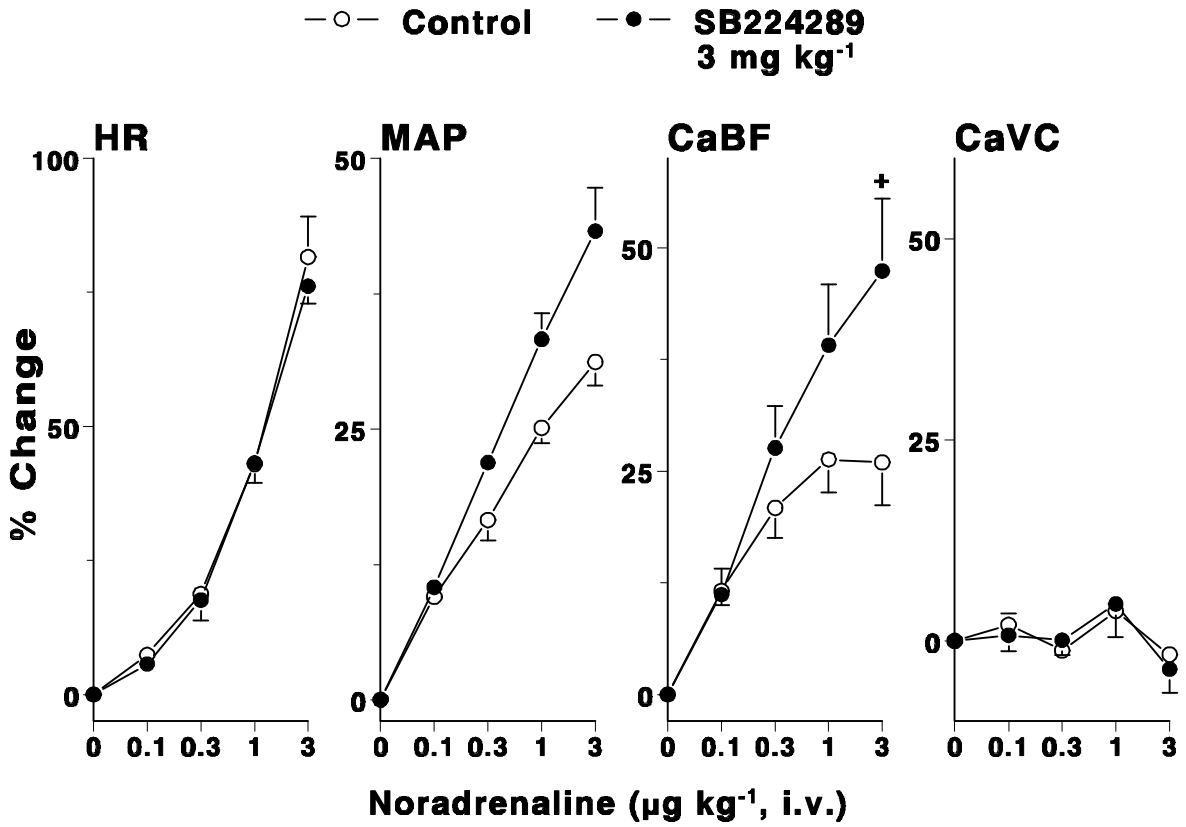

Figure 9.3 Effect of noradrenaline on heart rate, mean arterial blood pressure (MAP), carotid blood flow (CaBF) and carotid vascular conductance (CaVC) before and after SB224289 (3 mg kg^{-1}, i.v.). All values are presented as the mean±s.e.mean. +, P<0.05 after vs before.

9.4 Discussion

9.4.1 General

We have previously shown that sumatriptan decreases porcine carotid blood flow by a selective vasoconstriction of cranial arteriovenous anastomoses (Chapter 4; Den Boer *et al.*, 1991b). The drug seems to exert this response via 5-HT$_{1B/1D}$ receptors, since GR127935, a selective antagonist at these 5-HT$_1$ receptor subtypes

(see Chapter 3; Clitherow *et al*., 1994; Pauwels, 1996; Skingle *et al*., 1996), was able to abolish the sumatriptan-induced responses (Chapter 4). The recent availability of silent selective antagonists for the 5-HT_{1B} (SB224289) and 5-HT_{1D} (BRL15572) receptors led us to further analyse the nature of the receptors mediating these responses. The present study in anaesthetised pigs clearly showed that the sumatriptan-induced carotid arteriovenous anastomotic constriction was potently and specifically antagonised in a dose-dependent manner by the selective 5-HT_{1B} receptor antagonist SB224289, but not by the selective 5-HT_{1D} receptor antagonist BRL15572. Apart from the implications discussed below, these data indicate that the vasoconstrictor response to sumatriptan on porcine carotid arteriovenous anastomoses is mainly mediated by 5-HT_{1B} receptors.

9.4.2 Systemic and carotid haemodynamic effects of vehicle, SB224289 and BRL15572

Statistically significant changes were noticed only in the group that received the lower dose (1 mg kg^{-1}) of SB224289. A moderate increase in mean arterial blood pressure and a vasoconstrictor effect in the carotid vasculature, confined to the arteriovenous anastomotic fraction, were observed. We do not have a clear explanation for this, but it is interesting to note that the 5-$HT_{1B/1D}$ receptor antagonist GR127935 also constricts porcine arteriovenous anastomoses, but, in contrast to SB224289, decreases blood pressure (Chapter 4). Whereas the GR127935-induced carotid vasoconstriction may be related to its intrinsic activity at the h5-HT_{1B} receptor (Pauwels, 1996; Selkirk *et al*., 1998), this seems unlikely in the case of SB224289, because this compound rather behaves as an inverse agonist at this receptor (Selkirk *et al*., 1998).

9.4.3 Systemic haemodynamic effects induced by sumatriptan in animals treated with vehicle, SB224289 or BRL15572

Sumatriptan caused a small bradycardiac and hypotensive effect, similar to that reported earlier (Chapter 4). This seems to be a class effect of the drug, since several other 5-$HT_{1B/1D}$ receptor agonists exert this effect (Chapter 6). The mechanism involved in the hypotensive and bradycardiac action of sumatriptan is not clear, but may involve sympathoinhibition either at the level of ganglia, neurovascular junction (Jones *et al*., 1995; Villalón *et al*., 1998) or perhaps within the central nervous system (Saxena & Villalón, 1990); the latter mechanism seems unlikely on the basis of poor

central penetration of sumatriptan. The hypotensive response to sumatriptan was unaffected by GR127935 (Chapter 4), but potentiated by the 5-HT$_{1B}$ receptor antagonist SB224289 (Table 9.3), suggesting that sumatriptan simultaneously stimulates a systemic vasodilator and vasoconstrictor mechanism, of which the latter is amenable to blockade by SB224289. The sumatriptan-induced systemic dilatation may involve sympathoinhibition mediated by the 5-HT$_{1F}$ receptor, for which SB224289 displays a very low and GR127935 only a moderate affinity (see Table 9.1); this possibility requires further investigation. Whatever the mechanism, this effect of sumatriptan is clinically of little relevance. Sumatriptan (MacIntyre *et al*., 1993), but also other 5-HT$_{1B/1D}$ receptor agonists, such as rizatriptan (Sciberras *et al*., 1997), alniditan (Goldstein *et al*., 1996) and zolmitriptan (Seaber *et al*., 1996) produce increases rather than decreases in blood pressure in humans.

9.4.4 Carotid haemodynamics

Sumatriptan decreased total carotid blood flow, due to a potent vasoconstrictor action on the cephalic arteriovenous anastomoses. The effect was similar to that observed earlier with this antimigraine agent (see Chapter 4; Den Boer *et al*., 1991b). In animals treated with 1 mg kg^{-1} of the 5-HT$_{1B}$ receptor ligand SB224289, the sumatriptan-induced decreases in total carotid and carotid arteriovenous anastomotic blood flow were not much affected; only at 300 µg kg^{-1} of sumatriptan a significant attenuation was observed (see Figure 9.1). However, as described above, in the presence of SB224289 an enhancement of the sumatriptan-induced hypotension was observed. The latter will decrease the carotid perfusion pressure and, consequently, may exaggerate decreases in blood flow, thereby masking possible inhibition of the sumatriptan-induced effects by SB224289. Indeed, the decreases in total carotid and carotid arteriovenous anastomotic conductance (where the changes in blood flow are corrected for changes in blood pressure) were potently antagonised, although not completely eliminated, in animals treated with 1 mg kg^{-1} SB224289 (Figure 9.3). In contrast, 1 mg kg^{-1} of the 5-HT$_{1D}$ receptor antagonist BRL15572 did not affect the carotid vascular effects of sumatriptan in any way. As SB224289 and BRL15572 display similar affinities at their respective receptors (Table 9.1), the lack of inhibitory effects by BRL15572, combined with the potent blockade by SB224289 at similar

doses, clearly indicates that 5-HT_{1B}, but not 5-HT_{1D} receptors, are involved in the vasoconstriction of carotid arteriovenous anastomoses.

Since a part of the sumatriptan-induced carotid arteriovenous anastomotic constriction persisted after 1 mg kg^{-1} of SB224289, it may suggest that other receptors/mechanisms may be involved. Notwithstanding, we have previously shown that 0.5 mg kg^{-1}, but not 0.25 mg kg^{-1} of the 5-$HT_{1B/1D}$ receptor antagonist GR127935 was needed for a complete blockade of the sumatriptan-induced porcine carotid vasoconstrictor effects (Chapter 4). In view of the 10-fold higher affinity displayed at the h5-HT_{1B} receptor by GR127935 compared to SB224289 (Table 9.1), it can be expected that higher doses of SB224289 are needed to bring about a complete blockade. Indeed, 3 mg kg^{-1} of SB224289 completely abolished all sumatriptan-induced carotid vascular effects. In keeping with this, we have previously shown that 3- to 10-fold higher concentrations of SB224289 (Chapter 8) than GR127935 (Villalón *et al*., 1996) were required to completely inhibit the canine external carotid vasoconstriction by sumatriptan.

In order to ascertain the specificity of SB224289 (3 mg kg^{-1}), we decided to study cardiovascular responses to noradrenaline before and after administration of the antagonist. As expected, noradrenaline produced short-lasting increases in mean arterial blood pressure, which were accompanied by increases heart rate, as the baroreflex mediated bradycardia was absent due to vagotomy (Hoffman & Lefkowitz, 1996). Moreover, noradrenaline increased carotid blood flow, which was mainly due to the hypertensive effect, as carotid vascular conductance did not change in response to the drug, as shown earlier (Verdouw *et al*., 1984a). SB224289 did not attenuate these effects; in fact, the noradrenaline-induced hypertension was potentiated, probably resulting in an enhancement of the increases in carotid blood flow. Therefore, it is concluded that 3 mg kg^{-1} of SB224289 most likely produced a specific antagonism against the sumatriptan-induced effects. In keeping with this, SB224289 displays low affinities at α- and β-adrenoceptors (Gaster *et al*., 1998).

Taking the above into account, the present results imply that sumatriptan constricts porcine arteriovenous anastomoses via the 5-HT_{1B} receptor, which in contrast to the 5-HT_{1D} receptor is abundantly expressed on vascular smooth muscle (Ullmer *et al*., 1995; Bouchelet *et al*., 1996; Longmore *et al*., 1997). Moreover, the results obtained in this study imply that the so-called 5-HT_1-like receptor mediating vascular smooth muscle contraction (Saxena *et al*., 1998b), including porcine carotid

arteriovenous anastomotic constriction (Den Boer *et al*., 1991b), is identical to the 5-HT$_{1B}$ receptor. This is also shown in the isolated human temporal artery (Verheggen *et al*., 1998) and the canine external carotid vascular bed (Chapter 8). Additionally, in view of the complete blockade by SB224289 and the highly selective nature of the compound, it seems unlikely that the other known 5-HT$_1$ subtypes (5-HT$_{1A}$, 5-ht$_{1E}$ and 5-HT$_{1F}$) are involved in the sumatriptan-induced carotid vascular effects. Thus, intracarotid infusions of LY344864 (1-3100 µg min^{-1}), a selective 5-HT$_{1F}$ receptor agonist (Phebus *et al*., 1997), does not produce vasoconstriction in the canine external carotid vascular bed (Chapter 7). Also, *m*RNAs for 5-HT$_{1A}$ and 5-ht$_{1E}$ receptor have not been detected in the vascular smooth muscle (Ullmer *et al*., 1995).

In conclusion, the results of the present experiments show that the constriction of porcine carotid arteriovenous anastomoses by the 5-HT$_1$ receptor agonist sumatriptan, being antagonised by the selective 5-HT$_{1B}$ receptor antagonist SB224289, but not by the 5-HT$_{1D}$ receptor ligand BRL15572, is mediated by 5-HT$_{1B}$ receptors. In view of the putative pathophysiological role of arteriovenous anastomotic dilatation in migraine (Heyck, 1969; Saxena, 1995), the constriction of these non-nutrient vessels by sumatriptan via a 5-HT$_{1B}$ receptor mechanism may be, at least partly, responsible for the therapeutic effect of the drug in migraine.

Chapter 10

Nature of 5-HT_1-like receptors mediating depressor responses in vagosympathectomised rats; close resemblance to the cloned 5-ht_7 receptor

Summary It has been suggested that the late hypotensive response to 5-HT in vagosympathectomised rats is mediated by 5-HT_1-like receptors since this effect is mimicked by 5-CT, is not modified by cyproheptadine, ketanserin or MDL72222, but it is blocked by methysergide. The present study was set out to reanalyse this suggestion in terms of the classification schemes proposed in 1994 and 1996 by the NC-IUPHAR subcommittee on the classification and nomenclature of 5-HT receptors. I.v. bolus injections of 5-CT (0.01-0.3 μg kg^{-1}), 5-HT (1-30 μg kg^{-1}) and 5-MeO-T (1-30 μg kg^{-1}) produced dose-dependent hypotensive responses with a rank order of agonist potency: 5-CT >> 5-HT ≥ 5-MeO-T with sumatriptan (30-1000 μg kg^{-1}) inactive. The depressor responses to 5-HT and 5-CT were not attenuated by i.v. GR127935 (300-3000 μg kg^{-1}) or equivalent volumes of saline. In contrast, lisuride, methiothepin, mesulergine, metergoline and clozapine dose-dependently antagonised the responses to 5-HT and 5-CT; the rank order of apparent pA_2 values against 5-HT and 5-CT, respectively, was: lisuride (7.7; 7.8) > methiothepin (6.8; 7.0) ≥ mesulergine (6.4; 6.6) > clozapine (5.7; 5.8); metergoline displayed variable potencies (5.6; 6.4). Except for lisuride, which also affected isoprenaline-induced hypotension, the antagonism by the other drugs was specific. Based upon the above rank order of agonist potency, the blockade by a series of drugs showing high affinity for the cloned 5-ht_7 receptor and the lack of blockade by GR127935, our results indicate that the 5-HT receptor mediating hypotension in vagosympathectomised rats is operationally similar to other putative 5-ht_7 receptors mediating vascular and non-vascular responses (e.g. relaxation of the rabbit femoral vein, canine coronary and external carotid arteries and guinea-pig ileum as well as feline tachycardia).

10.1 Introduction

First noticed by Page and McCubbin (1953) and later further characterised (Kalkman *et al*., 1984; Saxena & Lawang, 1985; Martin *et al*., 1987), i.v. administration of 5-HT produces a triphasic blood pressure response in anaesthetised rats with intact vagus; this response consists of an initial hypotension associated with

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a brief, but intense, bradycardia via the von Bezold-Jarisch reflex (mediated by 5-HT$_3$ receptors located on afferent vagal fibres), followed by a vasopressor effect (mediated by vascular 5-HT$_{2A}$ receptors) and, finally, a longer-lasting hypotension (see Chapter 3; Saxena & Villalón, 1990; 1991). After bilateral vagosympathectomy and 5-HT$_2$ receptor blockade, using ritanserin, in a dose proven to be sufficient to block the pressor phase (Chapter 3), 5-HT exclusively produces the late depressor response. This latter response, also observed in the cat (Saxena *et al*., 1985b; Connor *et al*., 1986), has previously been ascribed to an action at vascular 5-HT$_1$-like receptors (Saxena & Lawang, 1985; Saxena & Villalón, 1990; 1991), on the basis of agonism by 5-HT, being mimicked by 5-CT, potent antagonism by the mixed 5-HT$_{1/2}$ receptor antagonists (methiothepin and methysergide) and the lack of inhibitory action by 5-HT$_2$ (ketanserin and cyproheptadine) and 5-HT$_3$ (MDL7222) receptor antagonists. Notwithstanding, it has been recommended that the receptor involved should be referred to as an atypical 5-HT$_1$-like or even "orphan" 5-HT receptor (Hoyer *et al*., 1994), mainly on the basis of the lack of agonist effect of the 5-HT$_1$-like receptor agonist sumatriptan (Feniuk *et al*., 1989; Perren *et al*., 1989) and contradictory transductional properties. Thus, according to current nomenclature, members of the 5-HT$_1$ family are negatively coupled to adenylyl cyclase, leading to a decrease in cAMP which is usually associated with vasoconstrictor rather than vasodilator and concomitant hypotensive responses (Rand *et al*., 1987; Hoyer *et al*., 1994). In keeping with this, evidence has emerged that sumatriptan-sensitive 5-HT$_1$-like receptors mediating vasoconstriction resemble 5-HT$_{1B/1D}$ receptors (Chapter 4; Villalón *et al*., 1996), of which the 5-HT$_{1B}$ receptor seems to be primarily involved (see Chapters 8 and 9; Verheggen *et al*., 1998). On the other hand, the recently cloned 5-ht$_7$ receptor (e.g. Bard *et al*., 1993; Ruat *et al*., 1993b) seems to be a suitable candidate for responses mediated by the atypical, sumatriptan-insensitive 5-HT$_1$-like receptors. Indeed, the cloned 5-ht$_7$ receptor is coupled positively to adenylyl cyclase and binding studies show that, as for 5-HT$_1$-like receptors, the rat 5-ht$_7$ receptor displays high affinity for 5-HT and 5-CT as well as for methiothepin, but low affinity for sumatriptan and ketanserin (see Tables 10.1 and 10.2).

Therefore, in the present study, we have further characterised the late hypotensive response to 5-HT in vagosympathectomised rats, with particular emphasis on verifying if these receptors are operationally similar to the cloned 5-ht$_7$ receptor. Although, until now, no selective 5-ht$_7$ receptor ligands are available, we made use of

several compounds that, according to current literature, display high affinity for, and in some cases relative selectivity at cloned rat 5-ht$_7$ receptors (see Tables 10.1 and 10.2).

Table 10.1 pK_i values at human (except when stated otherwise) 5-HT receptors of the agonists used in the present study.

	5-CT	***5-HT***	***5-MeO-T***	***Sumatriptan***
5-HT$_{1A}$	10.3[a]	9.2[a]	8.0[b] (pig)	6.4[c]
5-HT$_{1B}$	8.9[d] (rat)	8.6[d] (rat)	8.2[d] (rat)	7.3[d]/ 6.3[e] (rat)
	8.8[d]	8.5[d]	8.2[d]	8.1[d]/ 7.8[c]
5-HT$_{1D}$	9.2[f]	8.4[f]	8.3[f]	8.5[c]
5-ht$_{1E}$	5.1[g]	8.0[g]	5.5[g]	5.8[c]/5.6[g]
5-HT$_{1F}$	6.1[g]	8.0[g]	5.9[g]	7.9[c]/7.6[g]
5-HT$_{2A}$	4.7[b] (rat)	5.5[b] (rat)	5.5[b] (rat)	<5.5[h]
5-HT$_{2B}$	8.0[i] (rat)	8.6[i] (rat)	8.8[i] (rat)	<4.5[i] (rat)
				6.9*
5-HT$_{2C}$	6.2[b] (pig)	7.5[b] (pig)	7.6[b] (pig)	<5.5[h]
5-HT$_3$	Inactive[j] (rat)	6.7[j] (rat)	Inactive[j] (rat)	<5.5[h] (mouse)
5-HT$_4$	<5.0[k] (rat)	6.8[k] (rat)	6.4[k] (rat)	<5.5[h] (guinea-pig)
5-ht$_{5A}$	9.5[j] (rat)	8.1[j] (rat)		<5.5[h] (rat)
5-ht$_{5B}$	7.4[j] (rat)	6.6[j] (rat)		
5-ht$_6$	6.1[l] (rat)	6.8[l] (rat)	7.4[l] (rat)	<5.5[h]
5-HT$_7$	9.5[m] (rat)	8.7[m] (rat)	8.7[m] (rat)	5.9[h]

*, Gupta, P, personal communication. [a], Newman-Tancredi *et al.* (1997); [b], Hoyer (1989); [c], Leysen *et al.* (1996); [d], Beer *et al.* (1998); [e], Adham *et al.* (1992); [f], Weinshank *et al.* (1992); [g], Adham *et al.* (1993); [h], Napier *et al.* (1999); [i], Baxter *et al.* (1994), pEC_{50} values; [j], Hoyer *et al.* (1994); [k], Gerald *et al.* (1995); [l], Monsma *et al.* (1993); [m], Shen *et al.* (1993).

Table 10.2 pK_i values at human (except when stated otherwise) 5-HT receptors of the antagonists used in the present study.

	Lisuride	***Mesulergine***	***Metergoline***	***Methiothepin***	***Clozapine***	***GR127935***
5-HT$_{1A}$	9.7[a]	6.8[b] (rat)	8.1[f] (pig)	7.7*	6.9[d] (rat)	6.9[e] (rat)
5-HT$_{1B}$	6.7[f] (rat)	5.9[b] (rat)	8.3[g] (rat)	7.2[g] (rat)	6.2[d] (rat)	8.5[e] (rat)
			8.6[g]	7.6[g]	6.2[d]	9.9[e]
5-HT$_{1D}$			8.7[h]	7.7[h]	6.4[d]	8.9[e]
5-ht$_{1E}$			6.0[i]	6.7[j]	6.4[d]	6.2[e]
5-HT$_{1F}$		<5.0[j]	6.5[j]	6.2[j]	6.9[d]	7.3[e]
5-HT$_{2A}$	8.3[f] (rat)	9.1[c]** (rat)	8.5[c]** (rat)	9.0[c]** (rat)	8.5[d] (rat)	7.2[e] (rat)
5-HT$_{2B}$		7.9[k] (rat)				<6.0[k] (rat)
5-HT$_{2C}$	7.7[f] (pig)	9.1[c]** (pig)	10.6[c]** (pig)	8.2[c]** (pig)	7.9[d] (pig)	6.2[e] (pig)
5-HT$_3$		<5.0[b] (rat)			7.0[d] (mouse)	5.2[e] (rat)
5-HT$_4$		Inactive[c] (rat)		Inactive[c] (rat)		<5.0[e] (guinea-pig)
5-ht$_{5A}$		<6.0[c] (rat)	<6.0[c] (rat)	7.0[c] (rat)		5.2[e]
5-ht$_{5B}$		<6.0[c] (rat)	<6.0[c] (rat)	7.8[c] (rat)		
5-ht$_6$	8.1[c] (rat)	5.8[c] (rat)	7.5[c] (rat)	8.7[c] (rat)	8.4[d] (rat)	5.8[l]
5-HT$_7$	9.1[m] (rat)	7.7[m] (rat)	8.2[m] (rat)	9.4[m] (rat)	7.4[m] (rat)	6.2[l]

*, Pauwels, P.J., personal communication; **, pK_B value. [a], Newman-Tancredi *et al.* (1997); [b], Van Wijngaarden *et al.* (1990); [c], Hoyer *et al.* (1994); [d], Schotte *et al.* (1996); [e], Pauwels (1996); [f], Hoyer (1989); [g], Beer *et al.* (1998); [h], Pauwels *et al.* (1996); [i], McAllister *et al.* (1992); [j], Adham *et al.* (1993); [k], Baxter *et al.* (1994), pA_2 value; [l], Price *et al.* (1997); [m], Shen *et al.* (1993).

10.2 Methods

10.2.1 General

Experiments were carried out in 98 male Wistar rats (300-350 g). After initial anaesthesia with ether, the trachea was cannulated and a catheter was placed in the right external jugular vein. At this point, ether anaesthesia was stopped and, subsequently, the animals received i.v. bolus injections of sodium pentobarbital (30-40 mg kg^{-1}). Hereafter, both vagus nerves and accompanying cervical sympathetic trunks were cut to avoid the bradycardia and hypotension via the von Bezold-Jarisch reflex (Paintal, 1973). The right carotid artery was cannulated for the recording of blood pressure, using a pressure transducer (Combitrans disposable pressure transducer, Braun, Melsungen, Germany). The rats were artificially ventilated with a mixture of oxygen and room air using a respiratory pump (Infant ventilator MK3, HoekLoos, The Netherlands) at a rate of 40 strokes min^{-1} (volume: 20 ml kg^{-1}). Heart rate was measured with a tachograph (CRW, Erasmus University, Rotterdam, The Netherlands) triggered from electrocardiogram signals. Both blood pressure and heart rate were recorded simultaneously on a polygraph (CRW, Erasmus University, Rotterdam, The Netherlands). It should be noted that three experiments were carried out simultaneously; because of limited channels for heart rate, this variable was determined in only some experiments.

10.2.2 Experimental protocols

After a stabilisation period of about 30 min, baseline values of arterial blood pressure and heart rate were determined. Then the animals received consecutive i.v. bolus injections, every 5-10 min, of either 5-HT (1, 3 and 10 µg kg^{-1}; n=44, of which 17 animals received an additional dose of 30 µg kg^{-1}), 5-CT (0.01, 0.03, 0.1 and 0.3 µg kg^{-1}; n=42), 5-MeO-T (1, 3, 10 and 30 µg kg^{-1}; n=6) or sumatriptan (10, 30, 100, 300 and 1000 µg kg^{-1}; n=6) and the peak changes in mean arterial blood pressure and heart rate were noted.

The animals receiving 5-HT were pretreated with ritanserin (50 µg kg^{-1}, i.v.) in order to block pressor responses. Additionally, after each curve to 5-HT, consecutive bolus injections of isoprenaline (0.01, 0.03 and 0.1 µg kg^{-1}, i.v.) were given. For the sake of clarity, the results with isoprenaline are shown separately. Subsequently, this group of animals was subdivided into seven treatment groups receiving (over a 1-2 min period) either physiological saline (0.5 ml three times; n=5),

lisuride (10, 30 and 100 µg kg^{-1}; n=6), mesulergine (100, 300, 1000 and 3000 µg kg^{-1}; n=6), metergoline (100, 300, 1000 and 3000 µg kg^{-1}; n=6), methiothepin (100, 300 and 1000 µg kg^{-1}; n=9), clozapine (300, 1000 and 3000 µg kg^{-1}; n=6) or GR127935 (300, 1000 and 3000 µg kg^{-1}; n=6). Before each antagonist or saline treatment, the animals received small i.v. bolus injections of pentobarbital (20-30 mg kg^{-1}), necessary to establish equal baseline values of blood pressure. The responses to 5-HT and isoprenaline were elicited again about 10 min after each dose of saline and antagonists.

Similarly, the animals receiving 5-CT were subdivided into 7 treatment groups (n=6 each) as specified above, except that the doses of lisuride used were 3, 10 and 30 µg kg^{-1}, those of mesulergine 30, 100, 300 and 1000 µg kg^{-1} and those of methiothepin were 30, 100 and 300 µg kg^{-1}.

10.2.3 Calculation of agonist ED_{30} and apparent antagonist pA_2 values

Hypotensive responses produced by each dose of 5-HT and 5-CT before and after each antagonist dose were subjected to linear regression analysis to calculate ED_{30} values (i.e. the dose needed to decrease mean arterial blood pressure by 30 mmHg). With the baseline dose-ratio value set to 1, agonist dose ratios after the different antagonist doses were computed in each experiment. The conversion of the drug doses into nmol kg^{-1} allowed us to perform Schild analysis to determine apparent pA_2 values of antagonists.

10.2.4 Data presentation and statistical analysis

All data in the text and illustrations are presented as the mean±s.e.mean. The changes from baseline in blood pressure caused by 5-HT, 5-CT and isoprenaline before and after the different doses of antagonists were compared by the use of Duncan's new multiple range test, once an analysis of variance (randomised block design) had revealed that the samples represented different populations (Steel & Torrie, 1980). A P-value of 0.05 or less (two-tailed) was considered statistically significant.

10.2.5 Drugs

Apart from the anaesthetics, ether (Lab-scan, Dublin, Ireland) and pentobarbitone sodium (Apharmo, Arnhem, The Netherlands), the drugs used in this study were: GR127935 and sumatriptan succinate (GlaxoWellcome Research Ltd., Stevenage,

UK); 5-HT creatinine sulphate (Sigma Chemical Company, St. Louis, MO, USA); 5-MeO-T hydrochloride, 5-CT maleate, ritanserin and R(+)lisuride hydrogen maleate (all from RBI, Natick, USA); mesulergine hydrochloride and clozapine (Sandoz Pharma Ltd., Basel, Switzerland); methiothepin maleate (Hoffman La Roche b.v., Mijdrecht, The Netherlands); metergoline (Farmitalia, Milan, Italy); isoprenaline sulphate (Pharmacy Department, Erasmus University, Rotterdam, The Netherlands) and heparin sodium (Leo Pharmaceuticals, Weesp, The Netherlands) to prevent clotting of the catheters. GR127935 was solubilised according to the instructions of the supplier by the dispersion in distilled water to about 70°C for 10 s and then allowing to cool down to room temperature. The other antagonists were dissolved in distilled water with the following additives: 30% methanol (ritanserin and lisuride), 30% ethanol (metergoline), 4% ascorbic acid (clozapine), 25% propylene glycol (methiothepin); these vehicles had no effect on the haemodynamic variables or agonist-induced responses. The agonist drugs were dissolved in physiological saline. All doses refer to the respective salts, whereas those of 5-HT, 5-MeO-T and 5-CT refer to the free base.

10.3 Results

10.3.1 Blood pressure and heart rate responses to 5-HT, 5-CT, 5-MeO-T, sumatriptan and isoprenaline

Baseline values of mean arterial blood pressure and heart rate were 106±1 mmHg (n=98) and 361±9 beats min^{-1} (n=27), respectively. As depicted in Figure 10.1 (*upper panel*), i.v. bolus injections of 5-HT, 5-CT, 5-MeO-T and isoprenaline, but not of sumatriptan, produced dose-dependent decreases in mean arterial blood pressure. The blood pressure effects, except with the highest dose of 5-CT, lasted 5-10 min. The rank order of agonist potency was 5-CT >> 5-HT ≥ 5-MeO-T, with sumatriptan being inactive. Heart rate was increased by isoprenaline, but no consistent changes were observed with the other four agonists (Figure 10.1; *lower panel*). Heart rate changes were not analysed further.

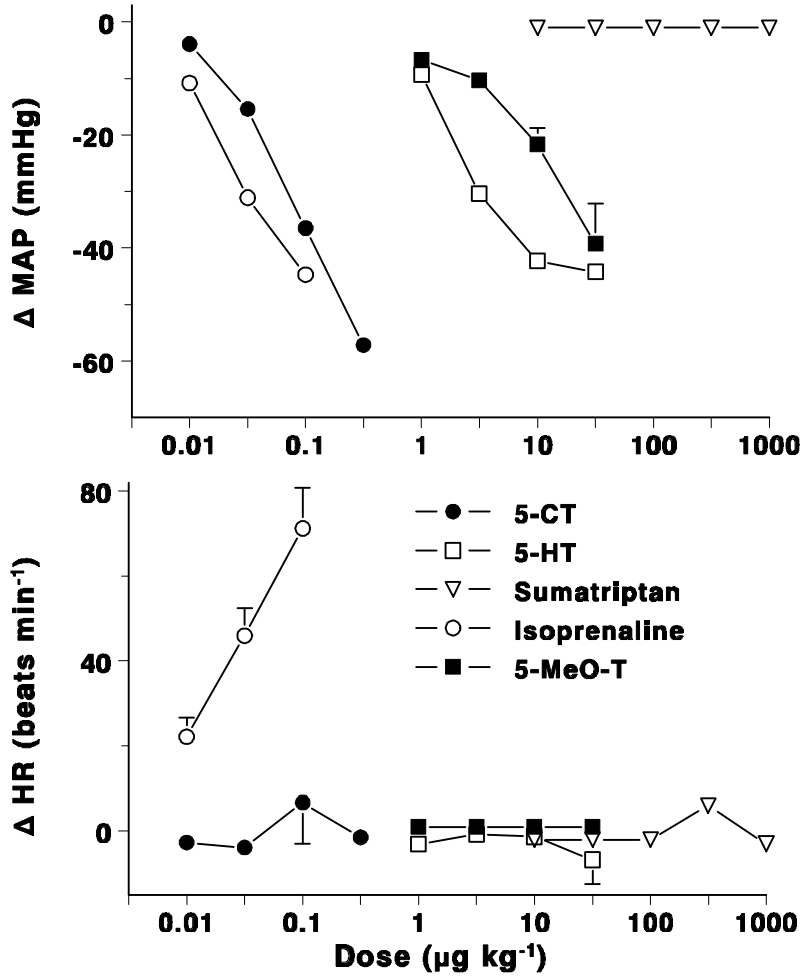

Figure 10.1 Peak changes in mean arterial blood pressure (MAP) and heart rate (HR; with group sizes for MAP and HR changes in brackets) by i.v. bolus injections of 5-CT (n=42 and 7), 5-HT (n=44 and 12), sumatriptan (n=6 and 2), isoprenaline (n=44 and 12) and 5-MeO-T (n=6 and 6) in vagosympathectomised, anaesthetised rats. Animals receiving 5-HT and isoprenaline were pretreated with 50 µg kg^{-1} of ritanserin (i.v.).

10.3.2 Hypotensive responses to 5-HT, 5-CT and isoprenaline in animals treated with physiological saline

As shown in Figure 10.2, the hypotensive responses induced by 5-HT and 5-CT were not much affected by treatment with physiological saline, although after the third administration of saline the changes induced by the lower doses of 5-CT were slightly, but significantly, attenuated. The isoprenaline-induced changes in blood pressure were significantly attenuated, especially after the third administration of saline.

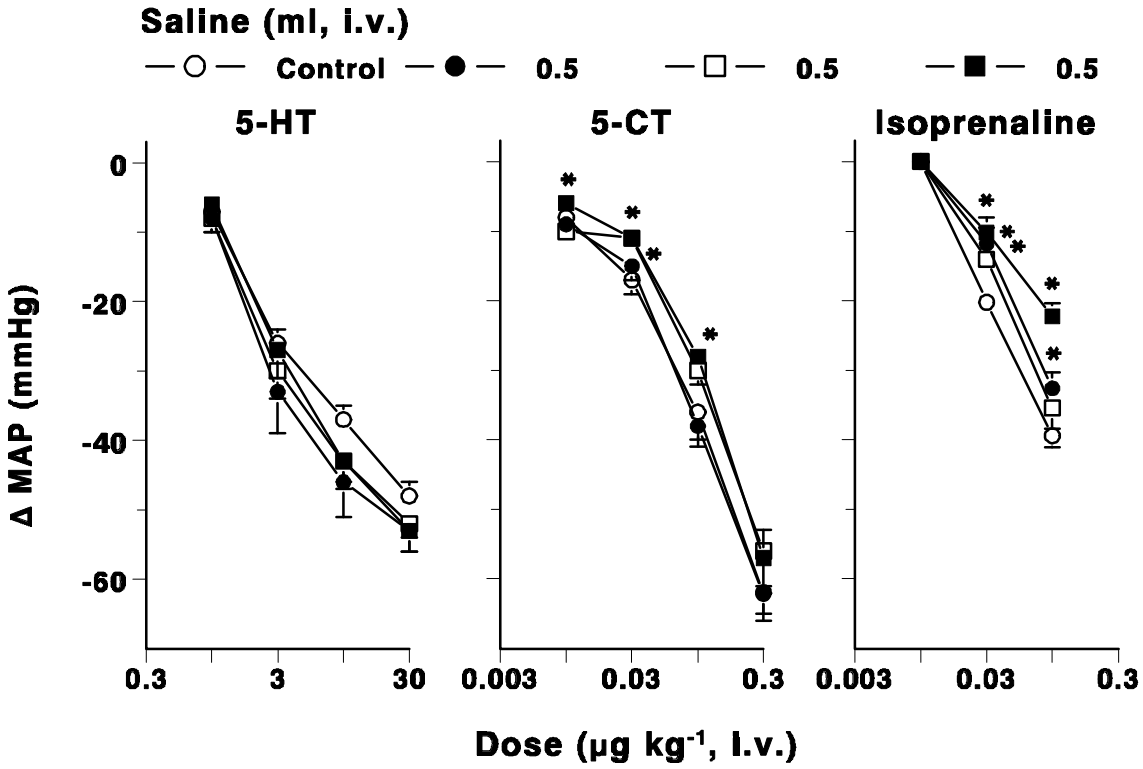

Figure 10.2 Hypotensive responses to 5-HT (n=5), 5-CT (n=6) and isoprenaline (n=5) before (Control) and after 3 i.v. administrations of 0.5 ml of saline in vagosympathectomised, anaesthetised rats. Animals receiving 5-HT and isoprenaline were pretreated with 50 µg kg^{-1} of ritanserin (i.v.). *, $P<0.05$ vs control.

10.3.3 Effects of saline or 5-HT receptor antagonists per se on systemic haemodynamics

While three consecutive i.v. administrations of saline (0.5 ml) or GR127935 (300-3000 µg kg^{-1}) did not produce any changes in blood pressure or heart rate, lisuride, mesulergine, metergoline, methiothepin and clozapine elicited a variable pressor response only in animals receiving 5-CT (data not given). This increase in blood pressure is apparently related to the antagonism of the long-lasting hypotensive effect of the highest dose (0.3 µg kg^{-1}) of 5-CT injected before the antagonists, since such an increase was not observed in animals receiving 5-HT or those treated with GR127935, which did not antagonise the effects of 5-CT (see below).

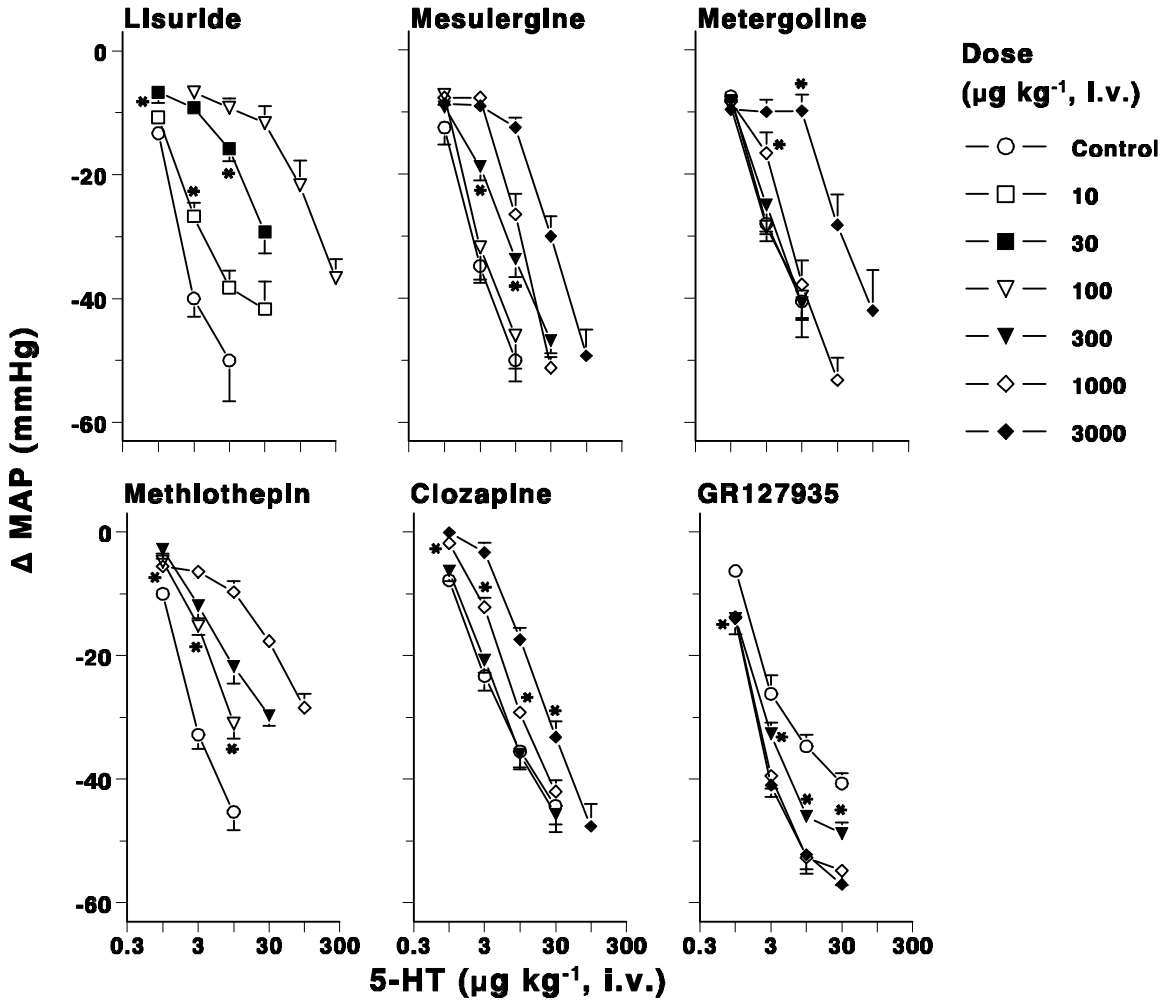

Figure 10.3 Hypotensive responses to 5-HT before (Control) and after different doses of 5-HT receptor antagonists (n=6 each, except for methiothepin n=9) in vagosympathectomised, anaesthetised rats, pretreated with 50 µg kg^{-1} of ritanserin (i.v.). *, P<0.05 vs control. All the graphs after the starred (*) graph are also significantly different from control.

10.3.4 Effects of several antagonists on the 5-HT-induced hypotensive responses

As shown in Figure 10.3, the responses to 5-HT were antagonised to varying degrees by lisuride, mesulergine, metergoline, methiothepin and clozapine, but the 5-HT$_{1B/D}$ receptor antagonist GR127935 enhanced 5-HT-induced responses. The rank order of antagonist potency of the different ligands against 5-HT was: lisuride > methiothepin ≥ mesulergine > clozapine = metergoline, with GR127935 inactive (Table 10.3).

Table 10.3 Effect of saline or several antagonists (i.v.) on the dose of 5-HT needed to decrease blood pressure by 30 mmHg (ED_{30}). Dose ratios, with baseline values set to 1, are given in brackets.

Dose ($\mu g\ kg^{-1}$)	***Saline***[a]	***Lisuride***	***Mesulergine***	***Metergoline***	***Methiothepin***	***Clozapine***	***GR127935***
Baseline	5.8±0.7 (1)	2.3±0.2 (1)	3.0±0.5 (1)	4.9±0.9 (1)	3.8±0.6 (1)	7.8±1.7 (1)	4.9±0.3 (1)
10	4.3±1.3 (0.7±0.3)	5.3±0.8* (2.3±0.4)	-	-	-	-	-
30	4.6±1.0 (0.8±0.2)	45.5±13.2* (17.8±3.9)	-	-	-	-	-
100	4.7±0.8 (0.8±0.1)	175.9±50.3* (82±29.3)	4.3±0.8 (1.5±0.2)	4.9±0.7 (1.1±0.0)	11.8±2.4* (4.1±0.9)	-	-
300	-	-	7.6±0.5* (3.1±0.6)	6.5±1.7 (1.3±0.3)	28.4±6.3* (9.9±2.6)	7.8±1.1 (1.1±0.2)	5.1±0.4 (1.1±0.1)
1000	-	-	10.6±0.9* (4.4±1.1)	7.3±1.4* (1.6±0.1)	128.1±31.8* (34.8±9.3)	11.9±1.6 (1.8±0.4)	4.7±0.3 (1.0±0.1)
3000	-	-	32.6±4.4* (12.3±1.9)	51.6±15.9* (12.1±3.1)	-	22.8±3.1* (3.5±0.6)	4.4±0.2 (0.9±0.1)
"pA_2"	-	7.7±0.1	6.4±0.2	5.6±0.1	6.8±0.2	5.7±0.2	-

Values are presented as the mean±s.e.mean. *, $P<0.05$ vs Control. [a], 3 consecutive infusions of saline (0.5 ml)

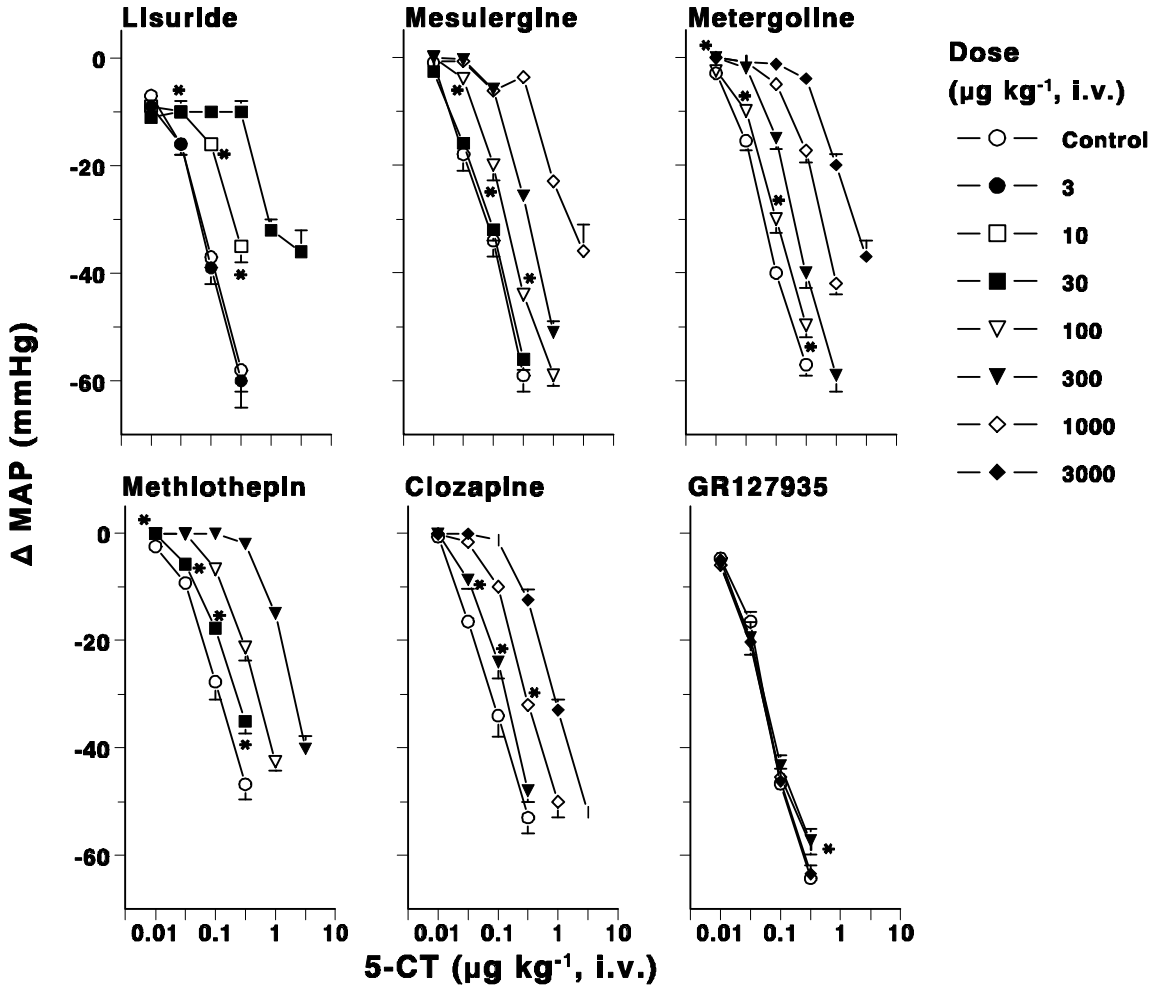

Figure 10.4 Hypotensive responses to 5-CT before (Control) and after different doses of 5-HT receptor antagonists (n=6 each) in vagosympathectomised, anaesthetised rats. *, P<0.05 vs control. All the graphs after the starred (*) graph are also significantly different from control.

10.3.5 Effects of several antagonists on the 5-CT-induced hypotensive responses

As shown in Figure 10.4, the responses to 5-CT were antagonised by lisuride, mesulergine, metergoline, methiothepin and clozapine, whereas GR127935 only slightly attenuated the effects of the highest dose of 5-CT. The rank order of antagonist potency against 5-CT was: lisuride > methiothepin ≥ mesulergine = metergoline > clozapine (Table 10.4).

Table 10.4 Effect of saline or several antagonists (i.v.) on the dose of 5-CT needed to decrease blood pressure by 30 mmHg (ED_{30}). Dose ratios, with baseline values set to 1, are given in brackets.

Dose ($\mu g\ kg^{-1}$)	***Saline***[a]	***Lisuride***	***Mesulergine***	***Metergoline***	***Methiothepin***	***Clozapine***	***GR127935***
Baseline	0.06±0.01 (1)	0.06±0.01 (1)	0.07±0.01 (1)	0.06±0.00 (1)	0.12±0.02 (1)	0.08±0.01 (1)	0.05±0.00 (1)
3	0.07±0.01 (1.2±0.2	0.05±0.01 (0.9±0.1)	-	-	-	-	-
10	0.09±0.01* (1.7±0.3)	0.2±0.03* (6.2±2.7)	-	-	-	-	-
30	0.09±0.01* (1.7±0.2)	2.79±1.04* (48.3±17.7)	0.07±0.00 (1.1±0.1)	-	0.28±0.04* (2.8±0.6)	-	-
100	-	-	0.16±0.01* (2.6±0.4)	0.09±0.01* (1.6±0.2)	0.47±0.05* (4.7±1.0)	-	
300	-	-	0.36±0.02* (5.9±0.8)	0.19±0.02* (3.3±0.3)	1.97±0.22* (19.3±2.7)	0.12±0.01* (1.5±0.1)	0.05±0.00 (1.1±0.1)
1000	-	-	1.63±0.2* (26.1±4.3)	0.56±0.05* (9.6±1.0)	-	0.30±0.02* (4.2±0.5)	0.05±0.00 (1.0±0.1)
3000	-	-	-	2.08±0.26* (36.0±4.9)	-	0.86±0.09* (12.4±2.3)	0.04±0.00 (1.0±0.1)
"pA_2"	-	7.8±0.1	6.6±0.1	6.4±0.1	7.0±0.1	5.8±0.1	-

Values are presented as the mean±s.e.mean. *, P<0.05 vs Control. [a], 3 consecutive infusions of saline (0.5 ml)

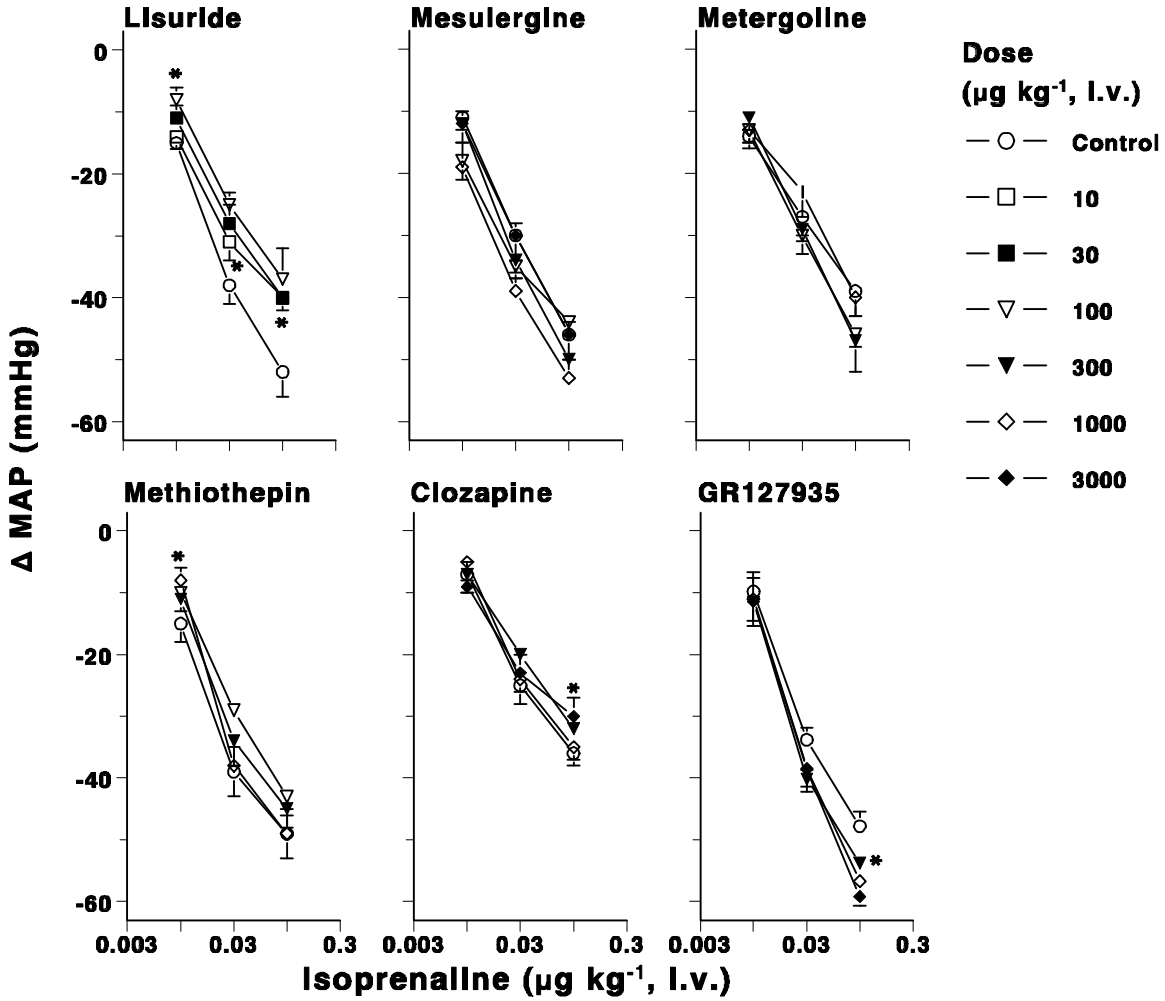

Figure 10.5 Hypotensive responses to isoprenaline before (Control) and after different doses of 5-HT receptor antagonists (n=6 each, except for methiothepin n=9) in vagosympathectomised, anaesthetised rats, pretreated with 50 µg kg^{-1} of ritanserin (i.v.). *, P<0.05 vs control. All the graphs after the starred (*) graph are also significantly different from control.

10.3.6 Effects of several antagonists on the isoprenaline-induced hypotensive responses

As shown in Figure 10.5, the isoprenaline-induced hypotensive responses were clearly attenuated only by lisuride; the other drugs, except for a slight reduction (at one agonist and antagonist dose) by methiothepin and clozapine, did not cause any reduction in the responses elicited by isoprenaline. As was the case with 5-HT, isoprenaline-induced responses were also slightly enhanced by GR127935.

10.4 Discussion

10.4.1 Effect of 5-HT receptor agonists on mean arterial blood pressure

As described earlier (Kalkman *et al*., 1984; Saxena & Lawang, 1985; Martin *et al*., 1987), the present results show that 5-HT receptor agonists dose-dependently decrease blood pressure; these responses were reproducible as they remained essentially unchanged in control animals receiving saline. The apparent rank order of potency was: 5-CT >> 5-HT ≥ 5-MeO-T. This characteristic is in keeping with the involvement of 5-HT_1-like receptors (see Table 10.1), which have been proposed to mediate depressor responses (Kalkman *et al*., 1984; Saxena & Lawang, 1985; Martin *et al*., 1987). Notwithstanding, it was already recognised before (Saxena & Lawang, 1985; Martin *et al*., 1987), that the responses mediated by 5-HT_1-like receptors could be subdivided into at least two operationally distinct receptors, mainly on the basis of their differential sensitivity to the action of 5-HT_1-like receptor agonists, such as 8-OH-DPAT, indorenate, RU24969 and/or sumatriptan (e.g. Saxena & Villalón, 1990; Den Boer *et al*., 1991b; Villalón *et al*., 1993b; 1995a; 1995c). Interestingly, recent studies have provided evidence that several of these 5-HT_1-like receptor-mediated responses are, in fact, identical to the 5-HT_{1B} receptor. The latter is mainly based on the agonism by sumatriptan and potent antagonism by the selective 5-$HT_{1B/1D}$ receptor ligand, GR127935 and the 5-HT_{1B} receptor antagonist, SB224289, but lack of effect by the 5-HT_{1D} receptor antagonist BRL15572 (see Chapters 4, 8 and 9; Villalón *et al*., 1996). In marked contrast, the present experiments show that sumatriptan did not mimic nor did it antagonise (data not shown) the effects of 5-HT and 5-CT, thereby excluding the involvement of 5-$HT_{1B/1D}$ receptors. Notwithstanding, the agonists in the present study display a pharmacological profile that closely parallels that of cloned 5-ht_7 receptors (see Table 10.1).

As shown in Chapter 3, 50 μg kg^{-1} of ritanserin proved to be sufficient to prevent the 5-HT-induced pressor responses. We have decided not to administer ritanserin to the animals receiving 5-CT, as the latter compound does not produce pressor responses in rats (Saxena & Lawang, 1985; Saxena & Villalón, 1990). However, in view of the appreciable affinity (pK_i: 7.2) displayed by ritanserin at 5-ht_7 receptors (Lovenberg *et al*., 1993), theoretically this compound could have affected the 5-HT-induced dose-response curves. Notwithstanding, this does not seem to be the case, since (i) 100 μg kg^{-1} of ritanserin did not affect the 5-ht_7 receptor-mediated

dilatation of the canine external carotid vasculature by 5-HT, 5-CT and 5-MeO-T (Villalón *et al*., 1997a), and (ii) 50 µg kg^{-1} of ritanserin did not affect rat hypotensive responses induced by 1, 3, 10 and 30 µg kg^{-1} of 5-MeO-T (4.2±1.1, 8.0±1.3, 18±0.6 and 28.8±3.6 mmHg before and 4.2±1.1, 5.2±0.5, 14.5±1.9 and 31.5±4.1 mmHg after ritanserin, respectively; Villalón, unpublished).

10.4.2 Do rat depressor responses involve any of the known 5-HT$_1$ receptor subtypes?

Although the above agonist profile already excludes sumatriptan-sensitive 5-HT$_1$-like as well as 5-HT$_{1B/1D}$ receptors (see above), the 5-HT$_1$ receptor family includes several other subtypes (5-HT$_{1A}$, 5-ht$_{1E}$ and 5-HT$_{1F}$, see Chapter 1). However, the involvement of the above 5-HT$_1$ subtypes in the present study is not likely because 5-CT (with a very high potency), but not sumatriptan, mimicked 5-HT in eliciting hypotension, whereas 5-CT and sumatriptan display low and high affinities at the 5-ht$_{1F}$ receptor (Table 10.1). This suggestion is reinforced when considering that: (i) mesulergine and clozapine, which specifically antagonised the responses to 5-HT and 5-CT in our experiments, do not bind potently to the 5-HT$_1$ receptor subtypes (Table 10.2) and (ii) the 5-HT$_1$ family is, by definition, negatively coupled to adenylyl cyclase (Hoyer *et al*., 1994), leading to a decrease in cAMP, which is usually associated with vasoconstriction and hypertension, not hypotension (Rand *et al*., 1987).

10.4.3 Lack of resemblance of the rat 5-HT receptor mediating hypotension with either 5-HT$_2$, 5-HT$_3$, 5-HT$_4$, 5-ht$_5$ or 5-ht$_6$ receptors

The pharmacological profile of the 5-HT receptors mediating hypotension in the vagosympathectomised, ritanserin-treated rat also seems to be inconsistent with a 5-HT$_2$, 5-HT$_3$, 5-HT$_4$ or 5-ht$_6$ classification on the basis of: (i) the high potency of 5-CT relative to 5-HT (present results; Saxena & Lawang, 1985), an order which is reversed for the aforementioned types (Table 10.1); (ii) insensitivity to antagonism by high doses of the 5-HT$_2$ receptor antagonists ketanserin, cyproheptadine (Saxena & Lawang, 1985) and ritanserin (De Vries *et al*., 1997a); (iii) resistance to antagonism by MDL72222 (Dalton *et al*., 1986) at doses that block the 5-HT$_3$ receptor-mediated von Bezold-Jarisch reflex; (iv) the ability of 5-MeO-T to induce depressor responses in rats, a compound that is inactive at 5-HT$_3$ receptors (Table 10.1); (v) the antagonism of hypotensive responses by methiothepin,

mesulergine and metergoline, which are inactive at 5-HT_4 receptors (see Table 10.2); (vi) the potent blockade by mesulergine, a compound displaying low affinity for 5-ht_6 receptors as compared to 5-ht_7 receptors (Table 10.2).

Interestingly, 5-CT (Table 10.1) and methiothepin (Table 10.2) display a relatively high affinity for the recombinant 5-ht_{5A} and 5-ht_{5B} receptors. This, therefore, raises the question whether the cloned 5-ht_5 receptors are related to the 5-HT receptors mediating depressor responses in the rat. However, the potent antagonism by mesulergine and metergoline, which do not seem to interact with 5-ht_{5A} and 5-ht_{5B} receptors (see Table 10.2), does not support this possibility. Consistent with the above, it is worthy of note that no *m*RNA expression for 5-HT_{1A}, 5-HT_{1D}, 5-HT_{1F}, 5-HT_{2C}, 5-HT_4, 5-$ht_{5A/B}$ and 5-ht_6 receptors was detected in rat blood vessels. In contrast, 5-ht_7 receptor *m*RNA, as well as that of 5-HT_{1B}, 5-$HT_{2A/B}$ receptors, was strongly expressed (Ullmer *et al*., 1995).

10.4.4 Resemblance of the rat 5-HT receptor mediating hypotension to cloned 5-ht_7 receptors

In keeping with the agonist profile found in the present study, and the unlikely involvement of 5-HT_1-5ht_6 receptors (see above), the different antagonists displayed a pharmacological profile against the 5-HT- and 5-CT-induced depressor responses that is consistent with the involvement of functional 5-ht_7 receptors. Indeed, all compounds used in the present study, displaying high affinities at cloned 5-ht_7 receptors, antagonised the depressor responses. Furthermore, we calculated apparent pA_2 values for the antagonists against 5-CT- and 5-HT-induced depressor responses. Admittedly, to apply Schild analysis to *in vivo* data, we had to assume equilibrium conditions of the compounds under study, for which we cannot provide supporting evidence in the present experimental set-up. However, recognising the limitations of calculating pA_2 values in an *in vivo* setting, we believe that it is still an appropriate and illustrative approximation, which provides a useful apparent rank order of antagonist potency. The rank order of antagonist potency (with apparent pA_2 values against 5-HT- and 5-CT-induced depressor responses, respectively) obtained in the present study was lisuride (7.7; 7.8) > methiothepin (6.8; 7.0) ≥ mesulergine (6.4; 6.6) > clozapine (5.7; 5.8), while metergoline displayed variable potencies (5.6; 6.4) and GR127935 was inactive. This order of potency resembles the order of pK_i values against cloned rat 5-ht_7 receptors, which is: methiothepin (9.4) ≥ lisuride (9.1) >

metergoline (8.2) > mesulergine (7.7) ≥ clozapine (7.4) >> GR127935 (6.2). Since the potencies of these compounds were almost equal in groups of animals receiving 5-HT or 5-CT, a common site of action is implied. However, metergoline seemed to be a more potent antagonist against 5-CT than it was against 5-HT-induced depressor responses (apparent pA_2 values of 6.4 and 5.6, respectively). Although we have no direct explanation for this, it should be noted that the reported pK_i values for metergoline against rat cloned 5-ht$_7$ receptors also vary (7.2-8.2) (Lovenberg *et al*., 1993; Ruat *et al*., 1993b; Shen *et al*., 1993).

Except for lisuride, which attenuated isoprenaline-induced decreases in blood pressure, all compounds acted as specific antagonists. GR127935, a selective 5-HT$_{1B/1D}$ receptor antagonist (Table 10.2), was devoid of blocking properties against the depressor responses. Combined with the lack of agonism by sumatriptan, this property clearly shows that the hypotensive responses are not mediated by 5-HT$_{1\text{-like}/1B/1D}$ receptors. Interestingly, GR127935 potentiated the depressor responses induced by 5-HT, most probably due to the moderate blocking properties of this compound at rat vasopressor 5-HT$_2$ receptors (Chapter 3). Although the rats were systematically pretreated with ritanserin, a part of the pressor response, though present, may have been overshadowed by the hypotensive effect. Admittedly, a (small, but significant) potentiating effect was also observed with isoprenaline, but to a much lesser extent. In keeping with the above explanation, GR127935 did not modify hypotensive responses induced by 5-CT, a compound devoid of interactions with 5-HT$_{2A}$ receptors.

In the light of the above lines of pharmacological evidence, the involvement of putative 5-ht$_7$ receptors mediating the late hypotensive response to 5-HT in vagosympathectomised rats is substantiated, as recently shown for other functional 5-ht$_7$ receptors, both in *in vitro* (Carter *et al*., 1995; Martin & Wilson, 1995; Leung *et al*., 1996; Hirst *et al*., 1997) as well as in *in vivo* (see Chapter 12; Villalón *et al*., 1997a; 1997c) studies.

10.4.5 Considerations of other possible mechanisms involved in the 5-HT-induced late hypotensive response in vagosympathectomised rats

In the present experiments we deliberately made use of an intact rat model; only bilateral vagosympathectomy was performed to avoid the von Bezold-Jarisch reflex (Paintal, 1973). The advantage of this preparation, compared to the frequently utilised

pithed rat model, is that a more physiological (normotensive) condition is approximated. On the other hand, the main disadvantage is that several mechanisms could contribute simultaneously to the hypotensive action of 5-HT receptor agonists. Indeed, four major mechanisms are believed to play a role in 5-HT receptor-mediated vasorelaxation and hypotension, namely (i) inhibition of central vasomotor *loci*, (ii) endothelial NO release, (iii) prejunctional inhibition of sympathetic outflow, and (iv) direct vascular smooth muscle relaxation (Saxena & Villalón, 1990). Nevertheless, as 5-HT, 5-MeO-T and 5-CT are not able to cross readily the blood-brain barrier, the first of these mechanisms can be excluded in the present experimental set-up. The second mechanism is similarly unlikely to play a major role, as the NO synthetase inhibitor L-NAME does not attenuate 5-HT- or 5-CT-induced depressor responses in pithed rats (Van Gelderen & Saxena, 1992). Additionally, the 5-HT receptors found to be involved in endothelium dependent vasorelaxation (Martin *et al*., 1987; Schoeffter & Hoyer, 1990; Glusa & Richter, 1993) display different pharmacological profiles, compared to the one found in the present study. Thirdly, the inhibition of sympathetic vasopressor responses in pithed rats by 5-HT is mediated via prejunctional 5-HT_1-like receptors probably resembling 5-$HT_{1A/1B/1D}$ receptors, on the basis of agonism by sumatriptan, as well as antagonism of this response by GR127935 (Chapter 13) and, consequently, this mechanism does not seem to be involved in dilatation of the rat systemic vasculature either. Taking the above into account, it is suggested that the hypotensive response to 5-HT in vagosympathectomised, normotensive rats is predominantly mediated directly via vascular smooth muscle receptors, that closely resemble cloned 5-ht_7 receptors. This suggestion is strengthened when considering that (i) the receptor mediating vasorelaxation in endothelium denuded blood vessels displays similar pharmacological profiles as obtained in the present study (Martin *et al*., 1987; Martin & Wilson, 1995; Leung *et al*., 1996) and, most significantly (ii) 5-ht_7 receptor *m*RNA is strongly expressed in vascular smooth muscle cells (Ullmer *et al*., 1995). Finally, it is interesting to note that the fall in systemic peripheral resistance during this putative 5-ht_7 receptor-mediated hypotension is almost exclusively confined to the skeletal muscle vascular beds (Chapter 11).

In conclusion, on the basis of the results presented in this study, the late depressor response in vagosympathectomised, normotensive rats, previously described to be

mediated by 5-HT$_1$-like receptors (Saxena & Lawang, 1985; Martin *et al*., 1987), appear to be mediated by 5-ht$_7$ receptors, most probably located on vascular smooth muscle. Our findings are in agreement with other *in vivo* studies, where functional 5-ht$_7$ receptors have been described, including tachycardia in cats (Chapter 12) and canine external carotid vasodilatation (Villalón *et al*., 1997a), responses that were previously attributed to 5-HT$_1$-like receptors. Since these responses represent functional correlates of the 5-ht$_7$ gene product, the 5-HT$_7$ (upper case) receptor appellation is reinforced.

Chapter 11

Changes in systemic and regional haemodynamics during 5-HT_7 receptor mediated depressor responses in rats

Summary The 5-HT-induced late depressor response in rats is mainly mediated by vascular 5-HT_7 receptors. The present study was devoted to determine the systemic and regional haemodynamic changes during this response, with particular emphasis on localising vascular beds that may contribute to the increase in total systemic vascular conductance. In vagosympathectomised, pentobarbital anaesthetised rats pretreated with the 5-HT_2 receptor antagonist ritanserin (50 µg kg^{-1}, i.v.), 5-HT (1, 3 and 10 µg kg^{-1} min^{-1} during 10 min; i.v.) produced a dose-dependent decrease in mean arterial blood pressure by up to 46±3%. This decrease was accompanied by increases in systemic vascular conductance by up to 83±15%; cardiac output was unaffected. 5-HT increased regional vascular conductance in skeletal muscle, carcass, mesentery/pancreas and adrenals by up to 740±141, 117±18, 135±26 and 88±22%, respectively, but decreased "lung" (mainly arteriovenous anastomotic) conductance by up to 81±2%. Pretreatment with R(+)lisuride (100 µg kg^{-1}, i.v.) abolished all 5-HT-induced systemic and regional haemodynamic effects. In contrast, i.v. pretreatment with S(-)lisuride (100 µg kg^{-1}) or GR127935 (300 µg kg^{-1}) did not affect the 5-HT-induced systemic haemodynamic changes. The above results suggest that hypotension induced via 5-HT_7 receptor activation was exclusively caused by vasodilatation of the systemic vasculature, confined to skeletal muscle, carcass, mesentery/pancreas and adrenal vascular beds. Furthermore, this study shows that blockade of vasorelaxant 5-HT_7 receptors by lisuride is stereoselective.

11.1 Introduction

The complexity of cardiovascular effects (bradycardia or tachycardia, hypotension or hypertension and vasodilatation or vasoconstriction) produced by 5-HT has been explained by its capacity to interact with specific receptors (see Saxena & Villalón, 1990; 1991; Martin, 1994; Jones *et al.*, 1995). These receptors include 5-HT_1 (5-HT_{1A}, 5-HT_{1B} and/or 5-HT_{1D} subtypes), 5-HT_2 (5-HT_{2A}, 5-$HT_{2B/2C}$), 5-HT_3, 5-HT_4 as well as 5-HT_7 receptors (see Chapter 1).

The cardiovascular response induced by 5-HT in the rat serves as an example to illustrate the above complexity. Thus, i.v. administration of 5-HT produces a triphasic blood pressure response in anaesthetised rats with intact vagus nerves. This

Based on: De Vries, P., De Visser, P.A., Heiligers, J.P.C., Villalón, C.M., & Saxena, P.R. (1999). Changes in systemic and regional haemodynamics during 5-HT_7 receptor mediated depressor responses in rats. *Br. J. Pharmacol.*, **359**, 331-338.

response consists of an initial hypotension associated with a brief, but intense, bradycardia via the von Bezold-Jarisch reflex (mediated by 5-HT$_3$ receptors located on cardiac vagal afferents), followed by a vasopressor effect (mediated by vascular 5-HT$_{2A}$ receptors) and, finally, a longer-lasting hypotension (see Chapter 3; Kalkman *et al*., 1984; Saxena & Lawang, 1985). After bilateral vagosympathectomy and pretreatment with ketanserin, ritanserin or cyproheptadine (5-HT$_{2A}$ receptor antagonists), 5-HT exclusively produces the late depressor response (see Chapters 3 and 10; Saxena & Lawang, 1985). Although this 5-HT-induced hypotensive response had initially been ascribed to an action at vascular 5-HT$_1$-like receptors (Martin *et al*., 1987; Saxena & Villalón, 1990), it was subsequently concluded that these receptors closely resemble the pharmacological profile of the 5-HT$_7$ receptors (Chapter 10). This conclusion was based, amongst other findings, on: (i) the inactivity of the 5-HT$_{1B/1D}$ receptor agonist, sumatriptan; (ii) the blockade by mesulergine and clozapine, compounds that do not interact with the 5-HT$_1$ receptor family (see Table 10.2); and (iii) the resistance to blockade by GR127935, a selective 5-HT$_{1B/1D}$ receptor antagonist (Skingle *et al*., 1996). In the light of these findings, the present study was designed to further analyse this late hypotensive response, with particular emphasis on ascertaining the regional blood flows responsible for the 5-HT-induced increase in systemic vascular conductance in the rat. For this purpose, the distribution of cardiac output to the different tissues during 5-HT-induced increase in systemic vascular conductance was determined using the radioactive microsphere technique (Saxena *et al*., 1980). In order to confirm the involvement of 5-HT$_7$ receptors, we decided to use: (i) the stereoisomers of lisuride [R(+) and S(-)], to investigate whether the blockade of 5-HT$_7$ receptors by this ergot derivative is stereoselective; and (ii) GR127935, which fails to antagonise 5-HT-induced hypotension in rats (Chapter 10).

11.2 Methods

11.2.1 General

Experiments were carried out in 26 male Wistar rats (300-330 g). After initial anaesthesia with ether, the trachea was cannulated and a catheter was placed in the right external jugular vein. At this point, ether anaesthesia was stopped and the animals received i.v. bolus injections of pentobarbital (30-40 mg kg^{-1}). Subsequently, the rats were artificially ventilated with a mixture of oxygen and room air using a

respiratory pump (Infant ventilator MK3, Hoekloos, The Netherlands) at a rate of 40 strokes min^{-1} (volume: 20 ml kg^{-1}). Bilateral vagosympathectomy was performed to avoid the bradycardia and hypotension caused by the von Bezold-Jarisch reflex (Paintal, 1973). The right common carotid artery was exposed and a catheter, connected to a pressure transducer (Combitrans disposable pressure transducer, Braun, Melsungen, Germany), was guided through the carotid artery into the left ventricle. The presence of the tip of the catheter in the left ventricle was confirmed by the observation of the sudden switch from an arterial to a ventricular pressure profile. Additionally, a catheter was placed into the left femoral artery and connected to another pressure transducer for the recording of blood pressure and for the withdrawal of reference blood samples. Heart rate was measured with a tachograph (CRW, Erasmus University, Rotterdam, The Netherlands) triggered from electrocardiogram signals. Both blood pressure and heart rate were recorded simultaneously on a polygraph (CRW, Erasmus University, Rotterdam, The Netherlands). The right external jugular vein was used for the administration of drugs.

11.2.2 Distribution of cardiac output

The distribution of aortic blood flow was determined with 15.5±0.1 (S.D.) μm diameter microspheres labelled with ^{141}Ce, ^{113}Sn, ^{103}Ru or ^{46}Sc (NEN Dupont, Boston, USA). For each measurement about 200,000 microspheres, suspended in 0.2 ml physiological saline and labelled with one of the isotopes, was mixed and injected into the left ventricle over a 15 sec period; the catheter was thoroughly flushed with 0.5 ml saline. Starting 10 sec before microsphere injection and lasting 70 sec, an arterial reference blood sample was drawn from the left femoral artery at a constant rate of 0.5 ml min^{-1}, using a withdrawal pump (Model 55, Harvard Apparatus, Natick, USA). At the end of the experiment the animal was sacrificed with an overdose of pentobarbital and all tissues and organs were dissected out, weighed and put in vials. The following tissues were studied: skeletal muscle, carcass (consisting of bone with skeletal muscle residue, fat, tail, eyes and urogenital tract), mesentery/pancreas (for practical reasons, these two tissues were not studied separately), adrenals, lungs, kidneys, skin, heart, liver, brain, gastrointestinal tract and spleen. The radioactivity in the reference blood samples and the tissues were counted for 5 min in a γ-scintillation counter (Packard, Minaxi Auto-Gamma 5000 series), using suitable windows

discriminating different isotopes. All data were processed by a set of specially designed computer programs (Saxena *et al*., 1980).

The cardiac output was calculated by multiplying the ratio of total and arterial blood sample radioactivity by the withdrawal rate of the arterial reference blood sample (0.5 ml min^{-1}). Accordingly, tissue blood flow was calculated by multiplying the ratio of tissue and total radioactivity by cardiac output (Saxena *et al*., 1980). Conductances were calculated as blood flow divided by the mean arterial blood pressure multiplied by hundred and expressed as 10^{-2} ml mmHg^{-1} min^{-1}.

11.2.3 Experimental protocols

The experiments were started after a stabilisation period of about 30 min. At this point, all animals were systematically pretreated with ritanserin (50 µg kg^{-1}, i.v.), which has been shown to be sufficient to block 5-HT$_{2A}$ receptor-mediated vasopressor responses (Chapter 3 and 10). The animals were then divided into 5 groups. In the first group (n=6), the effects of 3 consecutive 10-min i.v. infusions of vehicle (physiological saline; 0.1 ml min^{-1}) were studied to ascertain the stability of the preparation. The second group (control) received sequential 10-min i.v. infusions (at a rate of 0.1 ml min^{-1}) of 5-HT (1, 3 and 10 µg kg^{-1} min^{-1}; n=5), after pretreatment with saline (0.5 ml). In the last 3 groups (n=5 each), the effects of the above infusions of 5-HT were analysed in animals pretreated (i.v.) with either R(+)lisuride (100 µg kg^{-1}), S(-)lisuride (100 µg kg^{-1}) or GR127935 (300 µg kg^{-1}).

Fifteen min after respective pretreatments, baseline values of heart rate, mean arterial blood pressure, cardiac output and its distribution were determined. Subsequently, 10 min after the start of each of the infusions of vehicle or 5-HT all variables were reassessed. Between the different infusions, the animals were given about 15 min to recover. During this recovery period, the 5-HT-induced hypotension recovered completely (data not shown).

11.2.4 Data presentation and statistical analysis

All data are presented as the mean±s.e.mean. The changes induced by the different infusions of vehicle or agonists were calculated in each experiment. Data were compared to baseline values by the use of Duncan's new multiple range test, once an analysis of variance (randomised block design) had revealed that the samples represented different populations (Steel & Torrie, 1980). The changes caused by

5-HT in the R(+)lisuride-, S(-)lisuride- or GR127935-pretreated groups were compared to those in the control group by using Student's unpaired *t*-test. Statistical significance was accepted at $P<0.05$ (two-tailed).

11.2.5 Drugs

Apart from the anaesthetics, ether (Lab-scan, Dublin, Ireland) and pentobarbital (Apharmo, Arnhem, The Netherlands), the drugs used in this study were: GR127935 (Glaxo Group Research, Ware, UK; courtesy Dr. H.E. Connor); 5-HT creatinine sulphate (Sigma Chemical Company, St. Louis, MO, USA); ritanserin, S(-)lisuride and R(+)lisuride hydrogen maleate (RBI, Natick, USA); and heparin sodium (Leo Pharmaceuticals, Weesp, The Netherlands) to prevent clotting of the catheters. GR127935 was dissolved according to the instructions of the supplier by heating the dispersion in distilled water to about 70°C and then allowing to cool down to room temperature. All compounds were dissolved in physiological saline. When needed, 30% (v v^{-1}) methanol was added to dissolve ritanserin, S(-)lisuride and R(+)lisuride; this vehicle had no effect on the haemodynamic variables or the 5-HT-induced responses. All doses refer to the respective salts, whereas that of 5-HT refers to the free base.

11.3 Results

11.3.1 Baseline values of systemic and regional haemodynamic variables

Baseline values of systemic haemodynamic variables in the 26 vagosympathectomised rats pretreated with ritanserin were: heart rate, 231±5 beats min^{-1}; cardiac output, 111±5 ml min^{-1}; mean arterial blood pressure, 75±1 mmHg; diastolic arterial blood pressure, 56±1 mmHg; stroke volume, 0.48±0.02 ml; and systemic vascular conductance, 148±6 10^{-2} ml min^{-1} $mmHg^{-1}$. No significant differences were observed in these values between the 5 experimental groups. Furthermore, the i.v. pretreatment with ritanserin (50 µg kg^{-1}) as well as the subsequent i.v. administration of physiological saline (0.5 ml), R(+)lisuride (100 µg kg^{-1}), S(-)lisuride (100 µg kg^{-1}) or GR127935 (300 µg kg^{-1}) did not produce significant changes in these variables (data not shown).

Baseline values (n=26) of regional blood flow (ml min^{-1}; with the corresponding percentage of cardiac output in brackets) were: 15.4±1.6 (13.7±1.3%) for skeletal muscle, 26.2±1.5 (23.4±0.8%) for carcass, 1.6±0.1 (1.5±0.1%) for

mesentery, 0.16±0.01 (0.14±0.01%) for adrenals, 6.0±0.9 (5.3±0.7%) for lungs, 11.4±0.7 (10.6±0.8%) for kidneys, 8.1±0.4 (7.4±0.3%) for skin, 9.3±0.6 (8.3±0.4%) for heart, 2.4±0.2 (2.2±0.2%) for liver, 1.9±0.1 (1.7±0.1%) for brain, 27.1±1.6 (24.5±1.0%) for gastrointestinal tract (GIT) and 1.0±0.1 (0.9±0.1%) for spleen. No profound differences were observed in these values between the 5 experimental groups.

11.3.2 Effects of vehicle on systemic and regional haemodynamics

Three consecutive 10-min infusions of vehicle (saline; 0.1 ml min^{-1}) did not produce any changes in the systemic haemodynamic variables (Figure 11.1). Moreover, except for some decreases in the skin (data not shown) and skeletal muscles, vehicle infusion did not affect regional haemodynamics (Figure 11.1).

11.3.3 Effects of R(+)lisuride, S(-)lisuride and GR127935 on 5-HT-induced systemic haemodynamic changes

The effects of three consecutive 10-min i.v. infusions of 5-HT on systemic haemodynamics in the different experimental groups are depicted in Figure 11.2. 5-HT slightly decreased ($P<0.05$) heart rate (maximum change: -16±6%) and increased ($P<0.05$) stroke volume by up to 17±4%. These moderate changes were not significantly modified by pretreatment with R(+)lisuride, S(-)lisuride or GR127935. Cardiac output remained unchanged in all groups.

5-HT decreased mean and diastolic arterial blood pressures by up to 46±3% and 46±3%, respectively, and increased systemic vascular conductance (maximum change: 83±15%). These 5-HT-induced decreases in blood pressure and concomitant increases in systemic vascular conductance were essentially similar in animals pretreated with S(-)lisuride and GR127935, but, remarkably, were absent in animals pretreated with R(+)lisuride.

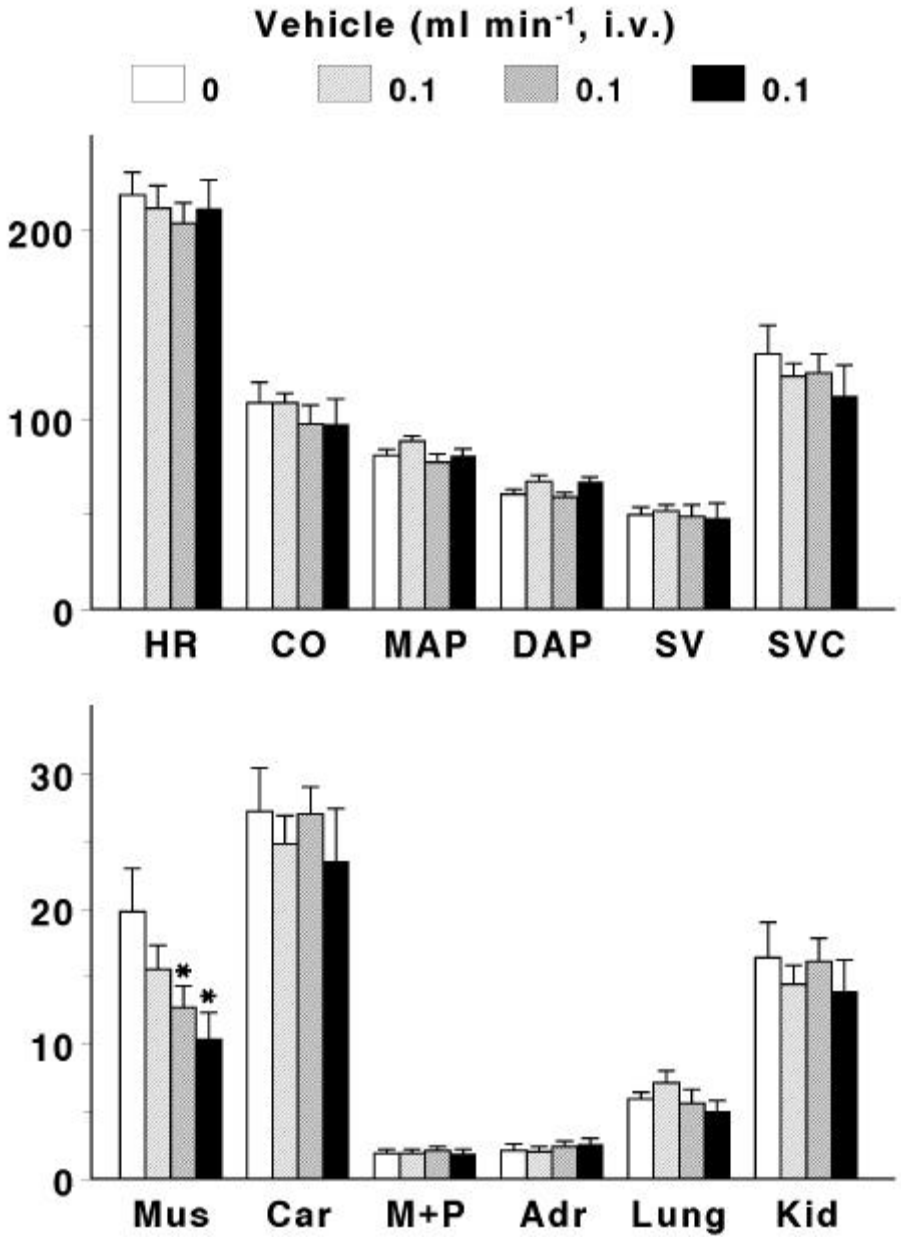

Figure 11.1 Effects of 3 consecutive 10-min i.v. infusions of vehicle (saline; 0.1 ml min^{-1}) on heart rate (HR; beats min^{-1}), cardiac output (CO; ml min^{-1}), mean arterial blood pressure (MAP; mmHg), diastolic arterial blood pressure (DAP; mmHg), stroke volume (SV; 10^{-2} ml), systemic vascular conductance (SVC; 10^{-2} ml $mmHg^{-1}$ min^{-1}) (*upper panel*) and skeletal muscle (Mus), carcass (Car), mesentery/pancreas (M+P), adrenal (Adr), lung and kidney (Kid) vascular conductances (10^{-2} ml $mmHg^{-1}$ min^{-1}) (*lower panel*) in anaesthetised rats systematically pretreated with ritanserin (50 µg kg^{-1}, i.v.). *, $P<0.05$ vs baseline. For the sake of clarity, adrenal vascular conductance values have been multiplied by a factor of 10.

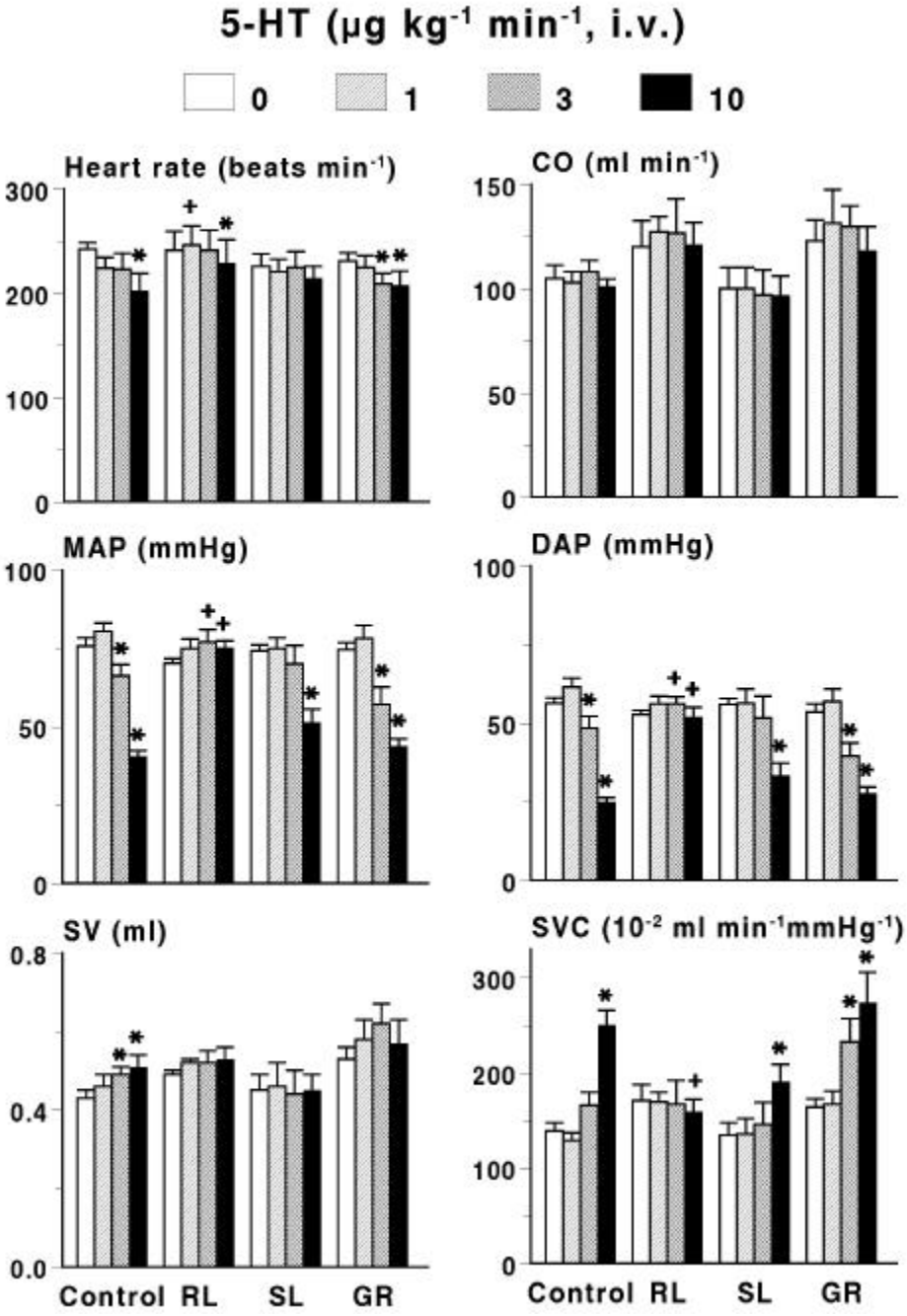

Figure 11.2 Effects of 10-min i.v. infusions of 5-HT on heart rate (HR), cardiac output (CO), mean arterial blood pressure (MAP), diastolic arterial blood pressure (DAP), stroke volume (SV) and systemic vascular conductance (SVC) in anaesthetised rats pretreated i.v. with either (n=5 each) saline (Control; 0.5 ml), R(+)lisuride (RL; 100 µg kg^{-1}), S(-)lisuride (SL; 100 µg kg^{-1}) or GR127935 (GR; 300 µg kg^{-1}). *, P<0.05 vs baseline. +, P<0.05 vs response by corresponding dose of 5-HT in control animals. All animals were systematically pretreated with ritanserin (50 µg kg^{-1}, i.v.).

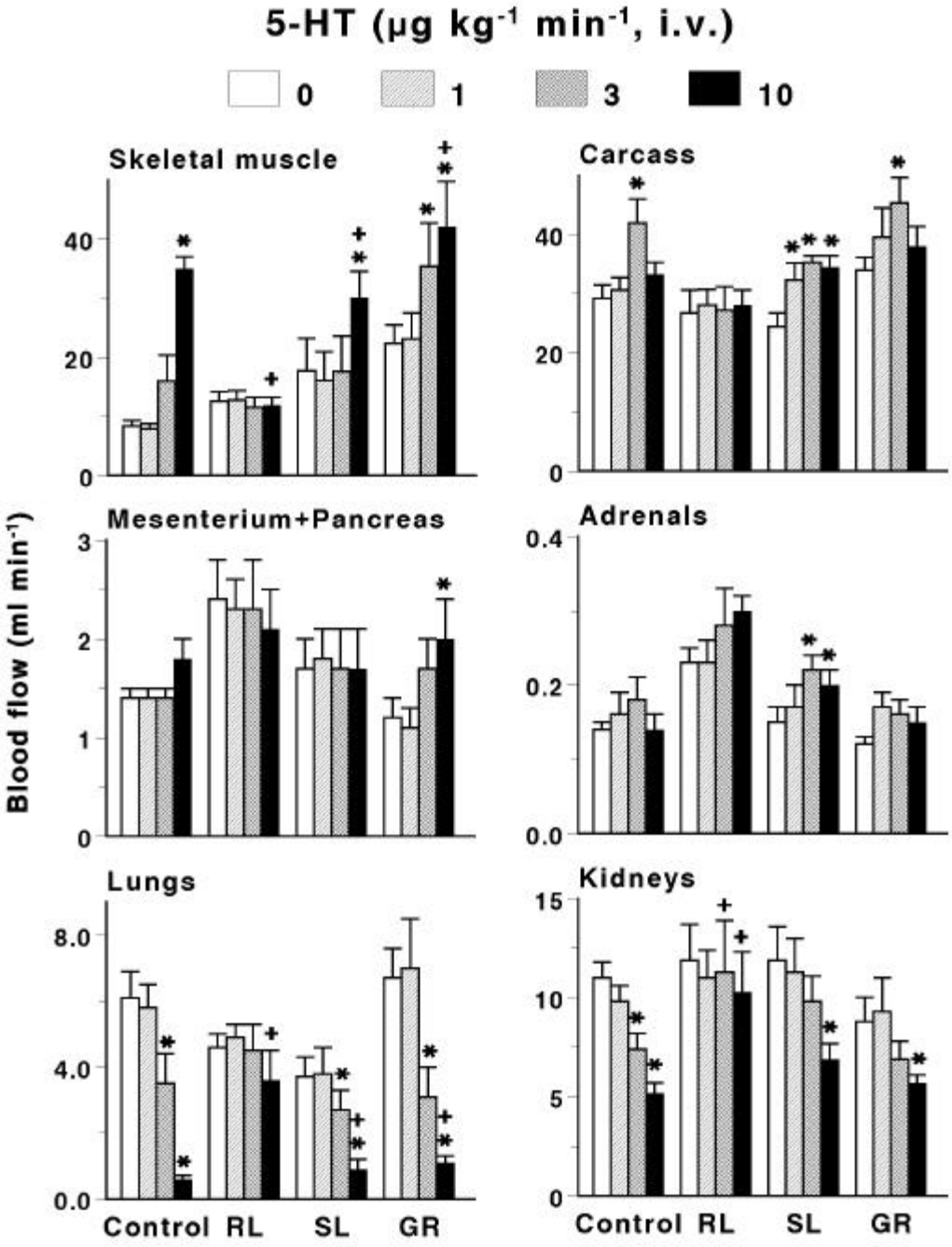

Figure 11.3 Effects of 10-min i.v. infusions of 5-HT on regional blood flow in anaesthetised rats pretreated i.v. with either (n=5 each) saline (Control; 0.5 ml), R(+)lisuride (RL; 100 µg kg^{-1}), S(-)lisuride (SL; 100 µg kg^{-1}) or GR127935 (GR; 300 µg kg^{-1}). *, $P<0.05$ vs baseline. +, $P<0.05$ vs response by corresponding dose of 5-HT in control animals. All animals were systematically pretreated with ritanserin (50 µg kg^{-1}, i.v.).

11.3.4 Effects of R(+)lisuride, S(-)lisuride and GR127935 on 5-HT-induced regional haemodynamic changes

The effects of three consecutive 10-min infusions (i.v.) of 5-HT (1, 3 and 10 μg kg^{-1} min^{-1}) on regional blood flows to a number of tissues in animals with or without (control) antagonist pretreatment are depicted in Figure 11.3. In control animals, 5-HT increased blood flows to skeletal muscle (maximum change: 346±67%) and carcass (maximum change: 47±19%), but decreased those to lungs (maximum change: 90±1%) and kidneys (maximum change: 53±6%). The increase in skeletal muscle blood flow by 5-HT may have been underestimated, since vehicle infusion by itself lowered skeletal muscle blood flow (see Figure 11.2). Blood flow changes elicited by 5-HT were potently blocked by pretreatment with R(+)lisuride, but remained essentially unchanged in animals pretreated with S(-)lisuride or GR127935.

The changes effected by 5-HT in vascular conductances in the above mentioned tissues are shown in Figure 11.4. 5-HT increased vascular conductance in the skeletal muscles (maximum change: 740±141%), carcass (maximum change: 117±18%), mesentery/pancreas (maximum change: 135±26%) and adrenals (maximum change: 88±22%). On the other hand, vascular conductance in the lungs (maximum change: 81±2%) and, to some extent, kidneys (23±4% at 3 μg kg^{-1} min^{-1}) was decreased by 5-HT. These 5-HT-induced changes in vascular conductances were substantially reduced or even abolished after pretreatment with R(+)lisuride. In contrast, pretreatment with S(-)lisuride, the 5-HT-induced changes in the skeletal muscles (maximum change: 250±120%) and mesentery/pancreas (maximum change: 50±23%) vascular conductances were slightly less, whereas the responses in carcass (maximum change: 107±16%), adrenals (maximum change: 96±13%) and lungs (maximum change: 68±5%) were not significantly modified. Pretreatment with GR127935 slightly attenuated the increase in muscle conductance (maximum change: 232±65%) without affecting the 5-HT-induced effects in carcass, mesentery/pancreas, adrenals and lungs (maximum changes: 94±21%, 190±50% and 126±43% and 73±3%, respectively).

Additionally, decreases in blood flow were observed in skin, heart, gastrointestinal tract, brain, spleen and liver, but the respective vascular conductance values remained unchanged, as the corresponding hypotensive responses were quantitatively similar (data not shown).

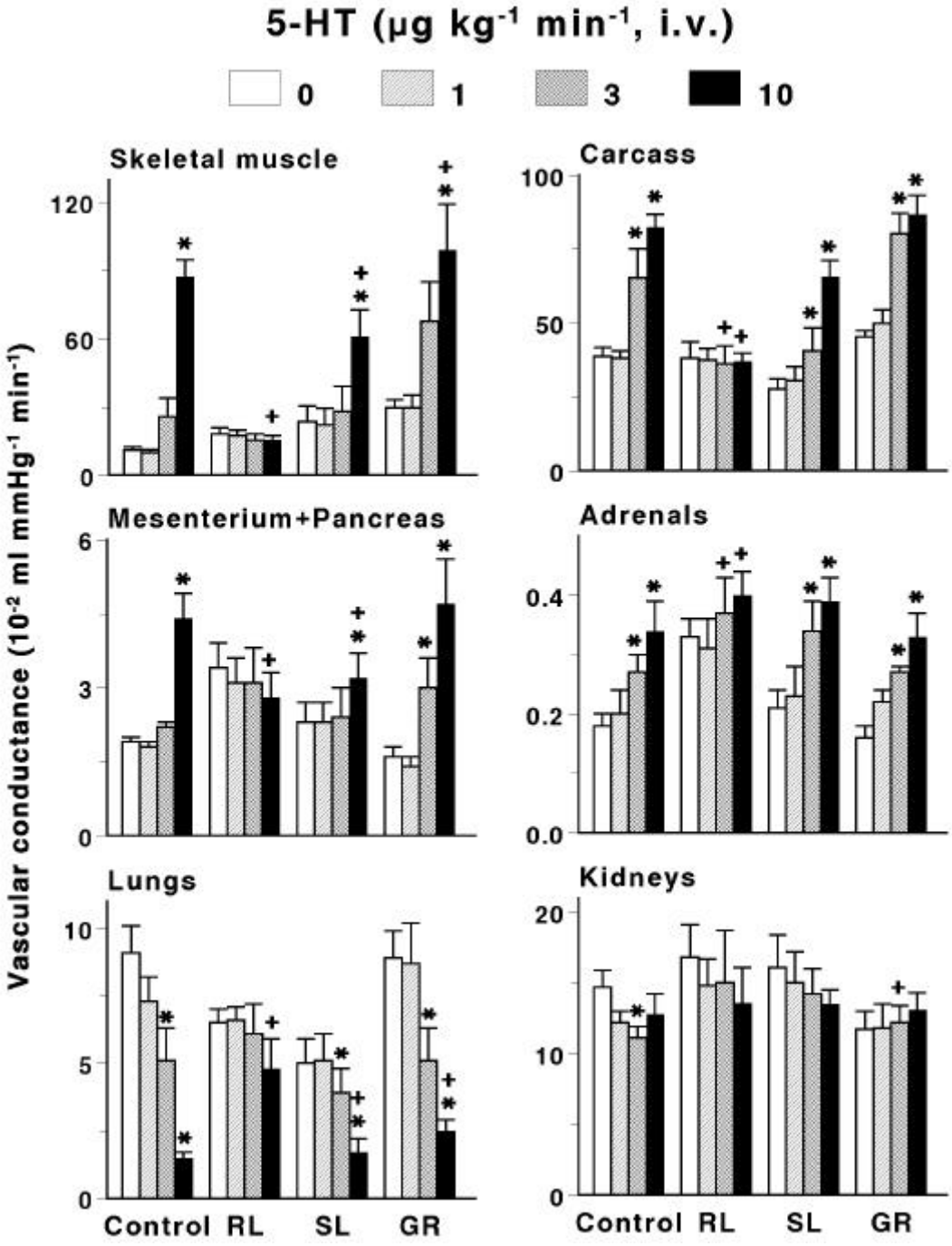

Figure 11.4 Effects of 10-min i.v. infusions of 5-HT on regional vascular conductance in anaesthetised rats pretreated i.v. with either (n=5 each) saline (Control; 0.5 ml), R(+)lisuride (RL; 100 µg kg^{-1}), S(-)lisuride (SL; 100 µg kg^{-1}) or GR127935 (GR; 300 µg kg^{-1}). *, P<0.05 vs baseline. +, P<0.05 vs response by corresponding dose of 5-HT in control animals. All animals were systematically pretreated with ritanserin (50 µg kg^{-1}, i.v.).

11.4 Discussion

11.4.1 General

It has previously been shown that i.v. bolus injection of 5-HT produces a triphasic blood pressure response in anaesthetised rats (Kalkman *et al*., 1984; Saxena & Lawang, 1985), of which exclusively a long lasting decrease in blood pressure persists after bilateral vagosympathectomy and 5-HT$_{2A}$ receptor blockade (Chapter 3). The receptor mediating this 5-HT-induced hypotension has recently been shown to resemble the 5-HT$_7$ receptor (Chapter 10). Thus, 5-HT-induced hypotension was: (i) mimicked, in decreasing order of agonist potency, by 5-CT, 5-HT and 5-MeO-T; sumatriptan (30-1000 µg kg^{-1}) was inactive; (ii) antagonised, in decreasing order of potency, by R(+)lisuride, methiothepin, mesulergine and metergoline, in agreement with their affinities at the 5-HT$_7$ receptor (see Table 10.2); and (iii) resistant to blockade by the 5-HT$_{1B/1D}$ receptor antagonist, GR127935 (Skingle *et al*., 1996), at doses that block 5-HT$_{1B/1D}$ receptor-mediated cardiovascular responses (see Chapter 4; Villalón *et al*., 1996). Undoubtedly, this pharmacological profile is identical to that displayed by other 5-HT$_7$ receptors mediating cardiovascular responses *in vitro* and *in vivo* (see Chapter 12; Eglen *et al*., 1997; Villalón *et al*., 1997a; 1997c; Saxena *et al*., 1998b). It must be recognised, however, that 5-HT-induced hypotension and/or vasodilatation may also involve secondary mechanisms (e.g. endothelial NO release, sympathoinhibition) which could have been overshadowed by the much more prominent effect on musculotropic 5-HT$_7$ receptors, as previously shown by several lines of evidence (Ullmer *et al*., 1995; De Vries *et al*., 1997b; Eglen *et al*., 1997; Saxena *et al*., 1998b). These considerations led us to choose lisuride (although, admittedly, a non-selective ligand; see Table 10.2) as antagonist of the 5-HT-induced hypotensive responses in the present study. This drug displays: (i) very high affinity (pK_i=9.1) at 5-HT$_7$ receptors; and (ii) the highest potency in blocking cardiovascular responses following 5-HT$_7$ receptor activation (see Chapter 10; Villalón *et al*., 1997a; 1997c).

Apart from the implications discussed below, the major findings of the present study in vagosympathectomised rats were that: (i) the 5-HT-induced hypotension was exclusively attributable to an increase in systemic vascular conductance, mediated by a lisuride-sensitive (most probably 5-HT$_7$) receptor; and (ii) the 5-HT-induced increase in systemic vascular conductance was confined to skeletal muscle, mesentery/pancreas, adrenal and carcass vascular beds.

11.4.2 Systemic haemodynamic changes induced by 5-HT

Only with its highest dose (10 µg kg^{-1} min^{-1}, i.v. for 10 min), 5-HT produced a slight bradycardia, for which we do have no clear-cut explanation. In our previous experiments, we reported that bolus injections of 5-HT (1-10 µg kg^{-1}) did not affect heart rate in vagosympathectomised, ritanserin-treated rats (Chapter 10), suggesting that the mode of administration (10-min infusions) could have played a role. In any case, 5-HT did not change cardiac output, as the 5-HT-induced bradycardia was accompanied by a compensatory increase in stroke volume. Additionally, 5-HT produced a dose-dependent decrease in blood pressure, which was exclusively caused by a dilatation of systemic vasculature, as cardiac output remained unaffected. The present results confirm previous reports, that this hypotension was mediated by 5-HT_7 receptors, as demonstrated in Chapter 10. Thus, R(+)lisuride completely blocked this response, whereas GR127935 hardly affected the 5-HT-induced hypotension. This suggestion is reinforced by the fact that 10-min i.v. infusions of 5-CT decreased blood pressure, with a higher potency than 5-HT (Saxena & Lawang, 1985). Moreover, the weak, if any, blockade by S(-)lisuride seems to indicate that this stereoisomer displays moderate affinity at the rodent 5-HT_7 receptor mediating hypotension.

11.4.3 Regional haemodynamic changes

In vagosympathectomised, ritanserin-pretreated, pentobarbital anaesthetised rats, the 5-HT_7 receptor-mediated decrease in blood pressure was solely caused by systemic vasodilatation. This vasodilatation was confined to the skeletal muscles, mesentery/pancreas, carcass and adrenals. As a result of the prominent vasodilatation in these vascular beds, cardiac output was redistributed, leading to an under-perfusion ("steal") in the other tissues/organs. Indeed, these decreases in regional blood flow, with the clear exception of lungs (see below), were due only to the corresponding decreases in blood pressure, as the respective conductance values remained essentially unmodified. Additionally, carcass vascular conductance increased in response to 5-HT, which most likely reflects changes in residual skeletal muscles, although a dilator action in fat, bone or urogenital tract vasculature cannot be excluded. Taking into account that skeletal muscle received the larger part of cardiac output, it is reasonable to assume that the dilatation in skeletal muscle arterioles contributed most to the dilatation of the total systemic vasculature, leading to hypotension. In keeping with this suggestion, the receptor mediating the dilatation to 5-HT in skeletal muscle

vasculature seems to resemble the pharmacological profile of the receptor mediating the hypotension to 5-HT. Thus, the 5-HT-induced skeletal muscle vasodilatation, as well as the concomitant decrease in blood pressure was abolished by R(+)lisuride, but not much affected by GR127935. On the same grounds, 5-HT$_7$ receptors seem to be primarily involved in the vasodilatation induced by 5-HT in the carcass, mesentery/pancreas, as well as adrenal vascular beds.

Contractile responses in several blood vessels, including the renal artery (Choppin & O'Connor, 1994; Whiting & Cambridge, 1995), cerebral artery (Deckert *et al*., 1994; Skingle *et al*., 1996) and gastrointestinal vasculature (Choppin & O'Connor, 1996), have been described to be mediated by 5-HT$_1$-like - most likely 5-HT$_{1B/1D}$ – receptors (Saxena *et al*., 1998b). Interestingly, however, little or no vasoconstrictor responses were observed in the corresponding vascular beds in the saline-pretreated animals. Similarly, although 5-HT$_7$ receptor *m*RNA has been shown to be abundantly expressed in renal and coronary arteries (Ullmer *et al*., 1995) and 5-HT$_7$ receptor-mediated vasorelaxant responses have been described in isolated coronary artery rings (Cushing *et al*., 1996), no dilator effects were observed in these tissues (heart and kidneys; see Figure 11.3). The absence of these vascular responses could be explained in terms of: (i) the presence of counter-regulatory mechanisms, such as tissue autoregulation (Saxena & Bolt, 1986); (ii) the fact that changes in tissue vascular conductance are mainly mediated by changes in smaller, more distal arterioles than those used in *in vitro* experiments; and (iii) a species difference. Admittedly, it is also possible that vasoconstrictor 5-HT$_{1B/1D}$ and vasodilator 5-HT$_7$ receptors were simultaneously activated by 5-HT, producing, as a consequence, an apparent lack of effect. However, this seems unlikely, as treatment with the 5-HT$_{1B/1D}$ receptor antagonist, GR127935, was not capable of unmasking a dilator effect.

In agreement with previous findings in rabbits, cats and pigs (Forsyth & Saxena, 1978; Saxena *et al*., 1978; Saxena & Verdouw, 1982; Den Boer *et al*., 1992b), 5-HT produced a dose-dependent decrease in lung vascular conductance values. This effect is representative of constriction of peripheral arteriovenous anastomoses (Saxena & Verdouw, 1982), as bronchial blood flow is only 1-1.5% of cardiac output (Baile *et al*., 1982). Interestingly, in the presence of ritanserin, this response was not much affected by GR127935, ruling out a major role for 5-HT$_{1B/1D}$ or 5-HT$_2$ receptors. Although it is tempting to attribute this effect to a novel (5-HT?) receptor, as recently shown in the porcine carotid circulation

(Chapter 5) and human brain (Castro *et al.*, 1997), we cannot categorically exclude the influence of baroreceptor-reflex-mediated increase in sympathetic activity (Saxena, 1986b).

In conclusion, the present investigation shows that 10-min i.v. infusions of 5-HT produce decreases in mean arterial blood pressure, exclusively caused by an increase in systemic vascular conductance. This systemic vasodilatation, confined to skeletal muscle, carcass, mesentery/pancreas and adrenal vascular beds, was mediated primarily by lisuride-sensitive 5-HT_7 receptors. Furthermore, this study confirms and extends our previous findings by showing that blockade of vasorelaxant 5-HT_7 receptors by lisuride is stereoselective. The present results may eventually be helpful in the future development of drugs producing local or systemic vasodilatation (Villalón *et al.*, 1997b). In this context, it has been shown that chronic administration of 5-CT in spontaneous hypertensive rats produced a sustained antihypertensive effect (Balasubramaniam *et al.*, 1993).

Chapter 12

Characterisation of putative 5-HT_7 receptors mediating tachycardia in the cat

Summary It has been suggested that the tachycardic response to 5-HT in the spinal-transected cat is mediated by 5-HT_1-like receptors since this effect, being mimicked by 5-CT, is not modified by ketanserin or MDL72222, but it is blocked by methiothepin, methysergide or mesulergine. The present study was set out to reanalyse this suggestion in terms of the IUPHAR 5-HT receptor classification schemes proposed in 1994 and 1996. Intravenous (i.v.) bolus injections of the tryptamine derivatives, 5-CT (0.01-30 μg kg^{-1}), 5-HT (3-30 μg kg^{-1}) and 5-MeO-T (3-30 μg kg^{-1}) as well as the atypical antipsychotic drug, clozapine (1000 and 3000 μg kg^{-1}) resulted in dose-dependent increases in heart rate, with a rank order of agonist potency of 5-CT >> 5-HT > 5-MeO-T >> clozapine. The tachycardic effects of 5-HT and 5-MeO-T were dose-dependently antagonised after i.v. administration of lisuride (30 and 100 μg kg^{-1}), ergotamine (100 and 300 μg kg^{-1}) or mesulergine (100, 300 and 1000 μg kg^{-1}); the highest doses used of these antagonists also blocked the tachycardic effects of 5-CT. Clozapine (1000 and 3000 μg kg^{-1}) did not affect the 5-HT-induced tachycardia, but attenuated, with its highest dose, the responses to 5-MeO-T and 5-CT; however, these doses of clozapine as well as the high doses of ergotamine (300 μg kg^{-1}) and mesulergine (300 and 1000 μg kg^{-1}) also attenuated the tachycardic effects of isoprenaline. In contrast, 5-HT-, 5-MeO-T- and 5-CT-induced tachycardia were not significantly modified after i.v. administration of physiological saline (0.1 and 0.3 ml kg^{-1}), the 5-$HT_{1B/1D}$ receptor antagonist, GR127935 (500 μg kg^{-1}) or the 5-$HT_{3/4}$ receptor antagonist, tropisetron (3000 μg kg^{-1}). I.v. injections of the 5-HT_1 receptor agonists, sumatriptan (30, 100 and 300 μg kg^{-1}) and indorenate (300 and 1000 μg kg^{-1}) or the 5-HT_4 receptor (partial) agonist cisapride (100, 300 and 1000 μg kg^{-1}) were devoid of effects on feline heart rate *per se* and failed to significantly modify 5-HT-induced tachycardic responses. Based upon the above rank order of agonist potency, the failure of sumatriptan, indorenate or cisapride to produce cardioacceleration and the blockade by a series of drugs showing high affinity for the cloned 5-ht_7 receptor, the present results indicate that the 5-HT receptor mediating tachycardia in the cat is operationally similar to other putative 5-HT_7 receptors mediating vascular and non-vascular responses. Since these responses represent functional correlates of the 5-ht_7 gene product, the 5-HT_7 receptor appellation is reinforced. Therefore, the present experimental model, which is not complicated by the presence of other 5-HT receptors, can be utilised to characterise and develop new drugs with potential agonist and antagonist properties at functional 5-HT_7 receptors.

Based on: Villalón, C.M., Heiligers, J.P.C., Centurión, D., De Vries, P. & Saxena, P.R. (1997). Characterization of putative 5-HT_7 receptors mediating tachycardia in the cat. *Br. J. Pharmacol.*, **121**, 1187-1195.

12.1 Introduction

5-HT elicits complex changes in the cardiovascular system comprising bradycardia or tachycardia, hypotension or hypertension and vasodilatation or vasoconstriction (for reviews see Saxena & Villalón, 1990; 1991; Saxena *et al.*, 1998b). In most species, bradycardia induced by 5-HT is mediated by 5-HT$_3$ receptors, via the activation of the von Bezold Jarisch reflex; in marked contrast, 5-HT-induced tachycardia is notoriously species-dependent and is mediated, directly or indirectly, either by 5-HT$_1$-like (cat), 5-HT$_2$ (rat, dog), 5-HT$_3$ (rabbit, dog) and 5-HT$_4$ (pig, human) receptors or by tyramine-like (guinea-pig) or unidentified mechanisms (Saxena, 1986a; Saxena & Villalón, 1990; 1991).

The so called 5-HT$_1$-like receptors mediating tachycardia in the cat are potently stimulated by 5-CT and blocked by methiothepin and methysergide (Saxena *et al.*, 1985b; Saxena, 1988). However, it is noteworthy that these receptors do not satisfactorily fulfil some classification requirements for the 5-HT$_1$ type, including insensitivity to stimulating doses of RU24969 and 8-OH-DPAT at typical 5-HT$_1$-like receptor-mediated responses (e.g. constriction of porcine carotid arteriovenous anastomoses; Saxena & Villalón, 1990), blockade by mesulergine (Saxena, 1988), an ergoline devoid of interactions with the 5-HT$_1$ receptor family (see Table 10.2), and inconsistency with a negative coupling to adenylyl cyclase (Hoyer *et al.*, 1994). These cardiac receptors, consequently, are different from the typical 5-HT$_1$-like receptors mediating vasoconstriction, a response usually associated with a decrease in cyclic AMP (Sumner *et al.*, 1992), but are similar to those mediating direct relaxation of vascular and non-vascular smooth muscle, a response that involves an increase in cyclic AMP (for references see Saxena & Villalón, 1990; Martin, 1994).

While evidence is emerging that sumatriptan-sensitive 5-HT$_1$-like receptors mediating vasoconstriction resemble 5-HT$_{1B}$ receptors (see Chapters 8 and 9), the recently cloned 5-ht$_7$ receptor (e.g. Bard *et al.*, 1993; Ruat *et al.*, 1993b) seems to be a suitable candidate for responses mediated by the atypical, sumatriptan-insensitive 5-HT$_1$-like receptors. Indeed, the cloned 5-ht$_7$ receptor is positively coupled to adenylyl cyclase (Chapter 1) and binding studies show that the cloned 5-ht$_7$ receptor displays high affinities for 5-CT, 5-HT, methiothepin, mesulergine and methysergide, but relatively low affinities for RU 24969, 8-OH-DPAT and sumatriptan (see Tables 10.1 and 10.2).

In the light of these findings, the present study was set out to investigate the operational characteristics of the 5-HT receptors mediating tachycardia in the spinal cat, with particular emphasis on verifying if these receptors display the pharmacological profile of the cloned 5-ht_7 receptor. Hence, the drugs employed included agonists and/or antagonists at 5-HT_1, 5-HT_2, 5-HT_3 and 5-HT_4 receptors, as well as some compounds with high affinity for the cloned 5-ht_5, 5-ht_6 and/or 5-ht_7 receptors (see Tables 10.1 and 10.2).

12.2 Methods

12.2.1 General

Forty one cats, not selected for breed or sex (2.5-4.0 kg) and fasted overnight, were initially anaesthetised with sodium pentobarbitone (30 mg kg^{-1}, i.v.). Left femoral vein and artery were cannulated for, respectively, drug injections and the measurement of aortic blood pressure using a Statham pressure transducer (P23 ID). Heart rate was obtained by triggering a tachograph (Grass Instruments Co., Quincy, MA, USA; model 7P4) with the ECG signals. Blood pressure and heart rate were recorded simultaneously by a model 7D Grass polygraph (Grass Instrument Co., Quincy, MA, USA). The animals were then given an additional dose of pentobarbital (10-15 mg kg^{-1}, i.v.) and intubated with an endotracheal tube for artificial ventilation with room air, using a Palmer ventilation pump (rate: 20 strokes min^{-1}; stroke volume: 13-16 ml kg^{-1}). Subsequently, both vagosympathetic trunks and the spinal cord (at the level of C_1-C_2) were sectioned in all animals, as previously reported (Saxena *et al*., 1985b; Saxena, 1988).

12.2.2 Experimental protocols

After the cats had been in a stable haemodynamic condition for at least 60 min, baseline values of blood pressure and heart rate were determined. Then, the animals were divided into two groups.

The first group (n=34) received consecutive i.v. bolus injections, every 5 to 10 min, of 5-HT (3, 10 and 30 µg kg^{-1}), 5-MeO-T (3, 10 and 30 µg kg^{-1}) and isoprenaline (0.01, 0.03 and 0.1 µg kg^{-1}) and the changes produced in blood pressure and heart rate were noted. At this point, the animals were divided into six subgroups. Four subgroups were treated, using a cumulative dose schedule, with either physiological saline (0.1 and 0.3 ml kg^{-1}; n=5), lisuride (30 and 100 µg kg^{-1}; n=6),

ergotamine (100 and 300 μg kg^{-1}; n=6) or mesulergine (100, 300 and 1000 μg kg^{-1}; n=6). The fifth subgroup (n=6) was given clozapine (1000 and 3000 μg kg^{-1}) following a sequential (not cumulative) dosing because it produced an immediate short-lasting tachycardic response by itself. The sixth subgroup (n=5) was treated with GR127935 (500 μg kg^{-1}) and, subsequently, with tropisetron (3000 μg kg^{-1}). The responses to i.v. injections of 5-HT, 5-MeO-T and isoprenaline, at the doses and sequence listed above, were elicited again 10 min after each dose of physiological saline or the above compounds.

The second group (n=7) received consecutive i.v. injections of 5-HT (3, 10 and 30 μg kg^{-1}) and isoprenaline (0.01, 0.03 and 0.1 μg kg^{-1}) and the changes in blood pressure and heart rate were noted. The animals were then divided into two subgroups. The first subgroup (n=4) was given, sequentially, i.v. injections of the 5-HT$_1$ receptor agonist, indorenate (300 and 1000 μg kg^{-1} spaced by 5 min) followed by the 5-HT$_4$ receptor (partial) agonist, cisapride (300 and 1000 μg kg^{-1} spaced by 5 min) and the second subgroup (n=3) was treated with sequential i.v. injections of the 5-HT$_1$ receptor agonist, sumatriptan (30, 100 and 300 μg kg^{-1} spaced by 5 min). After each treatment and dose, the responses to 5-HT and isoprenaline were reanalysed.

Subsequently, each of the above 8 subgroups received, at the end of their corresponding protocol, i.v. injections of 5-CT at cumulative total dose levels of 0.03, 0.1, 0.3, 1, 3, 10 and 30 μg kg^{-1} every 5-7 min. The changes in heart rate and blood pressure, produced after each dose of 5-CT, were noted. Lastly, in at least three cats of each of the above eight subgroups (see Results section), the highest dose used of saline or the corresponding drug was administered once again in an attempt to investigate which compounds could reverse the tachycardia elicited by the highest dose of 5-CT (30 μg kg^{-1}), as previously described for methysergide (Saxena *et al*., 1985b).

With the exception of 5-CT (see above), the dose-intervals between the different doses of agonists ranged between 1 and 10 min, as in each case we waited until the heart rate had returned to baseline values. For the antagonists, as well as for indorenate, cisapride and sumatriptan, a period of 10 min was allowed to elapse before the dose-response curves for the agonists were elicited again. The dosing with 5-HT, 5-MeO-T, isoprenaline, sumatriptan, indorenate, cisapride and clozapine was sequential, whilst that for 5-CT and the rest of antagonists was cumulative.

12.2.3 Data presentation and statistical analysis

All data in the text and figures are presented as the mean±s.e.mean and these were analysed using a computer program (Saxena, 1985). The agonist-induced increases in heart rate just before and after a particular dose of saline or antagonist drug within one group of animals were compared by Student Newman-Keuls' test, once an analysis of variance (randomised block design) had revealed that the samples represented different populations (Steel & Torrie, 1980). Furthermore, the increases in heart rate by 5-HT and isoprenaline before and after sumatriptan (430 µg kg^{-1}), indorenate (1300 µg kg^{-1}) or cisapride (1300 µg kg^{-1}), given cumulatively, were compared by using Student's paired *t*-test. Finally, the tachycardic responses to 5-CT in the different groups of animals were compared by using Student's unpaired *t*-test. A P-value of 0.05 or less (two-tailed) was considered statistically significant.

12.2.4 Drugs

Apart from the anaesthetic (sodium pentobarbitone), the drugs used in the present study (obtained from the sources indicated) were the following: 5-HT creatinine sulphate (Sigma Chemical Company, St. Louis, MO, USA); lisuride hydrogen maleate, isoprenaline hydrochloride, 5-MeO-T hydrochloride and 5-CT maleate (Research Biochemicals Int., Natick, MA, USA); sumatriptan succinate (gift: Prof. Dr. P.P.A. Humphrey, Glaxo Institute of Applied Pharmacology, Cambridge, UK); GR127935 (gift: Dr. M. Skingle, Glaxo Group Research Limited, Ware, Herts, UK); tropisetron, clozapine base, mesulergine hydrochloride and ergotamine tartrate (gift: Sandoz A.G., Basel, Switzerland); indorenate (gift: Prof. Dr. E. Hong, CINVESTAV-IPN, Mexico City, Mexico); and cisapride (gift: Janssen Pharmaceutica, Beerse, Belgium). All compounds were dissolved in distilled water. When needed, 4% (w v^{-1}) ascorbic acid (clozapine) or 5% (v v^{-1}) DMSO (lisuride) was added; these vehicles had no effect on the haemodynamic variables or the agonist-induced responses. The doses mentioned in the text refer to the salts of substances, except in the case of all agonists and clozapine, where they refer to the free base.

12.3 Results

12.3.1 Initial blood pressure and heart rate changes by 5-HT receptor agonists

Baseline values of mean arterial blood pressure and heart rate in the forty one cats were, respectively, 84±3 mmHg and 184±35 beats min^{-1}. The changes induced in mean arterial blood pressure by the agonist drugs were: 5-HT (-2±1, +3±4 and +15±5 mmHg after 3, 10 and 30 µg kg^{-1}, respectively; n=41), 5-MeO-T (-1±2, +7±4 and +23±4 mmHg after 3, 10 and 30 µg kg^{-1}, respectively; n=34), 5-CT (-1±17, -13±5, -17±6 and -20±7 mmHg after 0.03, 0.1, 0.3 and 1 µg kg^{-1}, with 3, 10 and 30 µg kg^{-1} producing no subsequent change; n=5, i.e. saline-treated animals), sumatriptan (-12±12, +17±9, +3±2 mmHg after 30, 100 and 300 µg kg^{-1}, respectively; n=3), indorenate (-5±7 and -3±5 mmHg after 300 and 1000 µg kg^{-1}, respectively; n=4), cisapride (-1±2 and -7±3 mmHg after 300 and 1000 µg kg^{-1}, respectively; n=4) and clozapine (-9±5 and -18±5 mmHg after 1000 and 3000 µg kg^{-1}, respectively; n=6). It should be pointed out that these effects were not evaluated further because in spinal cats the baseline blood pressure is low and, consequently, the hypotensive responses produced by the above agonists were smaller and not strictly dose-dependent (see above).

The onset of the increases in heart rate induced by the agonists under study was immediate. Figure 12.1 shows that isoprenaline, 5-CT, 5-HT, 5-MeO-T and clozapine caused dose-dependent increases in heart rate; in contrast, sumatriptan, indorenate and cisapride, at the doses tested, failed to increase feline heart rate. Isoprenaline was about 1.5 log units more potent than 5-CT, which was itself distinctly more potent (~1.5 log units) than 5-HT and 5-MeO-T; clozapine was the least potent. Thus, the apparent rank order of agonist potency was: isoprenaline >> 5-CT >> 5-HT > 5-MeO-T >> clozapine.

The duration of tachycardic effects of agonist drugs (except 5-CT) was relatively short: isoprenaline (3.9±0.2, 4.8±0.2 and 6.2±0.2 min after 0.01, 0.03 and 0.1 µg kg^{-1}, respectively), 5-HT (2.6±0.2, 4.6±0.2 and 7.0±0.3 min after 3, 10 and 30 µg kg^{-1}, respectively), 5-MeO-T (2.3±0.2, 3.5±0.2 and 5.2±0.2 min after 3, 10 and 30 µg kg^{-1}, respectively) or clozapine (2.1±1.0 and 3.0±1.0 after 1000 and 3000 µg kg^{-1}, respectively). Furthermore, the 5-CT-induced tachycardia has been shown to last longer than 1 h (Saxena *et al*., 1985b; Saxena, 1988) and, therefore, as expected, no recovery was observed with 5-CT during the dosing intervals (5-7 min) or for at least 10 min after the last dose.

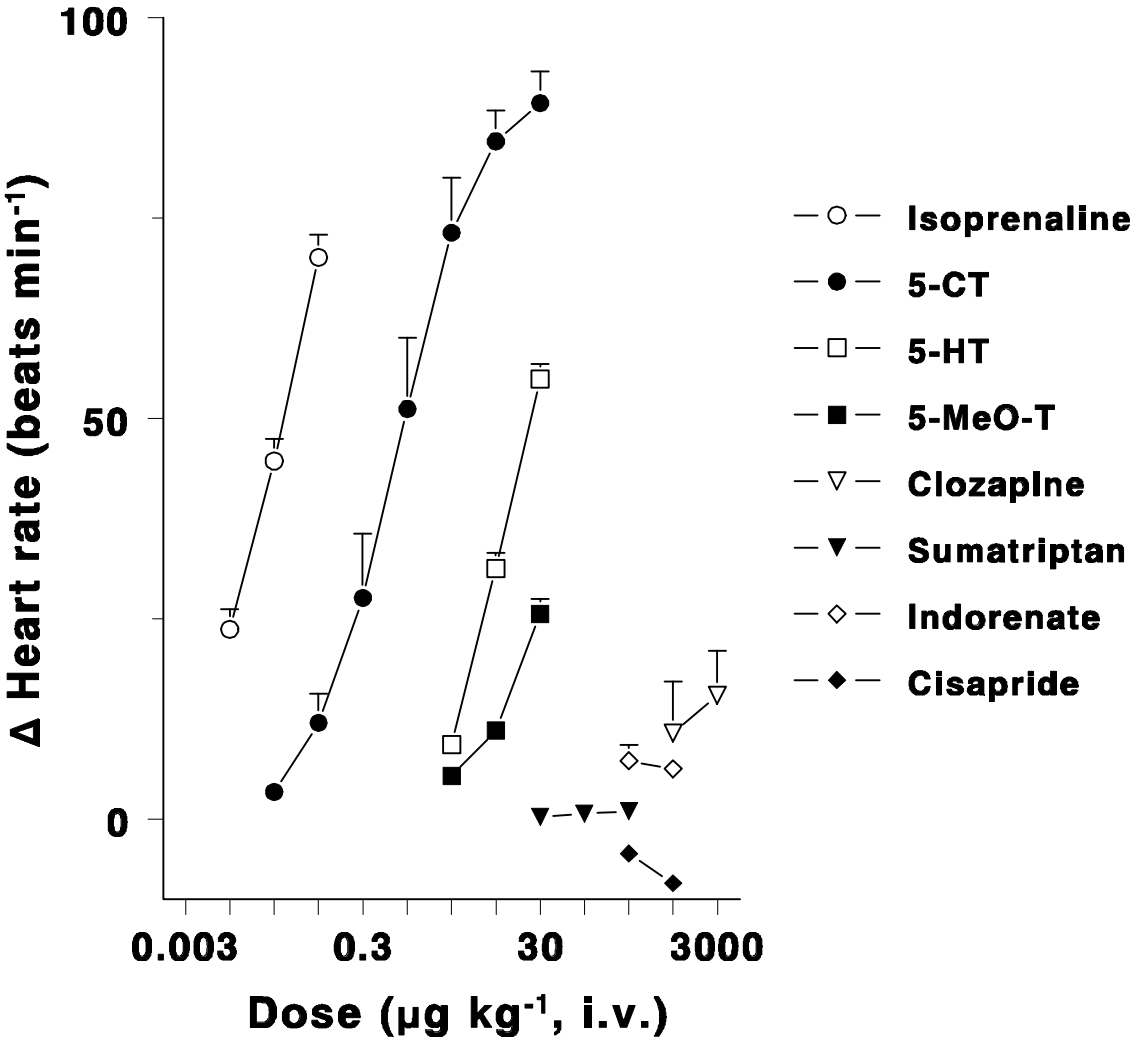

Figure 12.1 Comparative effects of i.v. bolus injections of isoprenaline (n=41), 5-CT (n=5), 5-HT (n=41), 5-MeO-T (n=34), clozapine (n=6), sumatriptan (n=3), indorenate (n=4) and cisapride (n=4) on heart rate in vagosympathectomised spinal cats.

12.3.2 Effect of physiological saline and 5-HT receptor antagonists on the tachycardic responses induced by 5-HT, 5-MeO-T and isoprenaline

The effects of physiological saline or lisuride on the tachycardic responses induced by 5-HT, 5-MeO-T and isoprenaline are depicted in Figure 12.2. No evidence of tachyphylaxis was observed since the responses to these agonists, at the doses and time intervals (330 min) used in the present study, were reproducible and remained essentially unchanged in control animals receiving two doses (0.1 and

0.3 ml kg^{-1}, i.v.) of physiological saline (Figure 12.2, *upper panels*). In contrast, the administration of low doses (30 and 100 µg kg^{-1}, i.v.) of lisuride potently and dose-dependently antagonised the tachycardic responses induced by 5-HT and 5-MeO-T; this blockade was specific as lisuride did not alter isoprenaline-induced tachycardia (Figure 12.2, *lower panels*).

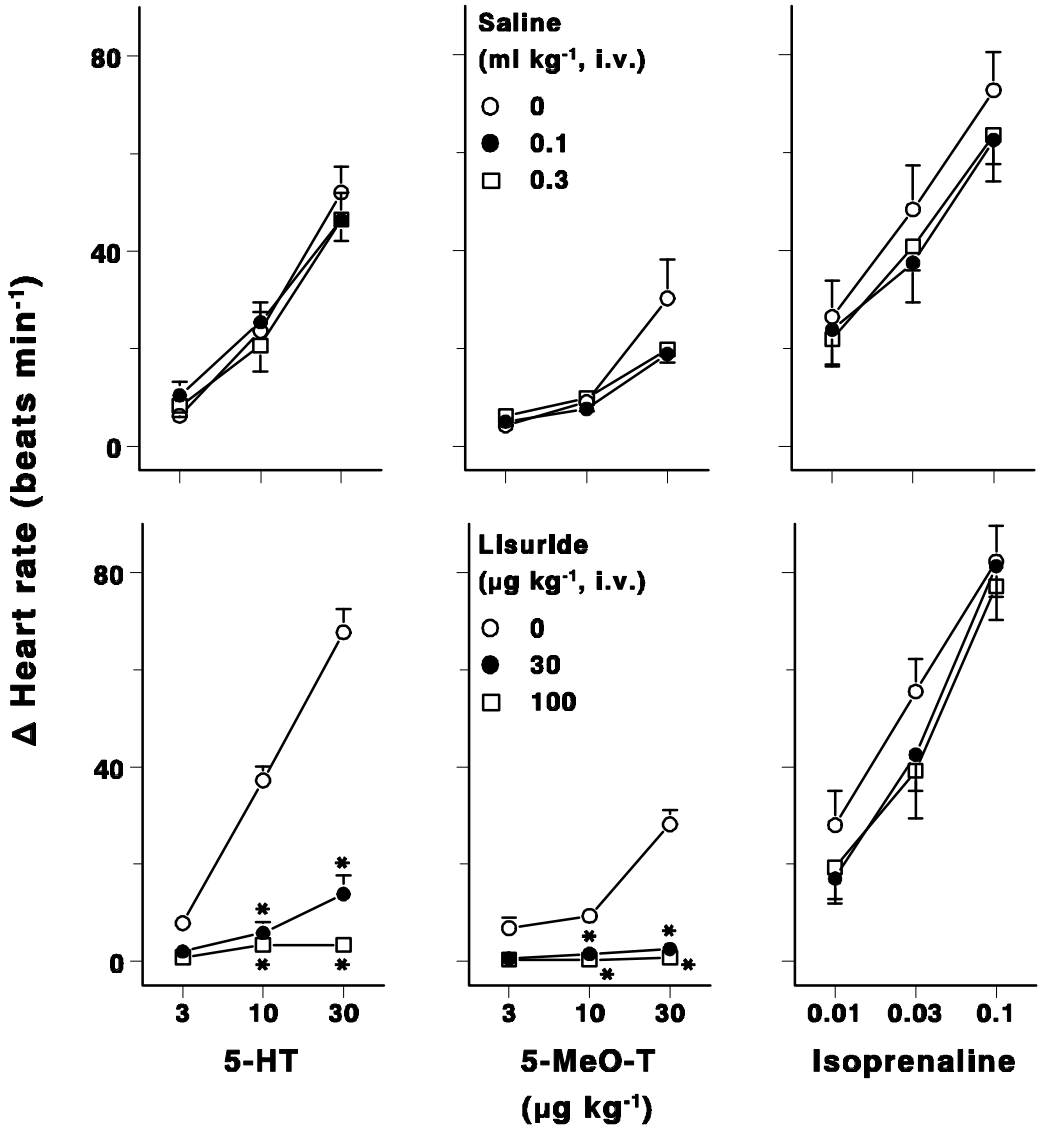

Figure 12.2 Effects of i.v. bolus injections of physiological saline (*upper panels*; n=5) or lisuride (*lower panels*; n=6) on tachycardic responses to 5-HT, 5-MeO-T and isoprenaline in vagosympathectomised spinal cats. *, P<0.05 vs the corresponding control response.

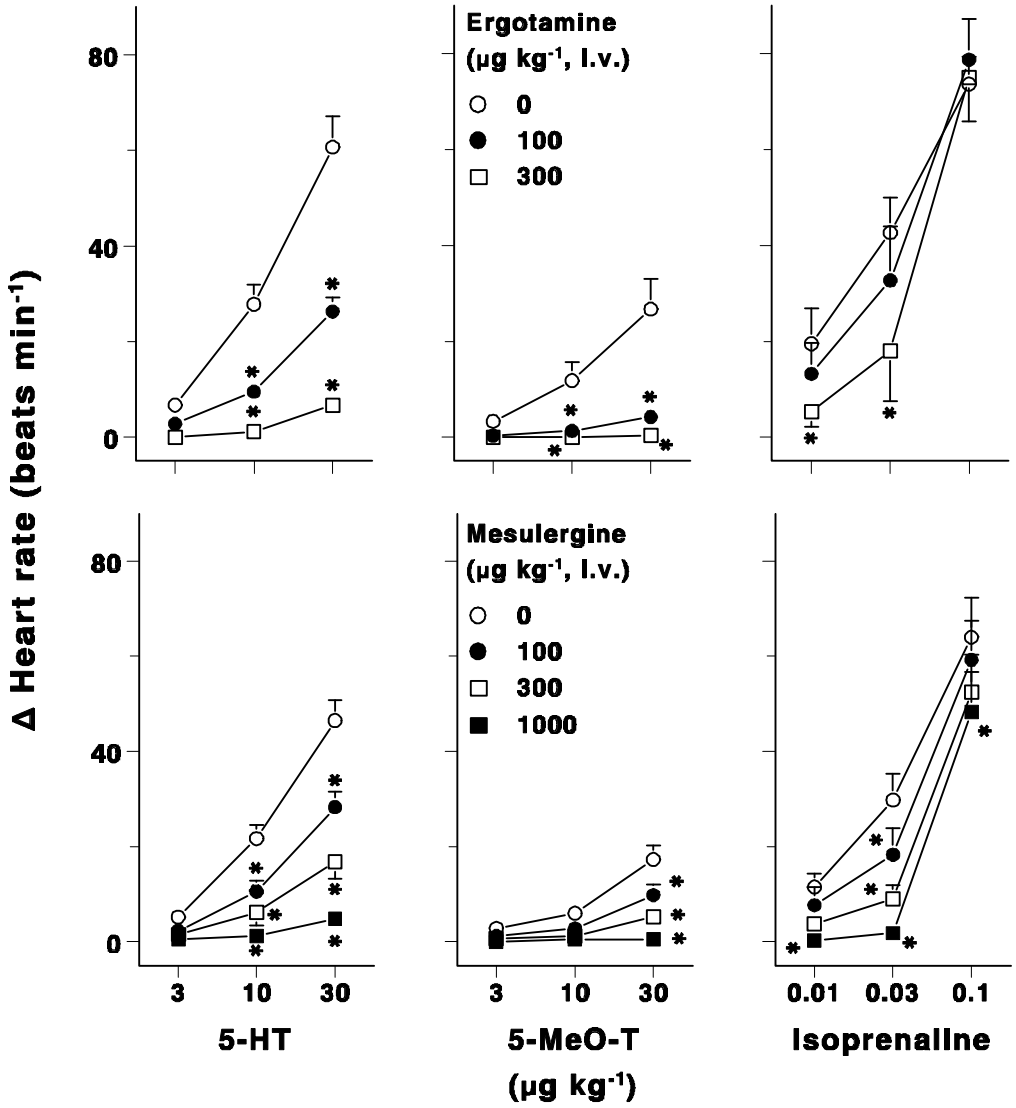

Figure 12.3 Effects of i.v. bolus injections of ergotamine (*upper panels*; n=6) or mesulergine (*lower panels*; n=6) on tachycardic responses to 5-HT, 5-MeO-T and isoprenaline in vagosympathectomised spinal cats. *, P<0.05 vs the corresponding control response.

Ergotamine (100 and 300 μg kg^{-1}) and mesulergine (100, 300 and 1000 μg kg^{-1}) also produced a dose-dependent blockade of the tachycardic responses to 5-HT and 5-MeO-T (Figure 12.3). However, the blockade produced by the high doses of ergotamine (300 μg kg^{-1}) and mesulergine (300 and 1000 μg kg^{-1}) was not specific as they also attenuated the tachycardic effects of isoprenaline (Figure 12.3). Moreover,

the atypical antipsychotic drug, clozapine (1000 and 3000 μg kg^{-1}), did not affect 5-HT-induced tachycardia, but significantly blocked, with its highest dose, the responses to 5-MeO-T; however, these doses of clozapine also attenuated the tachycardic effects of isoprenaline (Figure 12.4, *upper panels*).

The apparent order of potency for blockade of both 5-HT- and 5-MeO-T-induced tachycardic responses was ergotamine ≥ lisuride > mesulergine > clozapine. Finally, as shown in Figure 12.4 (*lower panels*), the tachycardic responses to 5-HT, 5-MeO-T and isoprenaline were not significantly modified after administration of the selective 5-HT$_{1B/1D}$ receptor antagonist, GR127935 or by the subsequent administration of tropisetron which, at 3000 μg kg^{-1}, is a 5-HT$_3$ and 5-HT$_4$ receptor antagonist (Chapter 1).

Except for an immediate and sustained increase in mean blood pressure by ergotamine (from 103±9 to 134±13 mmHg, after its highest dose; $P<0.05$), the values of mean blood pressure and heart rate before and 10 min after the administration of physiological saline or the above 5-HT receptor antagonists were not significantly different (data not shown).

12.3.3 Tachycardic effects of 5-HT before and after administration of indorenate, cisapride or sumatriptan

Since the 5-HT receptor agonists, indorenate, cisapride and sumatriptan, failed to mimic 5-HT in increasing heart rate (see Figure 12.1), we decided to investigate these compounds (1300 μg kg^{-1} each of indorenate and cisapride in one subgroup and 430 μg kg^{-1} of sumatriptan in another subgroup) as potential antagonists of the tachycardic responses to 5-HT. 5-HT (3, 10 and 30 μg kg^{-1}) elicited a dose-dependent tachycardia (first subgroup: 5±2, 26±10 and 46±11 beats min^{-1}, n=4, respectively; second subgroup:7±2, 17±6 and 43±7 beats min^{-1}, n=3, respectively). These responses as well as the tachycardia elicited by isoprenaline (0.01, 0.03 and 0.1 μg kg^{-1}) remained unaffected after administration of the above compounds (data not shown).

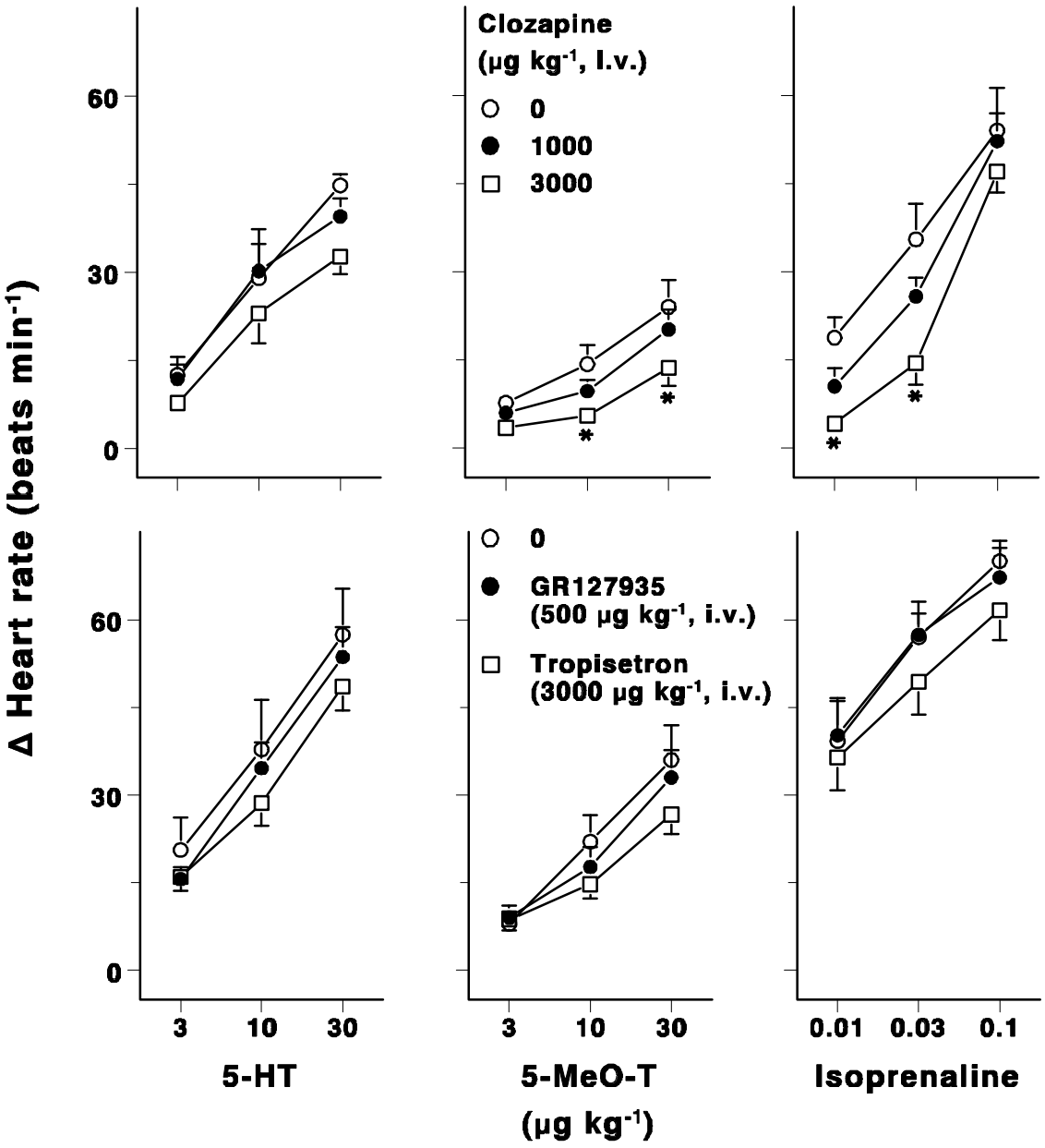

Figure 12.4 Effects of i.v. bolus injections of clozapine (*upper panels*; n=6) or GR127935 followed by tropisetron (*lower panels*; n=5) on tachycardic responses to 5-HT, 5-MeO-T and isoprenaline in vagosympathectomised spinal cats. *****, P<0.05 vs. the corresponding control response.

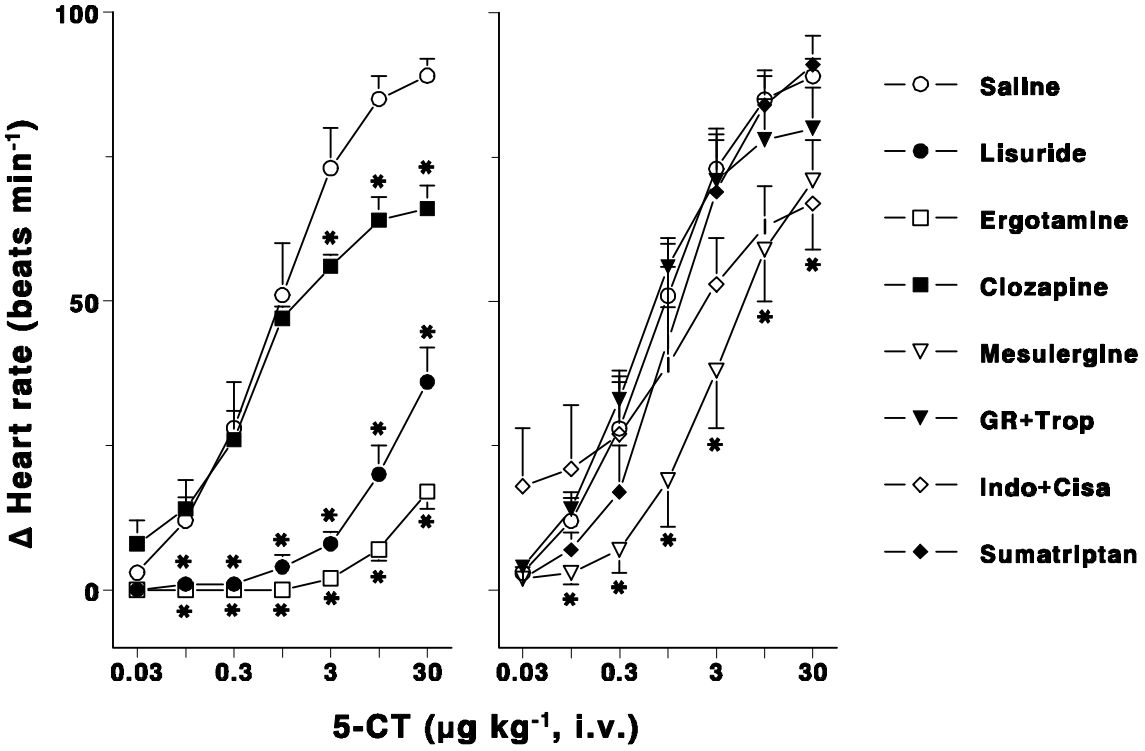

Figure 12.5 Effects of a number of drugs acting on 5-HT receptors on tachycardic responses to 5-CT in vagosympathectomised spinal cats. The cumulative doses of various drugs used were: saline (0.3 ml kg^{-1}; n=5), lisuride (100 µg kg^{-1}; n=6), ergotamine (300 µg kg^{-1}; n=6), clozapine (4000 µg kg^{-1}; n=6) in the *left panel*; mesulergine (1000 µg kg^{-1}; n=6), GR127935 (500 µg kg^{-1}) + tropisetron (3000 µg kg^{-1}; GR+Trop; n=5), indorenate (1300 µg kg^{-1}) + cisapride (1300 µg kg^{-1}; Indo+Cisa; n=4) and sumatriptan (430 µg kg^{-1}; n=3) in the *right panel*. *****, P<0.05 vs the corresponding control response in animals treated with saline, indicated in both panels.

12.3.4 Tachycardic effects of 5-CT after physiological saline or some compounds acting at 5-HT receptors

Figure 12.5 shows the comparative effects of different doses of 5-CT obtained in cats pretreated with cumulative doses of either saline (0.3 ml kg^{-1}), lisuride (100 µg kg^{-1}), ergotamine (300 µg kg^{-1}), clozapine (4000 µg kg^{-1}), mesulergine (1000 µg kg^{-1}), GR127935 (500 µg kg^{-1}) plus tropisetron (3000 µg kg^{-1}), indorenate (1300 µg kg^{-1}) plus cisapride (1300 µg kg^{-1}) or sumatriptan (430 µg kg^{-1}). Only lisuride, mesulergine, ergotamine and clozapine produced a significant blockade of the responses to 5-CT, with an apparent order of antagonist potency of ergotamine ≥

lisuride > mesulergine > clozapine. In addition, indorenate + cisapride slightly - though significantly - attenuated the response to the highest dose of 5-CT (30 µg kg^{-1}). The remaining compounds produced no significant effect (Figure 12.5).

After the tachycardic response to 30 µg kg^{-1} of 5-CT (given cumulatively) had been stable for at least 10 min, a subsequent administration of the same dose of saline, GR127935 + tropisetron, indorenate + cisapride or sumatriptan (n=3 each) in the corresponding subgroups produced no further change in heart rate (data not shown). In contrast, 5-CT-induced tachycardia was significantly ($P<0.05$) decreased from the prevailing values after i.v. administration of the same dose of lisuride (-17±1%; n=4), ergotamine (-8±2%; n=4), clozapine (-22±4%; n=3) or mesulergine (-25±2%; n=6).

12.4 Discussion

12.4.1 General

In previous studies, Saxena *et al.* (1985b) had proposed that the tachycardia induced by 5-HT in the cat, being potently mimicked by 5-CT and blocked by methysergide, is mediated by 5-HT_1-like receptors on the basis of the resistance to blocking doses of antagonists at 5-HT_2 (ketanserin, ritanserin) and 5-HT_3 (MDL72222) receptors. However, it is noteworthy that, in contrast to its constrictor effects via vascular 5-HT_1-like receptors (see Saxena & Villalón, 1990), methysergide did not show any agonist activity at the feline cardiac 5-HT_1-like receptors. Subsequently, Saxena (1988) showed that these atypical cardiac receptors were unrelated to the 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} (now 5-HT_{2C}) and 5-HT_{1D} subtypes based on: (i) the blockade, in decreasing order of potency, by methiothepin, mesulergine and metergoline, an order which does not match with their corresponding affinities for these receptors (see Table 10.2); (ii) the low agonist potency of the 5-HT_{1A} receptor agonist, 8-OH-DPAT; and (iii) the lack of activity of 5-$HT_{1A/1B}$ receptor agonist, RU 24969.

Certainly, the approach established by the IUPHAR 5-HT receptor classification scheme (Hoyer *et al*., 1994) lead us to conclude that the appellation 5-HT_1-like is no longer regarded as appropriate for the 5-HT receptors mediating tachycardia in the cat. The operational approach of the present study strengthens this view as the feline cardiac 5-HT receptors were not stimulated by sumatriptan or indorenate, which are both agonists at typical 5-HT_1-like receptors mediating vasoconstrictor responses (Saxena & Villalón, 1990; Villalón *et al*., 1990a; Hoyer *et al*., 1994) and could be blocked by a series of drugs showing high affinity for

cloned 5-ht$_7$ receptors. Thus, these cardiac receptors resemble those mediating smooth muscle relaxation and elevation of cyclic AMP in neonatal porcine vena cava (Trevethick *et al*., 1986; Sumner *et al*., 1989), hypotension in anaesthetised rats (Chapter 10) and cats (Connor *et al*., 1986) and vasodilatation in the canine external carotid bed (Villalón *et al*., 1997a). Apart from the implications discussed below, these data suggest that the 5-HT receptors mediating tachycardia in the cat may represent a functional correlate of the recombinant 5-ht$_7$ receptor.

12.4.2 Agonist action of some tryptamine derivatives on the feline heart 5-HT receptor

Admittedly, the rank order of agonist potency (5-CT >> 5-HT > 5-MeO-T) observed in our study is similar to that reported for the prejunctional sympatho-inhibitory 5-HT$_{1B/1D}$ receptors mediating canine external carotid vasodilatation (Villalón & Terrón, 1994a). Nevertheless, at these prejunctional 5-HT$_{1B/1D}$ receptors, indorenate and sumatriptan behaved as agonists (Villalón *et al*., 1993a; Villalón & Terrón, 1994a), as previously reported for other 5-HT$_{1B/1D}$ receptors (Saxena & Villalón, 1990; Villalón *et al*., 1990a; De Vries *et al*., 1996; 1996). Contrasting with this 5-HT$_{1B/1D}$ receptor operational profile, our results in the cat heart clearly show that sumatriptan and indorenate *did not* behave as agonists (or antagonists). The above rank order of agonist potency, therefore, closely resembles the pharmacological properties of the cloned 5-ht$_7$ receptor subtype; pK$_i$ values reported for recombinant 5-ht$_7$ receptors for 5-CT, 5-HT, 5-MeO-T and sumatriptan are 9.5, 8.7, 8.7 and 6.2, respectively (see Table 10.1).

12.4.3 Do feline heart 5-HT receptors correlate with any subtype of the 5-HT$_1$ receptor family?

Although the above rank order of agonist potency with indorenate and sumatriptan inactive already excludes the involvement of 5-HT$_{1A}$, 5-HT$_{1B/1D}$ and sumatriptan-sensitive 5-HT$_1$-like receptors, it is well known that the 5-HT$_1$ receptor family includes two additional subtypes, namely, the cloned 5-ht$_{1E}$ and 5-HT$_{1F}$ receptors (Chapter 1). However, the involvement of these subtypes in the present study is not likely because (i) 5-CT and 5-MeO-T, which display very low affinity for the recombinant 5-ht$_{1E}$ and 5-HT$_{1F}$ receptors (see Table 10.1), potently mimicked 5-HT in eliciting tachycardia; (ii) the tachycardic responses to 5-CT, 5-HT and

5-MeO-T were antagonised by mesulergine and clozapine, which do not interact with any subtype of the 5-HT_1 receptor family (see Table 10.2); (iii) these tachycardic responses were not antagonised by GR127935 at doses that are high enough to block 5-$HT_{1B/1D}$ receptors (De Vries *et al*., 1996; Skingle *et al*., 1996; Villalón *et al*., 1996) and (iv) the 5-HT_1 receptor family is, by definition, negatively coupled to adenylyl cyclase (Hoyer *et al*., 1994), a signal transduction system usually associated with vasoconstriction and bradycardia, not tachycardia (Rand *et al*., 1987; Saxena & Villalón, 1990; 1991; Sumner *et al*., 1992).

12.4.4 Lack of resemblance of the feline heart 5-HT receptor with either 5-HT_2, 5-HT_3, 5-HT_4, 5-ht_5 or 5-ht_6 receptors

The 5-HT receptors mediating tachycardia in the cat also seem to differ from the 5-HT_2, 5-HT_3, 5-HT_4 and 5-ht_6 receptor types on the basis of: (i) the high potency of 5-CT relative to 5-HT, an order which is reversed for the aforementioned types (see Table 10.1); (ii) insensitivity to antagonism by high doses of 5-HT_2 (ketanserin, ritanserin or cyproheptadine) and 5-HT_3 (MDL72222) receptor antagonists (Saxena *et al*., 1985b); (iii) the inactivity (as agonist or antagonist) of cisapride, a benzamide with partial agonist properties at the 5-HT_4 receptors mediating tachycardia in the pig (Villalón *et al*., 1991); (iv) resistance to antagonism by tropisetron at doses (3000 $\mu g\ kg^{-1}$) that block 5-HT_3 and 5-HT_4 receptors (Villalón *et al*., 1990b; 1991) and (v) the potent blockade by mesulergine, a compound showing selectivity for the cloned 5-ht_7 receptor (pK_i=7.7) over the cloned 5-ht_6 receptor (pK_i=5.8) (see Table 10.2). Furthermore, based on the above, the ability of 5-MeO-T to induce tachycardia in the cat, which is, in its own right, an additional criterion to exclude the participation of 5-HT_3 receptors (see Table 10.1), cannot be attributed to its agonist properties at 5-HT_4 receptors (Villalón *et al*., 1991).

Interestingly, 5-CT, methiothepin and ergotamine display a relatively high affinity for the recombinant 5-ht_{5A} and 5-ht_{5B} receptors (see Tables 10.1 and 10.2). This, therefore, raises the question whether the cloned 5-$ht_{5A/5B}$ receptors are related to the 5-HT receptors mediating tachycardia in the cat. However, this does not seem very likely because: (i) ergotamine failed to increase heart rate in the cat and (ii) methiothepin, which has a ten-fold lower affinity than ergotamine for 5-$ht_{5A/5B}$ receptors (see above), was more potent (not less potent as may be expected from their affinities for 5-$ht_{5A/5B}$ receptors) than ergotamine in blocking 5-HT-induced feline

tachycardia (present results; Saxena, 1988). Moreover, the antagonism by mesulergine and metergoline (Saxena, 1988), both with pK_i values of <6.0 at cloned 5-ht$_{5A/5B}$ receptors (see table 10.2), also points against these receptors mediating the 5-HT-induced tachycardia in the cat.

12.4.5 Resemblance of the feline heart 5-HT receptor to putative 5-HT$_7$ receptors

Since the involvement of 5-HT$_1$-5-ht$_6$ receptors seems improbable, the possibility has finally to be discussed that the 5-HT receptors mediating tachycardia in the cat resemble the cloned 5-ht$_7$ receptor. Indeed, the rank order of agonist potency of 5-CT >> 5-HT > 5-MeO-T >> clozapine to produce tachycardia in the cat, *with sumatriptan and indorenate inactive* parallels that reported at the mouse (Plassat *et al*., 1993), rat (Lovenberg *et al*., 1993; Ruat *et al*., 1993b; Shen *et al*., 1993) and human (Bard *et al*., 1993) cloned 5-ht$_7$ receptors (5-CT > 5-MeO-T ≥ 5-HT). Significantly, the above rank order of agonist potency is practically identical to that reported in other preparations where the relaxant effects of 5-HT have been ascribed to stimulation of musculotropic 5-HT$_7$ receptors. Some of these preparations include the rabbit femoral vein (Martin & Wilson, 1995), the *Cynomolgus* monkey isolated jugular vein (Leung *et al*., 1996), the guinea-pig ileum (Carter *et al*., 1995) and the canine coronary (Terrón, 1996b), external carotid (Villalón *et al*., 1997a) arteries and rat systemic vasculature (Chapter 10).

This suggestion gains weight when considering that the tachycardia induced by the 5-HT in the cat was blocked in previous studies (Saxena, 1988) by methiothepin, methysergide and metergoline, compounds that display high affinities for cloned 5-ht$_7$ receptors (see Table 10.2). On this basis, in the present study, we deliberately selected ergotamine, lisuride, clozapine and mesulergine as potential antagonists because they show either high affinity (the former three) or relative selectivity (the latter) for the cloned 5-ht$_7$ receptor (see Hoyer *et al*., 1994). Accordingly, all of these compounds blocked the 5-CT-, 5-HT- and 5-MeO-T-induced tachycardic responses, though this blockade appeared to be highly specific only for lisuride. Indeed, ergotamine, lisuride, mesulergine or clozapine have been reported to antagonise smooth muscle relaxant responses mediated by other functional 5-HT$_7$ receptors (see Chapter 10; Carter *et al*., 1995; Martin & Wilson, 1995; Terrón, 1996b; Villalón *et al*., 1997a).

Admittedly, there are no selective agonists and antagonists at cloned 5-ht_7 receptors available so far. Thus, although we recognise that the antagonists blocking 5-HT-induced feline tachycardia display varying degrees of affinity for receptors other than 5-HT, including α (α_1 and α_2)-adrenoceptors, H_2 histaminergic and muscarinic receptors (Leysen, 1985), the agonists used in the present study do not interact with these receptors; most significantly, these and other mechanisms in 5-HT-induced feline tachycardia (including stimulation of cardiac β-adrenoceptors and the indirect release of catecholamines) have already been excluded (Saxena *et al*., 1985b).

In conclusion, it is suggested that the tachycardic effect of 5-HT in the cat is mediated by a receptor similar to the cloned 5-ht_7 subtype. Since this cardiac 5-HT receptor represents another functional correlate of the 5-ht_7 gene product, the change of the appellation 5-ht_7 into 5-HT_7 is favoured (Eglen *et al*., 1997; Saxena *et al*., 1998b). Although species variations should not be dismissed (Saxena & Villalón, 1991), the present findings in the cat seem to be the first to show a tachycardic effect being mediated by the 5-HT_7 receptor. This *in vivo* experimental model, which is not complicated by the presence of other 5-HT receptors, can be utilised to characterise and develop, including the possibility of studying oral absorption, new drugs with potential agonist and antagonist properties at functional 5-HT_7 receptors.

Chapter 13

The 5-HT_1-like receptors mediating inhibition of sympathetic vasopressor outflow in the pithed rat: operational correlation with the 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} subtypes

Summary It has been suggested that the inhibition of sympathetically-induced vasopressor responses produced by 5-HT in pithed rats is mediated by 5-HT_1-like receptors. The present study has reanalysed this suggestion considering the classification schemes recently proposed by the NC-IUPHAR subcommittee on 5-HT receptors. Intravenous (i.v.) continuous infusions of 5-HT and the 5-HT_1 receptor agonists, 8-OH-DPAT (5-HT_{1A}), indorenate (5-HT_{1A}), CP93129 (5-HT_{1B}) and sumatriptan (5-$HT_{1B/1D}$), resulted in a dose-dependent inhibition of sympathetically-induced vasopressor responses. The sympatho-inhibitory responses induced by 5-HT, 8-OH-DPAT, indorenate, CP93129 or sumatriptan were analysed before and after i.v. treatment with blocking doses of the putative 5-HT receptor antagonists, WAY100635 (5-HT_{1A}), cyanopindolol (5-$HT_{1A/1B}$) or GR127935 (5-$HT_{1B/1D}$). Thus, after WAY100635, the responses to 5-HT and indorenate, but not to 8-OH-DPAT, CP93129 and sumatriptan, were blocked. After cyanopindolol, the responses to 5-HT, indorenate and CP93129 were abolished, whilst those to 8-OH-DPAT and sumatriptan (except at the lowest frequency of stimulation) remained unaltered. In contrast, after GR127935, the responses to 5-HT, CP93129 and sumatriptan, but not to 8-OH-DPAT and indorenate, were abolished. In additional experiments, the inhibition induced by 5-HT was not modified after 5-HT_7 receptor blocking doses of mesulergine. The above results suggest that the 5-HT_1-like receptors, which inhibit the sympathetic vasopressor outflow in pithed rats, display the pharmacological profile of the 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D}, but not that of 5-HT_7, receptors.

13.1 Introduction

The complexity of cardiovascular effects produced by 5-HT, including bradycardia or tachycardia, hypotension or hypertension and vasodilatation or vasoconstriction, has been explained by the capability of the monoamine to interact with specific receptors in the central nervous system (CNS), on the autonomic ganglia and postganglionic nerve endings, on vascular smooth muscle and endothelium and on the cardiac tissue (see Saxena & Villalón, 1990; 1991; Martin, 1994; Jones *et al*., 1995); in addition, the

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advent of selective 5-HT receptor agonists and antagonists has revealed that the cardiovascular effects of 5-HT may be mediated by 5-HT$_1$ (including the 5-HT$_{1A}$, 5-HT$_{1B}$ and/or 5-HT$_{1D}$ subtypes), 5-HT$_2$, 5-HT$_3$, 5-HT$_4$ and 5-HT$_7$ receptors (see Villalón *et al*., 1997b; Saxena *et al*., 1998b).

With respect to the capability of 5-HT to interfere with sympathetic transmission, it has been shown that the monoamine inhibits, via prejunctional 5-HT$_1$-like receptors, the contractile responses to adrenergic nerve stimulation in several blood vessels including, amongst others, the canine (Humphrey *et al*., 1988) and human (Göthert *et al*., 1990) saphenous veins, the canine external carotid bed (Villalón & Terrón, 1994a) and the rat vena cava (Molderings *et al*., 1987). The subsequent pharmacological analysis of these prejunctional 5-HT$_1$-like receptors (see Hoyer *et al*., 1994; Hartig *et al*., 1996) shows a correlation with either the 5-HT$_{1B}$ subtype (cyanopindolol-sensitive) in the case of rodents (e.g. Molderings *et al*., 1987) or the 5-HT$_{1B/1D}$ subtypes (GR127935-sensitive) in the case of non-rodent species (e.g. Molderings *et al*., 1990). These studies, however, do not prove if the above sympatho-inhibitory receptors are operative in the systemic vasculature; in this respect, we have shown, producing selective stimulation of the sympathetic vasopressor outflow in pithed rats, that 5-HT does indeed inhibit the sympathetically-induced vasopressor responses, but not those by exogenous noradrenaline (Villalón *et al*., 1995b). Since this sympatho-inhibitory response to 5-HT, being potently mimicked by 5-CT, is not modified by ritanserin, MDL72222 or tropisetron, but it is blocked by methysergide, we suggested the involvement of sympatho-inhibitory 5-HT$_1$-like receptors (Villalón *et al*., 1995a).

Nevertheless, the functional 5-HT$_1$-like receptors (see Hoyer *et al*., 1994) can, at present, be reclassified into 5-HT$_{1B/1D}$ receptors (stimulated by 5-CT and sumatriptan; blocked by GR127935, but not by mesulergine) and 5-HT$_7$ receptors (potently stimulated by 5-CT, but not by sumatriptan; blocked by mesulergine, but not by GR127935) (see Eglen *et al*., 1997; Saxena *et al*., 1998b). On this basis, the present study was carried out to reanalyse the pharmacological profile of the above sympatho-inhibitory 5-HT$_1$-like receptors. Hence, the drugs employed included, in addition to the endogenous ligand (5-HT), agonists and/or antagonists at 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1D}$ and 5-HT$_7$ receptors.

13.2 Methods

13.2.1 General

Experiments were carried out in a total of 164 male Wistar rats (250-300 g). After anaesthesia with ether and cannulation of the trachea, the rats were pithed by inserting a stainless steel rod through the orbit and foramen magnum into the vertebral foramen (Shipley & Tilden, 1947). The animals were artificially ventilated with room air using an Ideal Palmer pump (56 strokes min^{-1}; volume: 20 ml kg^{-1}). Subsequently, the pithing rod was replaced by an electrode, enamelled except for 1 cm length 9 cm from the tip, so that the uncovered segment was situated at T_7-T_9 of the spinal cord to stimulate the thoracic sympathetic nerves supplying the systemic vasculature (Gillespie *et al*., 1970). After bilateral vagotomy, catheters were placed in the left and right femoral veins, for the infusion of agonists and for the administration of antagonists respectively, and the left carotid artery, connected to a Statham pressure transducer (P23 ID), for the recording of arterial blood pressure. Heart rate was measured with a tachograph (7P4F, Grass Instrument Co., Quincy, MA, USA) triggered from the blood pressure signal. Both blood pressure and heart rate were recorded simultaneously by a model 7D Grass polygraph (Grass Instrument Co., Quincy, MA, USA). Prior to electrical stimulation, the animals received gallamine (25 mg kg^{-1}, i.v.) to avoid electrically-induced muscular twitching.

Since the sympatho-inhibitory effects of 5-HT are particularly more pronounced at lower frequencies of stimulation, all animals were systematically pretreated with 50 µg kg^{-1} (i.v.) of desipramine (to block reuptake of noradrenaline) before each stimulus-response curve (S-R curve). Under these conditions, as previously reported (Villalón *et al*., 1995a; 1995b): (i) the resulting vasopressor responses to lower frequencies of stimulation are greater in magnitude when compared to those elicited in rats without desipramine; and (ii) the potentiating effect of desipramine on the sympathetically-induced vasopressor responses does not wear off with time during the experiment.

13.2.2 Experimental protocol

After a stable haemodynamic condition for at least 30 min, baseline values of diastolic blood pressure and heart rate were determined. The sympathetic vasopressor outflow was stimulated by applying trains of 10 sec, consisting of monophasic rectangular pulses of 2 ms duration and 50 V, at increasing frequencies (0.03, 0.1, 0.3, 1 and

3 Hz); the S-R curve was completed in about 30 min. Then, one group of 20 animals (out of the 164) was subdivided into five subgroups (n=4 each) which received, respectively, i.v. continuous infusions of 5-HT (1.8, 3.0 and 5.6 μg kg^{-1} min^{-1}), 8-OH-DPAT, indorenate, CP93129 and sumatriptan (10, 30 and 100 μg kg^{-1} min^{-1} each) by a Harvard model 901 pump (Harvard Apparatus Co. Inc., Millis, MA, USA). Twenty min after the start of each infusion, a S-R curve was elicited as described above *during* the infusion of each agonist dose.

The remaining 144 animals were divided into 6 groups (see Figure 13.1) which received continuous i.v. infusions of physiological saline (0.01 ml min^{-1}; n=27), 5-HT (5.6 μg kg^{-1} min^{-1}; n=29), 8-OH-DPAT (30 μg kg^{-1} min^{-1}; n=20), indorenate (30 μg kg^{-1} min^{-1}; n=20), CP93129 (100 μg kg^{-1} min^{-1}; n=24) or sumatriptan (100 μg kg^{-1} min^{-1}; n=24) using the aforementioned pump. Twenty min after the start of the infusion, a S-R curve was elicited as previously described *during* the infusion of saline or the corresponding agonist. Once the S-R curve was completed (about 30 min), the infusions were stopped, except for the group receiving the infusion of 8-OH-DPAT, which was continuous until the end of the experiment (see Results section). Thus, in the 5 groups, where the infusion was stopped immediately after completing the S-R curve, the total period of infusion was of 50 min. At this point, the six groups were subsequently subdivided on the basis of treatment with antagonists (see Figure 13.1).

The first group, infused with saline, was subdivided into 5 treatment groups (see Figure 13.1) comprising i.v. bolus injections of: saline (0.3, 1, 3 and 10 ml kg^{-1}; n=7), WAY100635 (3, 10, 30 and 100 μg kg^{-1}; n=6), cyanopindolol (100, 300 and 1000 μg kg^{-1}; n=6), GR127935 (100, 300 and 1000 μg kg^{-1}; n=4) or mesulergine (300 μg kg^{-1}; n=4) in order to analyse their effects on the sympathetically-induced vasopressor responses *per se*. Ten min after each dose of saline or the corresponding antagonist, a S-R curve was elicited again. The second group, infused with 5-HT, was similarly subdivided into 5 treatment groups (see Figure 13.1) comprising i.v. bolus injections of: saline (0.3, 1 and 3 ml kg^{-1}; n=7), WAY100635 (10 μg kg^{-1}; n=6), cyanopindolol (100 μg kg^{-1}; n=6), GR127935 (100 μg kg^{-1}; n=6) or mesulergine (300 μg kg^{-1}; n=4); ten min after each treatment, a S-R curve was elicited again.

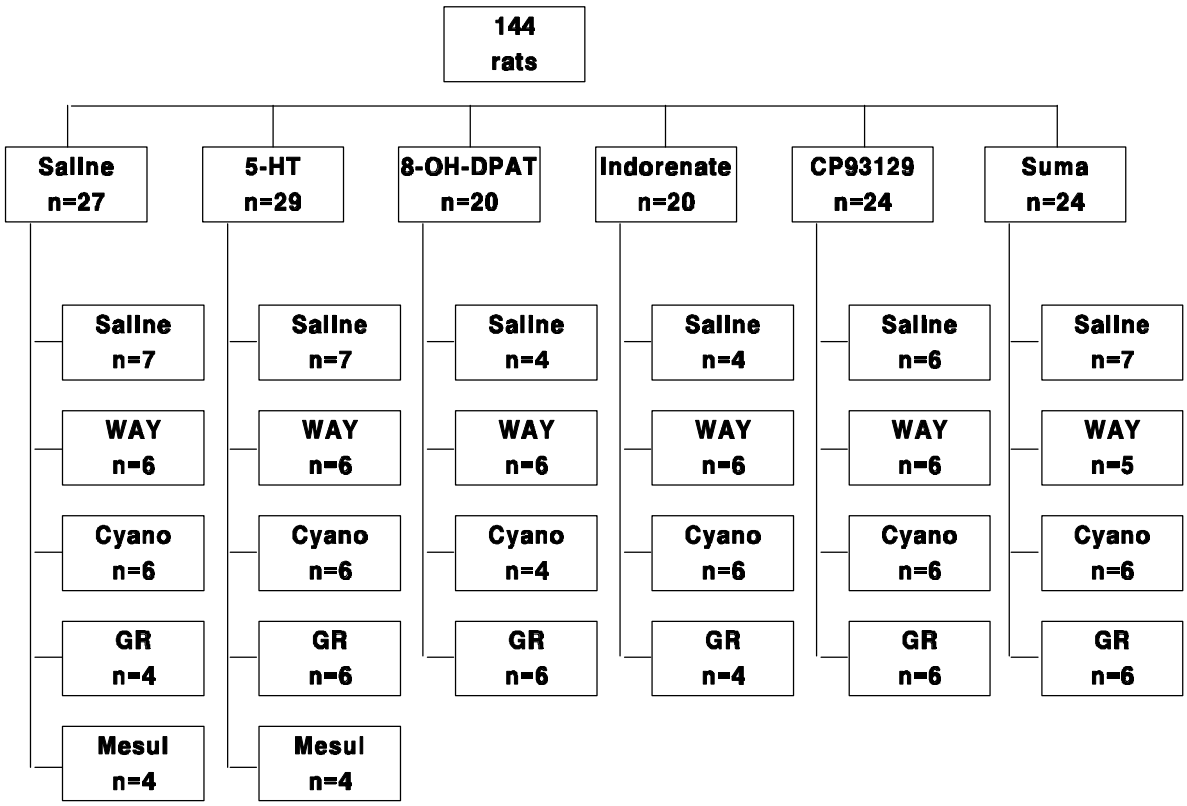

Figure 13.1 Experimental protocols showing the number of animals used in the 6 main groups (receiving infusions of physiological saline or agonist infusions) and the different subgroups (receiving i.v. bolus injections of physiological saline or antagonists).

As shown in Figure 13.1, each of the other four groups, infused with 8-OH-DPAT, indorenate, CP93129 or sumatriptan, was systematically subdivided into 4 treatment subgroups comprising, respectively, i.v. bolus injections of saline (0.3, 1 and 3 ml kg^{-1}), WAY100635 (10 µg kg^{-1} and, in some cases, 30 µg kg^{-1} when the preceding dose failed to block the sympatho-inhibition induced by the corresponding agonist), cyanopindolol (100 µg kg^{-1}) or GR127935 (100 µg kg^{-1}). Ten min after each treatment a S-R curve was elicited again (for number of experiments, see Figure 13.1). The reason for giving three or, as in the case of the first group, four different dose volumes of physiological saline as controls is that a single concentration of antagonist solution was used, so that increasing dose volumes were used to increase the dose of antagonist rather than using a fixed dose volume and increasing concentrations of antagonist solution.

The doses of 5-HT, indorenate, CP93129 or sumatriptan were infused at a rate of 0.01 ml min^{-1} during a total period of 50 min, at which time the corresponding S-R curve had already been completed. The dose of 8-OH-DPAT, although infused at the same rate, was continuous until the end of the experiment because in preliminary studies (not shown) we observed that the interruption of the infusion abolished the sympatho-inhibition produced by 8-OH-DPAT. The doses of 5-HT, 8-OH-DPAT, indorenate, CP93129 and sumatriptan were selected on the basis of results obtained from preliminary experiments, in which reproducible and consistent inhibitory effects on the S-R curves were elicited with no changes in baseline diastolic blood pressure or heart rate (Villalón *et al*., 1995a; 1995b). The dosing with all compounds used was sequential.

13.2.3 Drugs

Apart from the anaesthetic (diethyl ether), the drugs used in the study (obtained from the sources indicated) were: 5-HT creatinine sulphate and gallamine triethiodide (Sigma Chemical Co., St. Louis, MO, USA); indorenate hydrochloride (gift: Prof. Enrique Hong, CINVESTAV-IPN, Mexico City, Mexico); CP93129 (gift: Pfizer Inc., Groton, USA); 8-OH-DPAT, WAY100635 and desipramine hydrochloride (Research Biochemicals Int., Natick, MA, USA); GR127935 and sumatriptan succinate (gifts: Dr. Simon Lister and Dr. Helen Connor, GlaxoWellcome, Stevenage, Hertfordshire, UK); cyanopindolol and mesulergine hydrochloride (gifts: Sandoz AG, Basel, Switzerland). All compounds were dissolved in distilled water. When needed, 1% (w v^{-1}) ascorbic acid (CP93129) was added; this vehicle had no effect on baseline diastolic blood pressure or heart rate. The doses mentioned in the text refer to the salts of substances except in the case of 5-HT, 8-OH-DPAT, indorenate, CP93129 and sumatriptan, where they refer to the free base.

13.2.4 Data presentation and statistical analysis

All data in the text and figures are presented as the mean±s.e.mean. The peak changes in diastolic blood pressure produced by electrical stimulation in saline- and agonist-infused animals were determined. The difference between the changes in diastolic blood pressure within one subgroup of animals was evaluated with Student-Newman-Keuls' test, once an analysis of variance (randomised block design)

had revealed that the samples represented different populations (Steel & Torrie, 1980). A P-value of 0.05 or less (two-tailed) was considered statistically significant.

13.3 Results

13.3.1 Systemic haemodynamic variables

The baseline values of diastolic blood pressure and heart rate in the 164 rats were, respectively, 59.9±0.3 mmHg and 290±3.4 beats min^{-1}. Following the first i.v. bolus injection of desipramine (50 µg kg^{-1}) these values were transiently increased, but after 8 min they did not significantly differed from the baseline values (i.e. 61.2±0.6 mmHg and 292±3.3 beats min^{-1}, respectively).

The latter values in desipramine-pretreated rats were not significantly modified (data not shown) by: (i) the continuous infusion of physiological saline (0.01 ml min^{-1}), 5-HT (1.8, 3.0 and 5.6 µg kg^{-1} min^{-1}), 8-OH-DPAT, indorenate, CP93129 or sumatriptan (10, 30 and 100 µg kg^{-1} min^{-1} each); (ii) the i.v. bolus injections of saline (0.3, 1, 3 and 10 ml kg^{-1}), WAY100635 (3, 10, 30 and 100 µg kg^{-1}), GR127935 (100, 300 and 1000 µg kg^{-1}) or mesulergine (300 µg kg^{-1}); or (iii) the subsequent treatments with desipramine (50 µg kg^{-1} each).

In contrast, the administration of cyanopindolol (100, 300 and 1000 µg kg^{-1}, i.v.) to the control animals infused with saline (0.01 ml min^{-1}) resulted in changes in heart rate of, respectively, +20±7, +34±8 and -2±3 beats min^{-1}, but diastolic blood pressure was not significantly modified. Similarly, in the animals infused with the agonists at the above rates, cyanopindolol (100 µg kg^{-1}, i.v.) produced an increase in heart rate of, respectively, 37±2 (5-HT), 30±9 (8-OH-DPAT), 22±3 (indorenate), 28±5 (CP93129) and 22±8 (sumatriptan) beats min^{-1}.

13.3.2 Initial effects produced by electrical stimulation of the preganglionic (T_7-T_9) sympathetic nerves on blood pressure and heart rate

The onset of the responses induced by stimulation of the sympathetic vasopressor outflow was immediate and resulted in frequency-dependent increases in diastolic pressure (see Figures 13.2-13.5). These vasopressor responses are due to selective stimulation of the systemic vasculature since only negligible (if any) changes in heart rate were observed, as reported by other authors (e.g. Gillespie *et al.*, 1970; Flavahan *et al.*, 1985; Grant & McGrath, 1988).

13.3.3 Effect of 5-HT and the 5-HT$_1$ receptor agonists, 8-OH-DPAT, indorenate, CP93129 or sumatriptan, on the sympathetically-induced vasopressor responses

Figure 13.2 shows that continuous infusions of 5-HT (1.8, 3.0 and 5.6 µg kg^{-1} min^{-1}) produced a dose-dependent inhibition on sympathetically-induced vasopressor responses, as previously observed (Villalón *et al*., 1995b). This sympatho-inhibitory response to 5-HT was dose-dependently mimicked by the continuous infusions of the 5-HT$_1$ receptor agonists, 8-OH-DPAT, indorenate, CP93129 and sumatriptan (10, 30 and 100 µg kg^{-1} min^{-1} each; see Figure 13.2). This inhibition was, in all cases, significantly more pronounced at lower frequencies of stimulation (0.03-1 Hz). Comparatively, 5-HT was about 1 log unit more potent than the above agonists in its ability to produce sympatho-inhibition.

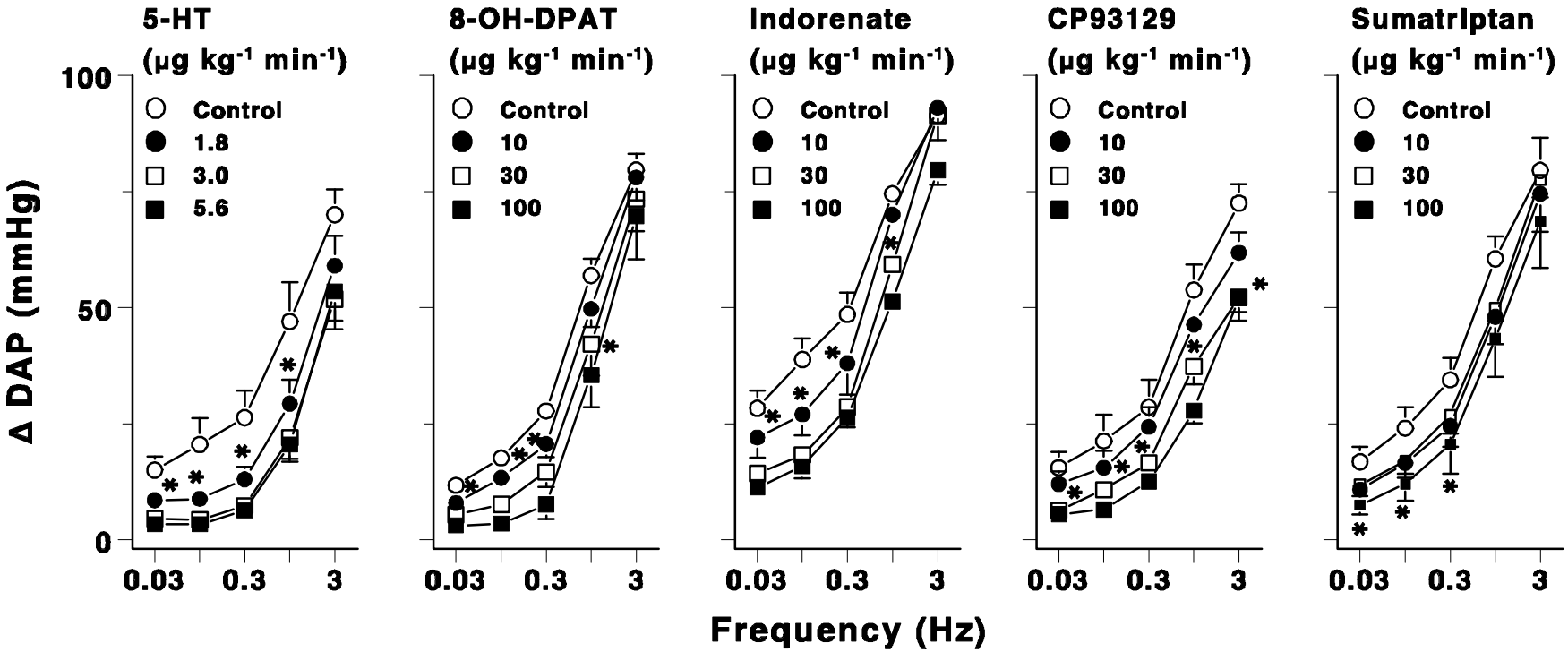

Figure 13.2 Increases in diastolic blood pressure (Δ DBP) produced by electrical stimulation of the sympathetic vasopressor outflow in pithed rats before (control) and during the i.v. continuous infusions of 5-HT, 8-OH-DPAT, indorenate, CP93129 and sumatriptan. *, P<0.05 vs. control. All the other graphs after the starred (*) graph are also significantly different from control.

13.3.4 Effect of physiological saline, WAY100635, cyanopindolol, GR127935 or mesulergine on the sympathetically-induced vasopressor responses per se and on the inhibition of sympathetically-induced vasopressor responses produced by 5-HT

Figure 13.3 (*upper panels*) shows the effects of i.v. bolus injections of physiological saline or the 5-HT receptor antagonists on the sympathetically-induced vasopressor responses *per se* in animals infused with saline (0.01 ml min^{-1} i.v. during 50 min). After stopping the infusion of saline, these vasopressor responses, which remained essentially unchanged in control animals receiving four subsequent i.v. bolus injections of saline (0.3, 1, 3 and 10 ml kg^{-1}), were not significantly modified by either the lower doses of WAY100635 (3, 10 and 30 µg kg^{-1}), cyanopindolol (100 and 300 µg kg^{-1}) or all doses tested of GR127935 (100, 300 and 1000 µg kg^{-1}) and mesulergine (300 µg kg^{-1}). In contrast, the highest doses of WAY100635 (100 µg kg^{-1}) and cyanopindolol (1000 µg kg^{-1}) produced a significant inhibition on the S-R curves which was, coincidentally, more pronounced at lower frequencies of stimulation (Figure 13.3, *upper panels*).

Figure 13.3 (*lower panels*) shows the effects produced by i.v. bolus injections of physiological saline (0.3, 1 and 3 ml kg^{-1}), WAY100635 (10 µg kg^{-1}), cyanopindolol (100 µg kg^{-1}), GR127935 (100 µg kg^{-1}) and mesulergine (300 µg kg^{-1}) on the inhibition of sympathetically-induced vasopressor responses produced by the infusion of 5-HT (5.6 µg kg^{-1} min^{-1} i.v. during 50 min). Under these experimental conditions, the sympatho-inhibition induced by the infusion of 5-HT was reproducible since even after stopping the infusion, the corresponding S-R curves remained without significant changes when the animals received three subsequent bolus injections of physiological saline (Figure 13.3, *lower panels*). In contrast, when analysing the effects of antagonists, the response to 5-HT was practically abolished after administration of WAY100635, cyanopindolol or GR127935, but was unaffected by mesulergine (Figure 13.3, *lower panels*).

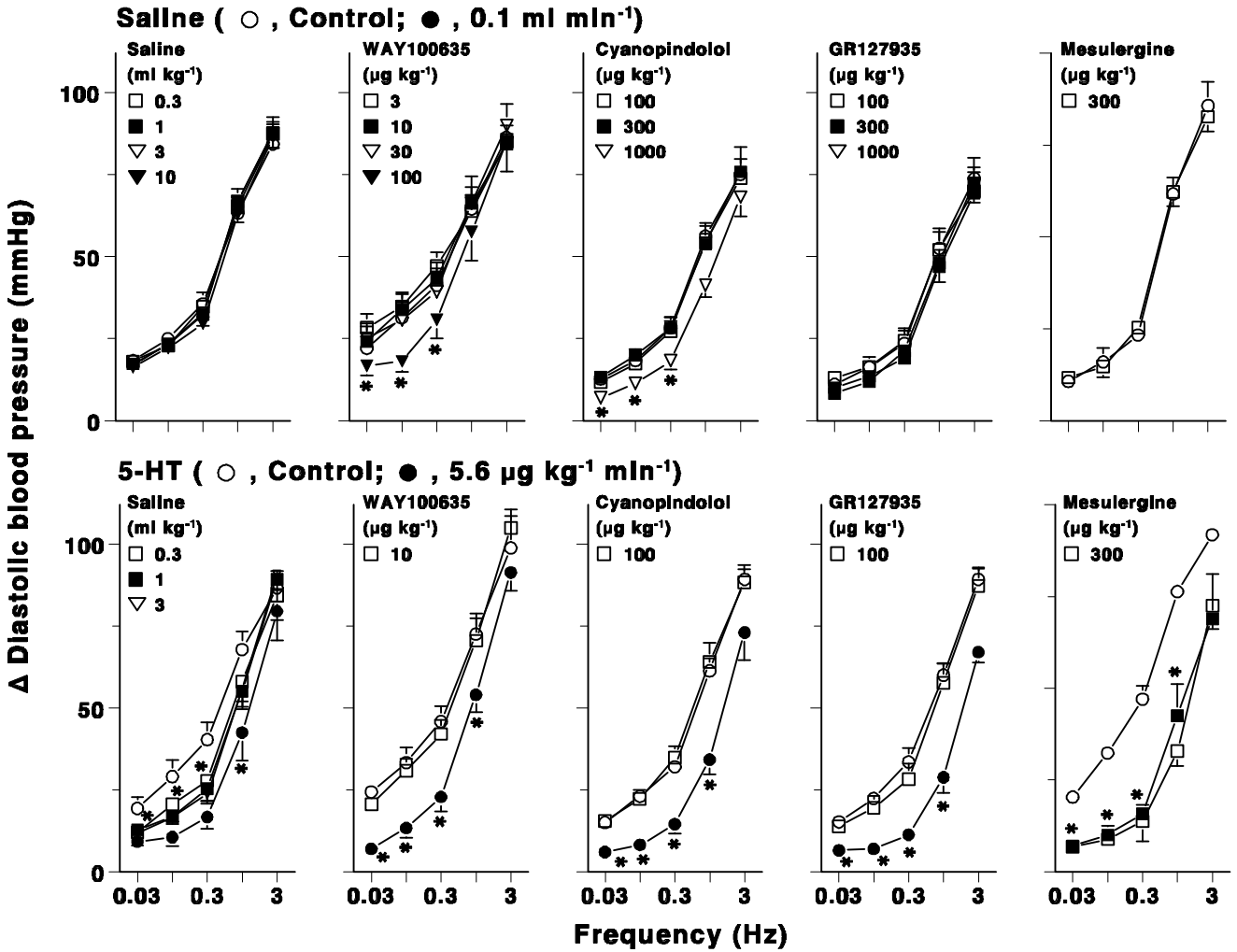

Figure 13.3 Effects of i.v. bolus injections of saline, WAY 100635, cyanopindolol, GR127935 or mesulergine on either: (*upper panels*) the increases in diastolic blood pressure by stimulation of the sympathetic vasopressor outflow in animals infused (i.v.) with saline (during 50 min); or (*lower panels*) the corresponding inhibition by the i.v. infusion of 5-HT (during 50 min) of sympathetically-induced vasopressor responses. The above doses of antagonists (or saline) were injected after stopping the infusion of saline (*upper panels*) or 5-HT (*lower panels*) and the S-R curves were elicited after each dose. *, $P<0.05$ vs control. All the other graphs after the starred (*) graph are also significantly different from control.

13.3.5 Effect of physiological saline, WAY100635, cyanopindolol or GR127935 on the inhibition of sympathetically-induced vasopressor responses produced by some 5-HT$_1$ receptor agonists

Figures 13.4 and 13.5 show the effects of physiological saline (0.3, 1 and 3 ml kg^{-1}), WAY100635 (10 and/or 30 µg kg^{-1}), cyanopindolol (100 µg kg^{-1}) or GR127935 (100 µg kg^{-1}) on the inhibition of sympathetically-induced vasopressor responses induced by 8-OH-DPAT (30 µg kg^{-1} min^{-1}; Figure 13.4, *upper panels*), indorenate (30 µg kg^{-1} min^{-1}; Figure 13.4, *lower panels*), CP93129 (100 µg kg^{-1} min^{-1}; Figure 13.5, *upper panels*) and sumatriptan (100 µg kg^{-1} min^{-1}; Figure 13.5, *lower panels*). As previously observed with 5-HT, the sympatho-inhibition produced by the continuous infusion (during 50 min) of indorenate, CP93129 and sumatriptan was reproducible since even after stopping the infusion, the corresponding S-R curves remained without significant changes when the animals received three subsequent bolus injections of physiological saline.

In the case of the inhibition produced by 8-OH-DPAT (Figure 13.4, *upper panels*), there was an important difference in the experimental protocol because in preliminary experiments (not shown) we observed that the infusion of 8-OH-DPAT, in contrast to that of the other agonists, produced a short-lasting sympatho-inhibition which was simply abolished when interrupting the infusion. That is why the infusion of 8-OH-DPAT had to be kept continuous until the end of the experiments. Under these conditions, the response to 8-OH-DPAT was reproducible since the corresponding S-R curves remained without significant changes when the animals received three subsequent bolus injections of physiological saline (Figure 13.4, *upper panels*).

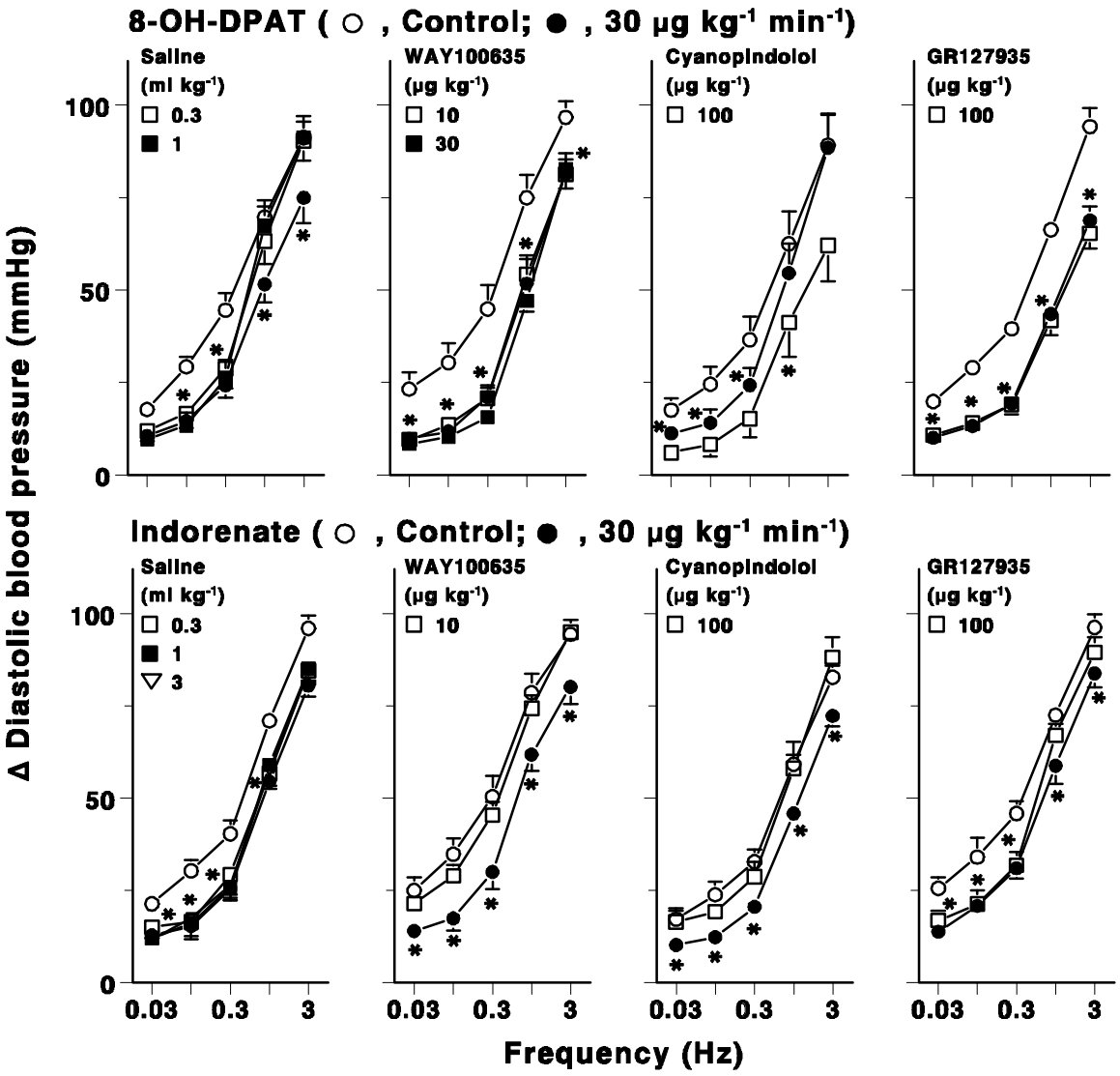

Figure 13.4 Effects of i.v. bolus injections of saline, WAY 100635, cyanopindolol or GR127935 on the inhibition of sympathetically-induced vasopressor responses induced by: (*upper panels*) 8-OH-DPAT (infused uninterruptedly); and (*lower panels*) indorenate (during 50 min). The above doses of antagonists (or saline) were injected either after concluding each S-R during the infusion of 8-OH-DPAT (*upper panels*) or after stopping the infusion of indorenate (*lower panels*); the S-R curves were elicited after each dose. *, $P<0.05$ vs control response. All the other graphs after the starred (*) graph are also significantly different from control.

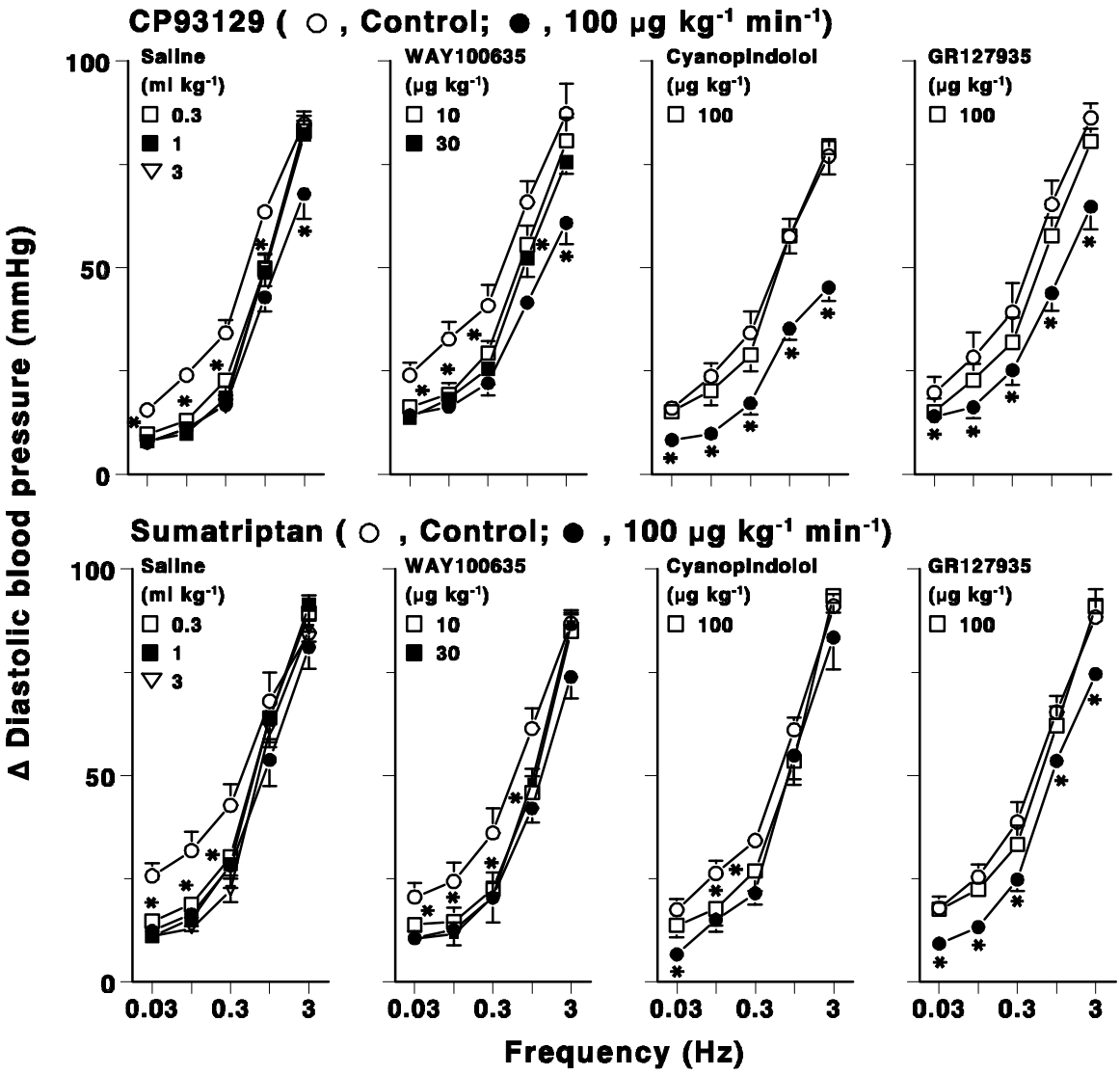

Figure 13.5 Effects of i.v. bolus injections of saline, WAY100635, cyanopindolol or GR127935 on the inhibition of sympathetically-induced vasopressor responses induced by the i.v. continuous infusion (during 50 min) of: (*upper panels*) CP93129; and (*lower panels*) sumatriptan. The above doses of antagonists (or saline) were injected after stopping the infusion of CP93129 or sumatriptan, and the S-R curves were elicited after each dose. *, P<0.05 vs control. All the other graphs after the starred (*) graph are also significantly different from control.

When analysing the effects of the antagonists, differential effects were observed on the sympatho-inhibition produced by each agonist. Thus, Figure 13.4 (*upper panels*) shows that the sympatho-inhibition produced by 8-OH-DPAT was practically resistant to blockade by WAY100635 (10 and 30 μg kg^{-1}), cyanopindolol (100 μg kg^{-1}) and GR127935 (100 μg kg^{-1}). Similarly, in additional experiments (not reported for the sake of clarity in the experimental protocol and in the figures), the inhibition by 8-OH-DPAT was not significantly modified after administration of the α_2-adrenoceptor antagonist, rauwolscine (1000 μg kg^{-1} i.v.), either alone (n=6) or in combination with WAY100635 (30 μg kg^{-1} i.v., n=4).

In contrast, the response to indorenate (Figure 13.4, *lower panels*) was virtually abolished by WAY100635 (10 μg kg^{-1}) and cyanopindolol (100 μg kg^{-1}), but not by GR127935 (100 μg kg^{-1}). With respect to the sympatho-inhibition produced by CP93129 and sumatriptan, it is evident that the responses to CP93129 (Figure 13.5, *upper panel*s) and sumatriptan (Figure 13.5, *lower panels*) were blocked by GR127935 (100 μg kg^{-1}), but remained unchanged after WAY100635 (10 and 30 μg kg^{-1}). Furthermore, after cyanopindolol, the responses to CP93129 were abolished and those to sumatriptan were blocked only at 0.03 Hz, but remained unaffected at the other frequencies studied (0.1-3 Hz).

13.4 Discussion

The aim of this study was to re-analyse the pharmacological profile of the "5-HT_1-like" receptors mediating the inhibition of sympathetically-induced vasopressor responses in pithed rats considering the classification schemes proposed by the NC-IUPHAR subcommittee on 5-HT receptors (see Hoyer *et al*., 1994; Hartig *et al*., 1996; Saxena *et al*., 1998b). Within this framework, we have to admit that our study did not measure sympathetic nerve activity directly, but the electrically-induced neurotransmitter release could be estimated indirectly by measurement of the evoked vasopressor response. Under these experimental conditions, the responses to 5-HT were considered to be (sympatho)-inhibitory on the basis that the monoamine is capable of inhibiting the vasopressor responses induced by preganglionic stimulation (T_7-T_9) of the sympathetic vasopressor outflow, but not those by exogenous noradrenaline (Villalón *et al*., 1995b).

Thus, our study shows that the inhibition of sympathetically-induced vasopressor responses induced by 5-HT: (i) can be mimicked by agonists at 5-HT_{1A},

such as indorenate and 8-OH-DPAT (Dompert *et al*., 1985; Hoyer *et al*., 1994), 5-HT$_{1B}$ (CP93129; Macor *et al*., 1990) and 5-HT$_{1B/1D}$ (sumatriptan; Hoyer *et al*., 1994) receptors; and (ii) can be blocked by antagonists at 5-HT$_{1A}$ (WAY100635), 5-HT$_{1A/1B}$ (cyanopindolol) or 5-HT$_{1B/1D}$ (GR127935) receptors, but not by antagonists at cardiovascular 5-HT$_7$ (mesulergine) receptors (Hoyer *et al*., 1994; Fletcher *et al*., 1996; Skingle *et al*., 1996; Villalón *et al*., 1997b).

Apart from the implications discussed below, the present results suggest that the 5-HT-induced inhibition of sympathetically-induced vasopressor responses in pithed rats could be mediated by "5-HT$_1$-like" receptors the pharmacological profile of which correlates with some subtypes (5-HT$_{1A/1B/1D}$) of the 5-HT$_1$ receptor family, but not with the 5-HT$_7$ receptor.

With respect to our experimental model, some features deserve further consideration. Thus, though we produced selective stimulation of the sympathetic vasopressor outflow, propranolol has been recommended to eliminate vasodilatation due to catecholamine release from the adrenal medulla (Flavahan *et al*., 1985). However, we deliberately avoided using propranolol since it has affinity for some 5-HT$_1$ binding sites (Hoyer, 1988) and, indeed, blocks some "5-HT$_1$-like" receptor-mediated functional responses in the rat (Martin, 1994), as we have shown with another ß-adrenoceptor antagonist (cyanopindolol) in the present study. Moreover, the possible influences arising from the CNS via 5-HT mechanisms can be ruled out, since pithed rats were used.

13.4.1 Systemic haemodynamic changes

Considering the short bursts of activity which characterize sympathetic nerves *in vivo*, our results showing the potentiation of sympathetic vasopressor responses after desipramine (compare Flavahan *et al*., 1985; Bulloch & McGrath, 1988; Villalón *et al*., 1995a; Villalón *et al*., 1995b) have relevance for the purpose of the present study, since the prejunctional inhibitory effects of 5-HT (and of any other agonist drug) are, coincidentally, more pronounced at lower frequencies of stimulation (Langer, 1980; Göthert *et al*., 1990).

Hence, it could be alternatively argued that the marked inhibitory effects of 5-HT, 8-OH-DPAT, indorenate, CP93129 and sumatriptan may be due to tachyphylaxis of the sympathetically-induced vasopressor responses. However, this seems unlikely since such responses remained essentially unchanged when the

animals received three subsequent i.v. bolus injections of physiological saline (Figures 13.2, 13.3 and 13.4), as previously reported for 5-HT (Villalón *et al*., 1995a; Villalón *et al*., 1995b).

On the other hand, it is interesting to note that higher doses of WAY100635 (100 µg kg^{-1}) and cyanopindolol (1000 µg kg^{-1}) produced a significant inhibition on the S-R curves *per se* (Figure 13.3, *upper panels*). Since this inhibitory effect was, coincidentally, more pronounced at lower frequencies of stimulation, as previously reported for 5-HT (Villalón *et al*., 1995a; 1995b) and other 5-HT_1 receptor agonists (see Figure 13.2), it is tempting to suggest that at higher doses these compounds may have stimulated the 5-HT_1 receptors mediating (sympatho)-inhibition. Admittedly, further experiments will be required to document and evaluate this possibility.

13.4.2 Possible involvement of (sympatho)-inhibitory 5-HT_{1A} receptors

The possible correlation of the (sympatho)-inhibitory 5-HT_1-like receptors with the 5-HT_{1A} receptor subtype is established, in the first instance, by the use of WAY100635, a highly potent and selective 5-HT_{1A} receptor antagonist (Fletcher *et al*., 1996) and cyanopindolol, a putative 5-$HT_{1A/1B}$ receptor antagonist (Hoyer *et al*., 1994). At doses devoid of effects on sympathetically-induced vasopressor responses *per se* (Figure 13.3, *upper panels*), these compounds were capable of blocking the (sympatho)-inhibition induced by both 5-HT (the endogenous ligand; Figure 13.3, *lower panels*) and indorenate (Figure 13.4, *lower panels*), a 5-HT_{1A} receptor agonist (Dompert *et al*., 1985). Accordingly, the (sympatho)-inhibition by indorenate being resistant to blockade by GR127935, an antagonist at 5-$HT_{1B/1D}$ receptors (previously described as, respectively, the 5-$HT_{1Dß/1Dα}$ subtypes of the 5-HT_{1D} receptor) (Hartig *et al*., 1996) may be explained in terms of its low affinity for 5-HT_{1A} receptors (Skingle *et al*., 1996). It must be emphasized that the doses of the 5-HT receptor antagonists, WAY100635, cyanopindolol and GR127935 used in the present study were higher than those required to abolish functional responses mediated by their respective receptors (see Martin, 1994; Fletcher *et al*., 1996; Skingle *et al*., 1996; Villalón *et al*., 1996).

8-OH-DPAT, another 5-HT_{1A} receptor agonist (Hoyer *et al*., 1994), presumably also produced (sympatho)-inhibition in the pithed rat (Figure 13.4, *upper panels*), but this response, interestingly, was *not* blocked by WAY100635, cyanopindolol or GR127935. Although this finding, apparently, does not support the

involvement of 5-HT$_{1A}$ (and 5-HT$_{1B/1D}$) receptors, it is tempting to consider that 8-OH-DPAT, unlike indorenate, behaves as a partial agonist at the α-adrenoceptors mediating vasopressor responses in the pithed rat (Castillo *et al.*, 1994). Thus, it could be argued that the agonist properties of 8-OH-DPAT, particularly at sympatho-inhibitory α_2-adrenoceptors (Borton *et al.*, 1991) might have masked its agonist properties at 5-HT$_{1A}$ receptors; however, our preliminary findings showing the failure of the α_2-adrenoceptor antagonist, rauwolscine, either alone or in combination with WAY100635, to antagonize the inhibition by 8-OH-DPAT (see Results section) does not support this hypothesis.

In view that the inhibition by 8-OH-DPAT was resistant to blockade by all the antagonists investigated in the present study, it is prudent to keep in mind that 8-OH-DPAT, in contrast to the other agonists, had to be infused continuously until the end of the experiments because its inhibitory response was abolished when interrupting the infusion (see Results section). Thus, it seems more likely that under conditions of an uninterrupted infusion, the level of 8-OH-DPAT in the biophase was so high that it may have overshadowed any potential antagonism towards 5-HT$_{1A}$ (and probably also 5-HT$_{1B/1D}$ and/or α_2) receptors. In any case, the pharmacological properties of 8-OH-DPAT emphasize the importance of being cautious when characterizing the operational profile of prejunctional autonomic 5-HT receptors, particularly in rats.

13.4.3 Possible involvement of (sympatho)-inhibitory 5-HT$_{1B}$ receptors

Evidence for the involvement of 5-HT$_{1B}$ receptors stems from the capability of cyanopindolol, a putative 5-HT$_{1A/1B}$ receptor antagonist (Hoyer *et al.*, 1994), to block the (sympatho)-inhibition induced by 5-HT (Figure 13.3, *lower panels*) and by the selective rodent 5-HT$_{1B}$ receptor agonist, CP93129 (Macor *et al.*, 1990, Figure 13.5, upper panels). Thus, the (sympatho)-inhibition by CP93129, being resistant to blockade by WAY100635 (at doses even higher than those required to block the (sympatho)-inhibition by 5-HT and indorenate; Figure 13.5, *upper panels*), confirms the high selectivity of the latter as a 5-HT$_{1A}$ receptor antagonist (Fletcher *et al.*, 1996). A relevant finding was also the blockade of CP93129-induced (sympatho)-inhibition by GR127935 since this compound is now apparently considered as an antagonist at non-rodent 5-HT$_{1B/1D}$ receptors (Hartig *et al.*, 1996; Villalón *et al.*, 1997b). Notwithstanding, binding data show that GR127935 has similar affinities for 5-HT$_{1Dα}$

(pK_i: 8.9; non-rodent 5-HT_{1D}), 5-$HT_{1Dß}$ (pK_i: 9.9; non-rodent 5-HT_{1B}) and 5-HT_{1B} (pK_i: 8.5; rodent 5-HT_{1B}) receptors (Skingle *et al*., 1996). Thus, the simplest interpretation suggests that the blockade of CP93129-induced (sympatho)-inhibition by GR127935 may be due to the high affinity of the latter for rodent 5-HT_{1B} receptors.

13.4.4 Possible involvement of (sympatho)-inhibitory 5-HT_{1D} , 5-ht_{1E} and/or 5-ht_{1F} receptors

Taking into consideration the classification criteria for 5-$HT_{1B/1D}$ receptors in rodent and non-rodent species (Hartig *et al*., 1996), our results with the 5-$HT_{1B/1D}$ receptor agonist, sumatriptan, suggest that rodent 5-HT_{1D} receptors could also be involved, as previously implied by Shepheard *et al.* (1997b). In keeping with this suggestion, the (sympatho)-inhibition produced by sumatriptan (see Figure 13.5, *lower panels*) was resistant to blockade by WAY100635 (at doses even higher than those required to block 5-HT and indorenate), a finding that excludes an action via 5-HT_{1A} receptors. Conversely, the 5-$HT_{1B/1D}$ receptor antagonist, GR127935, practically abolished the response to sumatriptan at all frequencies analysed (0.03-3 Hz), while cyanopindolol, which has a pK_D of 6.85 at 5-HT_{1D} compared with 8.28 at the 5-HT_{1B} receptor (Hoyer, 1988), had no significant effect at most of the frequencies studied (0.1-3 Hz). Although it is possible that, at this infusion rate (100 µg kg^{-1} min^{-1}), sumatriptan could have had a small effect, if any, at 5-HT_{1B} receptors, this was cyanopindolol-sensitive only at 0.03 Hz (Figure 13.5, *lower panels*).

In contrast with the above findings, the involvement of 5-ht_{1E} and 5-ht_{1F} receptors, although not categorically excluded due to the lack of potent and selective agonists and antagonists, seems unlikely on the basis of: (i) the relatively low affinity of WAY100635, cyanopindolol and GR127935 for these receptors (see Adham *et al*., 1993; Fletcher *et al*., 1996; Skingle *et al*., 1996); and (ii) the rank order of (sympatho)-inhibitory agonist potency of 5-CT>>5-HT>8-OH-DPAT (present results; Villalón *et al*., 1995a; 1995b). Except for 5-HT, which has the highest affinity for both these receptors, 5-CT and 8-OH-DPAT have very low affinity for 5-ht_{1E} or 5-ht_{1F} receptors (Adham *et al*., 1993).

13.4.5 Operational and transductional evidence against the involvement of 5-HT$_7$ receptors

Although there is no direct evidence that the inhibition produced by 5-HT, 8-OH-DPAT, indorenate, CP93129 and sumatriptan in our experiments involves inhibition of adenylyl cyclase, it is important to emphasize that all 5-HT$_1$ receptor subtypes are, by definition, negatively coupled to adenylyl cyclase (Hoyer *et al*., 1994), and this is a signal transduction system usually associated with the decrease in noradrenaline release from sympathetic neurones (Langer, 1980; Rand *et al*., 1987). Notwithstanding, given the above rank order of (sympatho)-inhibitory agonist potency of 5-CT>>5-HT>8-OH-DPAT, and the capability of methysergide, an antagonist at cardiovascular 5-HT$_1$ and 5-HT$_7$ receptors (Villalón *et al*., 1997b), to block the (sympatho)-inhibition induced by 5-HT (Villalón *et al*., 1995a), it could still be argued that 5-HT$_7$ receptors might be involved, as previously suggested for other cardiovascular 5-HT$_7$ receptors (De Vries *et al*., 1997b; Villalón *et al*., 1997a; 1997c). However, this is unlikely since mesulergine, an ergoline derivative devoid of interactions with the 5-HT$_1$ receptor family (Hoyer *et al*., 1994), at doses that are high enough to block cardiovascular 5-HT$_7$ receptors (De Vries *et al*., 1997b; Villalón *et al*., 1997a; 1997c), failed to antagonize the inhibition by 5-HT (Figure 13.3, *lower panels*). Consistent with this finding, the cardiovascular 5-HT$_7$ receptors, unlike 5-HT$_{1A/1B/1D}$ receptors are: (i) resistant to blockade by GR127935 and cyanopindolol and to the agonist action of indorenate, CP93129 and sumatriptan (De Vries *et al*., 1997b; Villalón *et al*., 1997a; 1997c); and (ii) positively coupled to adenylyl cyclase (Plassat *et al*., 1993), and this is a signal transduction system associated with an increase (not decrease) in the release of noradrenaline from sympathetic neurones (Langer, 1980; Rand *et al*., 1987).

13.4.6 Possible locus of the (sympatho)-inhibitory 5-HT$_{1A}$, 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors

Lastly, we would like to speculate upon the possible *locus* of the 5-HT$_{1A}$, 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors that mediate 5-HT-induced inhibition of sympathetically-induced vasopressor responses. In this context, although central mechanisms are not operative in our experimental model, we cannot categorically exclude an action of 5-HT and related agonists at both the sympathetic ganglia (Fozard, 1984b) and postganglionic

sympathetic neurons (Saxena & Villalón, 1990) which have modulatory 5-HT receptors.

Indeed, some studies suggest that 5-HT_1 receptors may be mediating inhibition (5-HT- and 5-CT-induced hyperpolarisation) of the sympathetic ganglionic transmission (Ireland & Jordan, 1987; Jones *et al*., 1995). These inhibitory ganglionic 5-HT_1 receptors closely resemble the 5-HT_{1A} subtype in rats (blocked by spiperone, cyanopindolol and 8-OH-DPAT) (Ireland & Jordan, 1987) or the 5-$HT_{1B/1D}$, but not the 5-HT_{1A}, subtypes in cats (blocked by GR127935, but resistant to WAY100635) (Jones *et al*., 1995). Although this apparent discrepancy may be due to a species difference, it must be highlighted that the possible role of inhibitory ganglionic 5-$HT_{1B/1D}$ receptors in rats will be unequivocally proven by the use of agonists (e.g. CP93129 and sumatriptan) and antagonists (e.g. cyanopindolol and GR127935) at these receptor subtypes.

Furthermore, the lines of pharmacological evidence available thus far have shown the existence of inhibitory 5-$HT_{1B/1D}$ (previously called 5-HT_1-like), but not 5-HT_{1A}, receptor subtypes on vascular sympathetic nerves (see Saxena & Villalón, 1990; Hoyer *et al*., 1994; Martin, 1994; Saxena *et al*., 1998b). With respect to the 5-$HT_{1B/1D}$ receptor subtypes (blocked by GR127935), one must keep in mind that species homologues of the same receptor can show major pharmacological differences.

For example, the rodent 5-HT_{1B} receptor (96% homology in the transmembrane region with the human 5-HT_{1B} receptor) displays the distinct pharmacology that has long been associated with the 5-HT_{1B} appellation (agonist: CP93129; antagonists: cyanopindolol and SDZ21009) (Hoyer *et al*., 1994; Hartig *et al*., 1996). Thus, on the basis of the moderate ability of ketanserin to discriminate between 5-HT_{1B} (ketanserin-resistant) and 5-HT_{1D} (ketanserin-sensitive) receptors, the inhibition of noradrenaline release from sympathetic neurons in human tissues has been attributed to both 5-HT_{1B} (e.g. the saphenous vein; Göthert *et al*., 1996) and 5-HT_{1D} (e.g. the right atrium; Molderings *et al*., 1996) receptors.

In contrast, in rat blood vessels, only 5-HT_{1B} receptors have been shown thus far to mediate the above inhibition of noradrenaline release (e.g. the vena cava; Molderings *et al*., 1987; Hoyer *et al*., 1994); to the best of our knowledge, there are no formally recognized functional roles for the 5-HT_{1D} receptor subtype on rat postganglionic sympathetic neurons. Hence, subtype-selective 5-HT_{1B} and 5-HT_{1D}

receptor ligands (see Saxena *et al*., 1998b) become crucial probes to explore whether or not sympathetic neuronal effects in the rat are mediated by the 5-HT$_{1D}$ subtype.

In conclusion, we suggest that the 5-HT-induced inhibition of sympathetically-induced vasopressor responses in the pithed rat is primarily mediated by 5-HT$_1$ receptors that resemble the pharmacological profile of the 5-HT$_{1A}$, 5-HT$_{1B}$ and 5-HT$_{1D}$ receptor subtypes, but not that of the 5-HT$_7$ receptor.

Chapter 14
General discussion

14.1 Nature of 5-HT receptors mediating porcine and canine carotid vasoconstrictor effects and sympathoinhibition in the rat *in vivo*

It has previously been demonstrated that 5-HT is able to induce a vasoconstriction within the external carotid vascular bed of the dog (Saxena *et al*., 1971; Saxena, 1972; Villalón *et al*., 1993b). Similarly, 5-HT constricts the porcine carotid vasculature. The resulting decrease in the carotid blood flow is exclusively confined to the arteriovenous anastomotic fraction (Saxena & Verdouw, 1982; Verdouw *et al*., 1984b). This 5-HT-induced cranial vasoconstriction in the dog and pig, but also the inhibition of sympathetically-induced blood pressure increases in the rat, were shown to be primarily mediated by sumatriptan-sensitive 5-HT_1-like receptors (Saxena *et al*., 1986; Den Boer *et al*., 1991b; Villalón *et al*., 1995a; 1995c). Although these 5-HT_1-like receptors showed some pharmacological resemblance to the 5-$HT_{1B/1D}$ receptor subtypes, this could not be substantiated mainly because of a lack of subtype selective agonists and/or antagonists. The development of selective 5-$HT_{1B/1D}$ receptor antagonists, in particular GR127935 (Clitherow *et al*., 1994; Pauwels, 1996; Skingle *et al*., 1996), proved to be of great importance. Indeed, the results of the experiments described in Chapter 3 clearly show that GR127935 is a potent antagonist at (5-HT_1) receptors mediating carotid vasoconstriction in the rabbit, but is devoid of interactions with functional 5-HT_3, 5-HT_4 or 5-HT_7 receptors. Consistent with its binding profile, however, GR127935 moderately interacts with 5-HT_2 receptors. As shown in Chapter 4, GR127935 completely antagonised the sumatriptan-induced vasoconstriction of porcine carotid arteriovenous anastomoses. Villalón *et al.* (1996) obtained similar results with GR127935 against the sumatriptan-induced decreases in canine external carotid blood flow. Therefore, these results imply that the sumatriptan-sensitive 5-HT_1-like receptors mediating cranial vasoconstriction are identical to the 5-$HT_{1B/1D}$ receptor subtypes. Additionally, GR127935 potently antagonised the porcine carotid vascular effects by the newer antimigraine agents, alniditan, eletriptan and GMC2021 (Chapter 6).

Subsequently, we showed that the receptors in the canine (Chapter 8) as well as porcine (Chapter 9) carotid vasculature were pharmacologically similar to the 5-HT_{1B} receptor, but not the 5-HT_{1D} receptor. This conclusion was based on the

findings that the selective 5-HT_{1B} receptor antagonist, SB224289 (Hagan *et al*., 1997), produced a complete blockade, whereas the selective 5-HT_{1D} receptor antagonist, BRL15572 (Schlicker *et al*., 1997), was devoid of effect. In keeping with the latter, 5-HT_{1B} receptors are abundantly expressed on vascular smooth muscle, whereas this is not the case for the 5-HT_{1D} receptor (Longmore *et al*., 1997; 1998). These findings represent the first *in vivo* evidence showing that vascular constriction induced by sumatriptan is mediated primarily via 5-HT_{1B}, but not 5-HT_{1D} receptors. Thus, SB224289 and BRL15572 seem to be excellent tools for further investigating the pharmacology of the 5-$HT_{1B/1D}$ receptors. Additionally, in view of the complete blockade by SB224289 and the highly selective nature of the compound, it seems unlikely that the other known 5-HT_1 subtypes (5-HT_{1A}, 5-ht_{1E} and 5-HT_{1F}) are involved in the sumatriptan-induced carotid vascular effects. Accordingly, LY344864, a selective 5-HT_{1F} receptor agonist (Phebus *et al*., 1997), does not produce vasoconstriction in the canine external carotid vascular bed (Chapter 7) or in isolated cranial arteries (Bouchelet & Hamel, 1999).

As described in Chapter 5, however, GR127935 did not completely block the ergotamine- or dihydroergotamine-induced constriction of porcine carotid arteriovenous anastomoses. In the dog carotid vasculature, we clearly show that the constriction induced by the above ergot derivatives is mediated by a combination of GR127935-sensitive 5-$HT_{1B/1D}$ receptors, as well as yohimbine-sensitive α_2-adrenoceptors (Chapter 7). These α_2-adrenoceptors may also have been involved in the GR127935-resistant part of the ergot-induced porcine carotid vascular effects. Indeed, Willems *et al.* (1999) have recently shown that stimulation of both α_1- and α_2-adrenoceptors results in potent vasoconstriction of porcine cranial arteriovenous anastomoses.

Interestingly, in Chapter 5 it was shown that 5-HT-induced constriction of porcine carotid arteriovenous anastomoses was not much affected by GR127935, using a dose that was capable of abolishing sumatriptan-induced effects. These results led us to conclude that 5-HT and sumatriptan act primarily via different receptor mechanisms. Thus, whereas sumatriptan causes cranial vasoconstriction via 5-HT_{1B} receptors, the receptor responsible for the effect of 5-HT cannot be classified using the current 5-HT receptor nomenclature scheme. This GR127935-insensitive receptor may correspond to the novel 5-HT receptor site found in human brain (see Chapter 1; Castro *et al*., 1997); obviously, this needs further investigation. In any case, since the

constriction of carotid arteriovenous anastomoses is of high predictive value for antimigraine efficacy (Chapter 2), the α-adrenoceptors, as well as the atypical receptor stimulated by 5-HT, may be considered as targets for future antimigraine drugs.

The sumatriptan-sensitive 5-HT_1-like receptors mediating inhibition of sympathetically-induced vasopressor responses in the rat also seem to resemble 5-$HT_{1B/1D}$ receptors, in view of the antagonism displayed by GR127935 (Chapter 13). In contrast to the vasoconstrictor 5-HT_1-like receptors (which appear to be identical exclusively to the 5-HT_{1B} receptor, see above), this sympathoinhibitory effect may also involve 5-HT_{1A} and 5-HT_{1D} receptors (Chapter 13).

14.2 Nature of 5-HT receptors mediating vasodilatation of the rat systemic vasculature and feline tachycardia

It has been demonstrated previously that 5-HT produces smooth muscle relaxation in several vascular and non-vascular preparations. Originally, these responses were ascribed to an action at vascular 5-HT_1-like receptors, which were also shown to be involved in the 5-HT-induced feline tachycardia (Saxena *et al*., 1985b; Saxena & Villalón, 1990; 1991). Notwithstanding, the IUPHAR Receptor Nomenclature Committee recommended that the receptor involved should be referred to as an atypical 5-HT_1-like or even "orphan" 5-HT receptor (Hoyer *et al*., 1994), mainly on the basis of the lack of agonist effect of the 5-HT_1-like receptor agonist sumatriptan and contradictory transductional properties (for details see Chapter 1). As described above, the sumatriptan-sensitive (vasoconstrictor) 5-HT_1-like receptors mainly resemble 5-HT_{1B} receptors. For several reasons, we considered the recombinant 5-ht_7 receptor site a suitable candidate for responses mediated by the atypical, sumatriptan-insensitive 5-HT_1-like receptors. The cloned 5-ht_7 receptor is coupled positively to adenylyl cyclase, a transductional system generally associated with smooth muscle relaxation and tachycardia (Langer, 1980; Rand *et al*., 1987). Moreover, binding studies show that the rat 5-ht_7 receptor displays high affinity for 5-HT and 5-CT as well as for methiothepin, but low affinity for sumatriptan and ketanserin (see Chapter 1).

Indeed, we have shown that the pharmacological profile of the sumatriptan-insensitive 5-HT_1-like or "orphan" receptor mediating decreases in blood pressure in the rat (Chapter 10) and tachycardia in the cat (Chapter 12) is similar to that of the cloned 5-ht_7 receptor. The 5-ht_7 receptor was also shown to mediate 5-HT-induced

carotid vasodilatation in the anaesthetised dog (Villalón *et al*., 1997a), relaxation of isolated canine cerebral arteries (Terrón & Falcón-Neri, 1999), as well as of several vascular and non-vascular smooth muscle preparations (see Chapter 1). These lines of evidence, coupled to the fact that *m*RNA for the 5-ht_7 receptors has been detected in vascular smooth muscle (Ullmer *et al*., 1995; Schoeffter *et al*., 1996), indicate that the "orphan" 5-HT receptors mediating direct smooth muscle relaxation and tachycardia in the cat resemble recombinant 5-ht_7 receptors structurally, transductionally and operationally. Therefore, the search for the "orphan" has successfully ended and the upper case 5-HT_7 receptor appellation is now clearly justified, although, admittedly, there are no selective agonists for 5-HT_7 receptors thus far. The recent availability of the selective 5-HT_7 receptor antagonists, SB258719 (Forbes *et al*., 1998; Thomas *et al*., 1998), which displays an at least 100 fold selectivity over other 5-HT receptors, will help the further characterisation of this 5-HT receptor class.

14.3 5-HT_1-like receptors: a time to bid goodbye

The present decade has indisputably witnessed a remarkable progress in the classification of 5-HT receptors. This achievement is due to several factors, including: (i) the discovery of new recombinant receptors, using molecular biology techniques; (ii) the possibility to express these recombinant receptors in cell lines, enabling the characterisation of their pharmacological profile; and (iii) the increasing knowledge concerning the receptor transductional properties. Additionally, the discovery of compounds acting selectively at 5-HT receptors proved to be of great importance. Accordingly, the findings discussed in this thesis show that the family of "5-HT_1-like" receptors, originally proposed by Bradley *et al.* (1986), has come a long way, ultimately yielding at least three pharmacologically distinct receptors (5-HT_{1B}, 5-HT_{1D} and 5-HT_7). Consequently, as proposed by Saxena *et al.* (1998b), the term "5-HT_1-like receptor" has now become redundant (see also Figure 1.1).

It should be kept in mind that not all responses that were previously ascribed to an action at these 5-HT_1-like receptors have been analysed using the presently available, selective 5-HT receptor agonists and antagonists. Therefore, we cannot categorically exclude the possibility that some responses are mediated by other 5-HT receptors or even by unknown, novel receptors/mechanisms. In fact, the 5-HT-induced porcine carotid arteriovenous anastomotic constriction was not effectively antagonised by GR127935, despite being mediated by 5-HT_1-like

receptors. This may also be true for the inhibition of plasma protein extravasation in guinea-pig dura mater (Yu *et al*., 1997) and contraction of isolated bovine middle cerebral artery (Roon *et al*., 1999).

Table 14.1 Efficacy, pharmacokinetics and affinity of sumatriptan, PNU142633, LY334370 and zolmitriptan.

	Sumatriptan 100 mg, p.o.	**Sumatriptan 6 mg, s.c.**	**PNU142633 50 mg, p.o.**	**LY334370 200 mg, p.o.**	**Zolmitriptan 5 mg, p.o.**
Therapeutic gain	28[a], 31[b], 33[c]	53[d]	-8%[e]	52[f]	47[g]
CNS penetration	Little	Little	Little[h]	?	Marked
C_{max} *(ng ml^{-1})*	54[i]	72[i]	?	388[j]	10[k]
	295		406	334	288
pK$_i$:					
5-HT$_{1B}$	7.8[l]		4.8[e]	6.9[m]	8.3[n]
5-HT$_{1D}$	8.3[l]		8.3[e]	6.9[m]	9.2[n]
5-HT$_{1F}$	7.9[l]		?	8.8[m]	7.2[n]

Data taken from: [a], Visser *et al.* (1996c); [b], Jackson (1998); [c], Goadsby (1998b); [d], The Subcutaneous Sumatriptan International Study Group (1991); [e], McCall (1999); [f], Goldstein *et al.* (1999a); [g], Dahlöf *et al.* (1998); [h], McCall, R.B., personal communication; [i], Fowler *et al.* (1991); [j], Pereira *et al.* (1999). The authors revealed at the International Headache Society Meeting at Barcelona, Spain (June 22-26, 1999) that the values listed in this abstract were 10-fold higher than the actual C_{max} values. The value in the Table has been derived from the actual C_{max}; [k], Peck *et al.* (1998); [l], Leysen *et al.* (1996); [m], Johnson *et al.* (1997); [n], Martin *et al.* (1997). Therapeutic gain is defined as headache response (decrease in headache from severe or moderate to mild or none) minus placebo response at 2 hours. ?, Data not available. CNS, Central nervous system.

14.4 Beyond the second-generation triptans

The results from the present thesis demonstrate that sumatriptan produces vasoconstriction in the carotid vascular bed. This cranial vasoconstrictor effect is exerted on the main capacitance arteries (Table 2.5) as well as arteriovenous

anastomoses (Table 2.4). Although sumatriptan displays high affinities at 5-HT_{1D} as well as 5-HT_{1F} receptors, several lines of pharmacological evidence indicate that the cranial vasoconstrictor activity of the antimigraine drug is mediated by the 5-HT_{1B} receptor. Therefore, assuming that vasoconstriction of dilated cranial blood vessels is responsible for the therapeutic efficacy of sumatriptan, it is implied that agonist action at the 5-HT_{1D} and/or 5-HT_{1F} receptor is not required for the antimigraine action. However, several other mechanisms, which do not seem to be mediated solely by the 5-HT_{1B} receptor, have also been implicated in migraine relief (see Chapter 2). These mechanisms include inhibition of the trigeminovascular system, either peripherally or centrally. Indeed, in an attempt to avoid the 5-HT_{1B} receptor-mediated moderate hypertension and coronary constriction noticed with these drugs, the therapeutic potential of selective 5-HT_{1D} or 5-HT_{1F} receptor agonists has been explored.

14.4.1 5-HT_{1D} receptor agonists

A series of isochroman-6-carboxamide derivatives, including PNU109291 and PNU142633, have been described as highly selective 5-HT_{1D} receptor agonists (see Tables 1.2 and 14.1; Ennis *et al*., 1998; McCall, 1999). PNU109291 did not produce vasoconstriction in human and bovine isolated cerebral arteries (Bouchelet & Hamel, 1999). Moreover, both compounds are devoid of carotid vasoconstrictor effects in the anaesthetised cat and do not decrease meningeal artery blood flow or canine coronary artery diameter (Ennis *et al*., 1998; McCall, 1999). Yet, these 5-HT_{1D} receptor agonists potently inhibit dural plasma protein extravasation in the guinea-pig (Ennis *et al*., 1998; McCall, 1999). Although inhibition of dural plasma protein extravasation by itself is not predictive of antimigraine activity, the 5-HT_{1D} receptor seems to be involved in the inhibition of the central component of the trigeminovascular system (see Chapter 2). Therefore, selective 5-HT_{1D} receptor agonists are valuable for probing migraine mechanisms (Ennis *et al*., 1998). Notwithstanding, as shown in Table 14.1, the 5-HT_{1D} receptor agonist, PNU142633, has not proved effective in the treatment of migraine (McCall, 1999), implying that the 5-HT_{1D} receptor is not involved in antimigraine effects. PNU109291 is active as an agonist in the guinea-pig hypothermia model and inhibits c-*fos* expression in the trigeminal nucleus caudalis, suggesting that the compound readily crosses the blood brain barrier (Waeber *et al*., 1997; Ennis *et al*., 1998). However, it remains to be established whether PNU142633 (the clinically evaluated compound) is able to gain

access to the central nervous system. More importantly, PNU109291 and, especially, PNU142633 display low intrinsic efficacy compared to 5-HT at primate 5-HT_{1D} receptors in GTPγ^{35}S binding assays (Pregenzer *et al.*, 1999). Therefore, at present we cannot categorically exclude the therapeutic importance of 5-HT_{1D} receptors.

14.4.2 5-HT_{1F} receptor agonists

Two potent 5-HT_{1F} agonists, LY344864 and LY334370 have been described (Table 14.1). Both compounds potently inhibit dural plasma protein extravasation (Johnson *et al.*, 1997; Phebus *et al.*, 1997). LY344864 is reported to be devoid of vasoconstrictor activity in human and bovine isolated cerebral blood vessels (Bouchelet & Hamel, 1999) and in the canine external carotid vasculature (see Chapter 7; Villalón *et al.*, 1999). LY334370 has been under clinical evaluation, but the trials were abandoned because of data from animal toxicology studies. Nevertheless, the results of this clinical trial suggest that LY334370 is therapeutically active in acute migraine (Table 14.1; Goldstein *et al.*, 1999a). However, it remains to be verified whether the clinically active doses of this compound are devoid of interaction with the 5-HT_{1B} receptor. In this context, it is important to note that LY334370 exhibits only a 10-fold lower affinity at the 5-HT_{1B} receptor compared to sumatriptan (Table 14.1), whereas the C_{max} values of therapeutic doses of LY334370 are about 10-fold higher than that of sumatriptan (Pereira *et al.*, 1999). We recognise that 5-HT_{1F} receptor stimulation does not mediate cerebral vasoconstriction and that LY344864 lacks vasoconstrictor effects, but, unfortunately, no information is available on the possible vasoconstrictor effect of the clinically active 5-HT_{1F} receptor agonist, LY334370, in isolated human cranial arteries.

Cohen and co-workers (Cohen *et al.*, 1998) have reported that both LY334370 and LY344864 lack vasoconstrictor activity in the rabbit isolated saphenous vein. However, it should be noted that a lack of vasoconstrictor activity in the isolated saphenous vein does not necessarily mean the absence of cranial vasoconstriction. For example, the antimigraine agent BMS181885 behaves as a silent competitive antagonist in the canine saphenous vein, yet the drug potently constricts isolated cephalic blood vessels (Yocca *et al.*, 1997) and the porcine (see Chapter 6; Saxena *et al.*, 1998a), feline and canine (Yocca *et al.*, 1997) carotid vasculature. Lastly, no information is available concerning the possible formation of active metabolites or plasma protein binding. In any case, the efficacy of LY334370 in

migraine has no bearing on the therapeutic importance of the 5-HT_{1B} receptor, in view of poor affinity displayed by the potent antimigraine drugs alniditan (Goldstein *et al*., 1996) and IS159 (Chaveau & Delaage, 1997; Dingemanse *et al*., 1999) at the 5-HT_{1F} receptor (Leysen *et al*., 1996).

14.5 Implications for future antimigraine therapy

As discussed above, it appears that the 5-HT_{1B} receptor is the predominant receptor mediating the therapeutic effects by sumatriptan, although, based on the currently available information, the role of 5-HT_{1D} and 5-HT_{1F} receptors can neither be completely discounted nor proven. In any case, the results presented in this thesis imply that mainly the 5-HT_{1B} receptor (not the other 5-HT_1 receptor subtypes) is responsible for the sumatriptan-induced cranial vasoconstriction. Accordingly, it is tempting to suggest that a direct vascular smooth muscle contraction of dilated cephalic blood vessels (possibly, including the carotid arteriovenous anastomoses) is the most important characteristic of acutely-acting antimigraine drugs. This suggestion gains weight when considering that: (i) sumatriptan poorly penetrates the central nervous system; (ii) inhibition of plasma protein extravasation is not predictive of antimigraine efficacy; and (iii) the 5-$HT_{1B/1D}$ receptor agonists alniditan and IS159, which have little 5-HT_{1F} receptor affinity, are at least as effective as sumatriptan in aborting acute migraine attacks (Goldstein *et al*., 1996; Chaveau & Delaage, 1997; Dingemanse *et al*., 1999). This hypothesis may be challenged by assessing the antimigraine efficacy of low brain-penetrant, selective 5-HT_{1B} receptor agonists; unfortunately, such compounds are not yet available. On the other hand, it is not yet known whether the integrity of the blood-brain barrier remains intact during migraine attacks. It should also be noted that the 5-HT_{1B} receptor is widely distributed not only on vascular smooth muscle, but also within the peripheral and central components of the trigeminal nerve.

The inhibition of central trigeminal neuronal pathways may be effected by the more lipophilic triptans, such as zolmitriptan, rizatriptan and eletriptan. This property may account for the somewhat higher efficacy observed with these second-generation triptans, zolmitriptan (Dahlöf *et al*., 1998), rizatriptan (Visser *et al*., 1996c) and eletriptan (Jackson, 1998), compared to sumatriptan (see Table 14.1). On the other hand, it can also be argued that the higher efficacy with these drugs is mainly due to their improved pharmacokinetics. In keeping with the latter is the fact that the highest

antimigraine efficacy to date is observed with subcutaneous injections of sumatriptan (Table 14.1). Moreover, at least one 5-HT_{1D} receptor agonist (PNU142633), which has been claimed to inhibit the central trigemino-vascular system, is ineffective in migraine (see Section 14.4.1).

As discussed in Chapter 2, low intensity stimulation of trigeminal afferents leads to dural vasodilatation, which is inhibited by sumatriptan, at doses devoid of dural vasoconstriction *per se*. This effect, which at least in the rat is mediated by 5-HT_{1B} receptors, could also be involved in the antimigraine effects by triptans.

Perhaps, to achieve maximal antimigraine efficacy, the drugs should be designed to act via several mechanisms. A drug, which selectively inhibits trigemino-vascular system (either central or peripheral) without cranial vasoconstriction, may not efficiently abort migraine attack. However, this remains a goal worth pursuing.

14.6 Possible mechanisms for future migraine prophylaxis

Although the advances made in the acute treatment of migraine are of major clinical importance, efforts should also be directed to develop prophylactic drugs that decrease the frequency of migraine attacks. Some β-adrenoceptor antagonists, the antiepileptic drug valproate and several other drugs are used in migraine prophylaxis, but the exact mechanisms are not well understood (Welch, 1993; Ferrari, 1998). As discussed below, some new theories have been put forward, which may be important targets for future migraine prophylaxis.

14.6.1 Blockade of cranial vasodilatation following cortical spreading depression

The role of cortical spreading depression in migraine (Lauritzen, 1994) is controversial because of difficulty of demonstrating it clinically. However, a promising technique using magnetic resonance imaging, developed by SmithKline Beecham scientists (Smith *et al*., 1998), offers new opportunities for investigating cortical spreading depression in humans. Meanwhile, animal studies show that cortical spreading depression can lead to cranial vasodilatation (Parsons, 1998). Inhibition of this vasodilator response appears to be a novel avenue for developing antimigraine drugs without vasoconstrictor action *per se*. Indeed, Chan *et al.* (1999) have recently reported a series of such compounds, in particular SB220453, which antagonises cortical spreading depression and blocks plasma protein

extravasation in rats, but does not interact with any of the known 5-HT receptors. Moreover, SB220453 blocks carotid vasodilatation in cats induced by trigeminal ganglion stimulation (Upton *et al*., 1999) and lacks vasoconstrictor effect on human isolated coronary artery or saphenous vein (MaassenVanDenBrink *et al*., 1999b). It will be interesting to know whether a drug that lacks vasoconstrictor properties, but prevents cortical spreading depression and/or the consequent cranial vasodilatation would be effective in migraine as a prophylactic and/or acute abortive agent.

14.6.2 Cerebral calcium channelopathy

Several investigators have shown that genetic factors are important in migraine (Russell & Olesen, 1995; Ophoff *et al*., 1996; Ferrari, 1998). Thus, mutations in the α_{1A} subunit of a brain specific P/Q type calcium channel were shown to be involved in familial hemiplegic migraine (Ophoff *et al*., 1996) and may also be involved in non-hemiplegic migraine (Terwindt *et al*., 1998). The full understanding of this calcium channelopathy may ultimately result in the development of migraine-specific prophylactic drugs.

14.6.3 5-HT$_7$ receptor antagonists

As discussed in this thesis, the main effect induced by 5-HT$_7$ receptor stimulation is vascular smooth muscle relaxation. This 5-HT$_7$ receptor-mediated vasorelaxant effect is also observed in canine (Terrón & Falcón-Neri, 1999) and porcine (Ueno *et al*., 1995) isolated cranial blood vessels, as well as in the canine external carotid vasculature (Villalón *et al*., 1997a). Interestingly, several currently used migraine prophylactic drugs display affinity at the 5-HT$_7$ receptor, such as methysergide (Herrmann *et al*., 1977; Silberstein, 1998) and lisuride (Herrmann *et al*., 1977; Somerville & Herrmann, 1978; Del Bene *et al*., 1983). Admittedly, these drugs are non-selective ligands. Notwithstanding, it may be argued that 5-HT$_7$ receptor-mediated cranial vasodilatation plays a role in the migraine headache phase. Consequently, it may be worth assessing the effectiveness of selective 5-HT$_7$ receptor antagonists in the prevention of migraine.

Chapter 15

Thesis summary

15.1 Summary in English

In **Chapter 1** an overview is given about the nomenclature and classification of 5-HT receptors. Firstly, the historical aspects of the discovery of 5-HT and the responses elicited by the ligand have been described, ultimately leading to the classification by Bradley and colleagues in 1986. At that time, the 5-HT receptors were divided into three classes, namely 5-HT_1-like, 5-HT_2 and 5-HT_3 receptors, mainly based on operational differences. With the adoption of structural and transductional characteristics as additional criteria in 1994, the NC-IUPHAR reclassified 5-HT receptors into 5-HT_1 (5-HT_1-like, 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-ht_{1E} and 5-ht_{1F}), 5-HT_2 (5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C}), 5-HT_3, 5-HT_4, recombinant (5-$ht_{5A/5B}$, 5-ht_6, 5-ht_7) and "orphan" receptors. Based on the latter classification schemes, each of these receptors has been discussed, updated with the most recent information available. Particular emphasis was put on the evolution of the so-called 5-HT_1-like receptor, which turned out to be identical to at least three different receptors, namely 5-HT_{1B}, 5-HT_{1D} and 5-HT_7 receptors.

Chapter 2 deals with several aspects of migraine. Firstly, the epidemiology and clinical features are shortly described. Thereafter, an attempt is made, given the current knowledge about migraine headaches, to explain the pathophysiology of the disease. Additionally, pharmacokinetic and pharmacodynamic characteristics of the acutely-acting antimigraine drug sumatriptan and the new second-generation triptans are discussed. Lastly, the pharmacology and relevance of the currently used experimental models, which are believed to be predictive of therapeutic potential, have been described in detail.

In **Chapter 3**, we investigated the selectivity of GR127935 against functional responses known to be mediated by 5-HT_1-like, 5-HT_2, 5-HT_3, 5-HT_4 or 5-HT_7 receptors in several *in vivo* preparations. GR127935 has recently been introduced as an experimental tool to antagonise 5-$HT_{1B/1D}$ receptor-mediated functional responses and indeed exhibits a very high affinity and selectivity for 5-$HT_{1B/1D}$ binding sites. We show that GR127935 potently antagonises the decreases in total carotid blood

flow as well as hypotensive responses in the rabbit induced by the 5-HT_1-like receptor agonist sumatriptan. GR127935 did not modify 5-HT-induced: (i) tachycardia in the pig (5-HT_4 receptor-mediated) and cat (5-HT_7 receptor-mediated); (ii) depressor effects in the rat and cat (5-HT_7 receptor-mediated); (iii) von Bezold-Jarisch reflex in the rat or the early phase of the urinary bladder contraction in the cat (both 5-HT_3 receptor-mediated). In contrast, high doses of GR127935 suppressed 5-HT-induced pressor responses in the rat and cat and urinary bladder contractions (secondary phase) in the cat as well as the DOI-induced pressor responses in the rat, which are all mediated by 5-HT_{2A} receptors. Lastly, GR127935 produced a methiothepin-resistant vasoconstriction of porcine carotid arteriovenous anastomoses *per se*.

In conclusion, we have demonstrated that GR127935 is a potent and selective 5-$HT_{1B/1D}$ receptor antagonist devoid of interactions at 5-HT_3, 5-HT_4 and 5-HT_7 receptors. However, GR127935 possesses a moderate 5-HT_{2A} receptor blocking property, which is consistent with its binding profile and seems to display intrinsic activity in the porcine carotid vascular bed.

In **Chapter 4** we have investigated whether GR127935 was able to antagonise the sumatriptan-induced porcine carotid vascular effects, which were previously shown to be mediated by 5-HT_1-like receptors. In animals pretreated with saline, sumatriptan reduced the total carotid and arteriovenous anastomotic blood flows in a dose-dependent manner. In contrast, sumatriptan increased blood flow to the skin, ears and fat, although the total capillary fraction was not significantly affected. These carotid vasoconstrictor responses to sumatriptan were dose-dependently antagonised by GR127935. Taken together, these results led us to conclude that arteriovenous anastomotic constriction and, possibly, arteriolar dilatation in the skin, ears and fat by sumatriptan are mediated by 5-$HT_{1B/1D}$ receptors. Therefore, vascular 5-HT_1-like receptors in the porcine carotid bed appear to be identical to 5-$HT_{1B/1D}$ receptors.

Chapter 5 was devoted to establish the contribution of 5-$HT_{1B/1D}$ receptors in the constriction of carotid arteriovenous anastomoses elicited by 5-HT (in presence of 5-HT_2 receptor blockade), ergotamine and dihydroergotamine (DHE) in anaesthetised pigs. It was demonstrated that 5-HT, ergotamine and DHE reduced arteriovenous anastomotic and increased nutrient blood flows and vascular conductances. The vasodilator response to 5-HT, observed mainly in the skin and ear, was much more

prominent than that of the ergot alkaloids. Treatment with the 5-$HT_{1B/1D}$ receptor antagonist GR127935 significantly attenuated the effects induced by both ergot derivatives, whereas the drug only slightly affected the carotid vascular effects by 5-HT. The results suggest that 5-HT constricts carotid arteriovenous anastomoses primarily via receptors, which seem to differ from those (5-$HT_{1B/1D}$) stimulated by sumatriptan. The ergot alkaloids produce arteriovenous anastomotic constriction for a substantial part via 5-$HT_{1B/1D}$ receptors, but also invoke unidentified receptors. Both these non-5-$HT_{1B/1D}$ receptors may be targets for the development of novel antimigraine drugs.

In **Chapter 6** we describe the vasoconstrictor effects of some second-generation antimigraine drugs on porcine carotid arteriovenous anastomoses, an effect that seems to be of high predictive value for antimigraine activity. Intravenous administrations of BMS181885, avitriptan, eletriptan, GMC2021 and alniditan decreased total carotid blood flow by a selective vasoconstriction of the carotid arteriovenous anastomoses. The apparent rank order of potency was: alniditan > sumatriptan > avitriptan > eletriptan > GMC2021, whereas BMS181885 seemed to have a lower intrinsic activity. The arteriovenous anastomotic constriction by alniditan, eletriptan and GMC2021 was potently antagonised by GR127935. After similar reductions in carotid blood flow following a single 100 $\mu g\ kg^{-1}$ intravenous dose at 30 min, the effect of BMS181885 lasted longer than that of sumatriptan. These results suggest that the new triptans selectively constrict porcine carotid arteriovenous anastomoses mainly via 5-$HT_{1B/1D}$ receptors and should be able to abort migraine headaches. The latter has indeed been confirmed in initial clinical studies in man. Lastly, the long-lasting effect of BMS181885 may reduce the recurrence rate in migraine.

In **Chapter 7** we show that intracarotid infusions of the antimigraine drugs methysergide, ergotamine and DHE produce selective vasoconstriction in the external carotid bed of vagosympathectomised, anaesthetised dogs, without affecting blood pressure and heart rate. Since the above drugs display affinity for several binding sites, including α-adrenoceptors and several 5-HT_1 and 5-HT_2 receptor subtypes, this study has analysed the mechanisms involved in the above responses. The reductions in external carotid blood flow (ECBF) by methysergide were abolished and even reversed to increases in animals pretreated with GR127935. The reductions in ECBF

by ergotamine and DHE remained unchanged in animals pretreated with prazosin, but were partly antagonised in animals pretreated with either GR127935 or yohimbine. Pretreatment with a combination of GR127935 and yohimbine abolished the responses to both ergotamine and DHE. The above doses of antagonists were shown to produce selective antagonism at their respective receptors. These results suggest that the external carotid vasoconstrictor responses to methysergide primarily involve 5-$HT_{1B/1D}$ receptors, whereas those to ergotamine and DHE are mediated by 5-$HT_{1B/1D}$ receptors as well as α_2-adrenoceptors.

In **Chapter 8**, we show results obtained with the newly developed, selective 5-HT_{1B} (SB224289) or 5-HT_{1D} (BRL15572) receptor antagonists. In vagosympathectomised dogs intracarotid infusions of 5-HT and sumatriptan dose-dependently decreased ECBF, without affecting mean blood pressure or heart rate. Treatment with the selective 5-HT_{1B} receptor antagonist SB224289 produced a potent, specific and dose-dependent blockade of this response, whereas the selective 5-HT_{1D} receptor antagonist BRL15572 was ineffective. It is concluded that mainly 5-HT_{1B}, but not 5-HT_{1D} receptors mediate the canine external carotid vasoconstriction by 5-HT and sumatriptan.

In **Chapter 9**, we discuss the effects of the selective 5-HT_{1B} (SB224289) and 5-HT_{1D} (BRL15572) receptor antagonists on the constriction of porcine carotid arteriovenous anastomoses by sumatriptan. Since the effects of sumatriptan were antagonised by SB224289, but not by BRL15572, we concluded that this effect was primarily mediated by 5-HT_{1B}, but not 5-HT_{1D} receptors.

In **Chapter 10** we have further characterised the pharmacological profile of the receptors involved in the 5-HT-induced late hypotensive response in rats. It has been suggested previously that this hypotensive response to 5-HT was mediated by "5-HT_1-like" receptors since this effect is mimicked by 5-CT, is not modified by cyproheptadine, ketanserin or MDL72222, but it is blocked by methysergide. Bolus injections of 5-CT, 5-HT and 5-MeO-T produced dose-dependent hypotensive responses with a rank order of agonist potency: 5-CT >> 5-HT ≥ 5-MeO-T with sumatriptan inactive. The depressor responses to 5-HT and 5-CT were not attenuated by GR127935 or equivalent volumes of saline. In contrast, lisuride, methiothepin,

mesulergine, metergoline and clozapine dose-dependently antagonised the responses to 5-HT and 5-CT; the apparent rank order of antagonist potency was: lisuride > methiothepin ≥ mesulergine > clozapine; metergoline displayed variable potencies. Based upon the above rank order of agonist potency, the blockade by a series of drugs showing high affinity for the cloned 5-ht_7 receptor and the lack of blockade by GR127935, our results indicate that the 5-HT receptor mediating hypotension in rats is operationally similar to other putative 5-ht_7 receptors mediating vascular and non-vascular responses (e.g. relaxation of the rabbit femoral vein, canine coronary and external carotid arteries and guinea-pig ileum as well as feline tachycardia). Since these responses represent functional correlates of the 5-ht_7 gene product, the upper case 5-HT_7 receptor appellation is reinforced.

In **Chapter 11**, we have investigated which vascular beds were responsible for the 5-HT-induced late depressor response in rats, which is mainly mediated by vascular 5-HT_7 receptors, as demonstrated in the previous chapter. In vagosympathectomised, pentobarbital anaesthetised rats, 5-HT produced a dose-dependent decrease in mean arterial blood pressure. This hypotension was accompanied by increases in systemic vascular conductance, but cardiac output was unaffected. Exclusively skeletal muscle, carcass, mesentery/pancreas and adrenal vascular beds contributed to the increase in total systemic vascular conductance. Pretreatment with R(+)lisuride abolished all 5-HT-induced systemic and regional haemodynamic effects. In contrast, pretreatment with S(-)lisuride or GR127935 did not affect the 5-HT-induced systemic haemodynamic changes. The above results suggest that hypotension induced via 5-HT_7 receptor activation was exclusively caused by vasodilatation of the systemic vasculature, confined to skeletal muscle, carcass, mesentery/pancreas and adrenal vascular beds. Furthermore, this study shows that blockade of vasorelaxant 5-HT_7 receptors by lisuride is stereoselective.

In **Chapter 12**, we have further characterised the receptors involved in the 5-HT-induced tachycardia in cats. It was previously shown that this response was mediated by 5-HT_1-like receptors. Based upon the rank order of agonist potency (5-CT >> 5-HT > 5-MeO-T >> clozapine), the failure of sumatriptan, indorenate or cisapride to produce cardioacceleration and the blockade by a series of drugs showing high affinity for the cloned 5-ht_7 receptor, the results indicated that the 5-HT receptor mediating

tachycardia in the cat was operationally similar to other putative 5-HT_7 receptors mediating vascular and non-vascular responses. Therefore, this experimental model, which is not complicated by the presence of other 5-HT receptors, can be utilised to characterise and develop new drugs with potential agonist and antagonist properties at functional 5-HT_7 receptors.

In **Chapter 13**, we have tried to establish which 5-HT_1 receptor subtypes were involved in the inhibition of sympathetically-induced vasopressor responses produced by 5-HT in pithed rats. Infusions of 5-HT and the 5-HT_1 receptor agonists, 8-OH-DPAT (5-HT_{1A}), indorenate (5-HT_{1A}), CP93129 (5-HT_{1B}) and sumatriptan (5-$HT_{1B/1D}$), resulted in a dose-dependent inhibition of sympathetically-induced vasopressor responses. The sympatho-inhibitory responses induced by 5-HT, 8-OH-DPAT, indorenate, CP93129 or sumatriptan were analysed before and after treatment with blocking doses of the putative 5-HT receptor antagonists, WAY100635 (5-HT_{1A}), cyanopindolol (5-$HT_{1A/1B}$) or GR127935 (5-$HT_{1B/1D}$). After WAY100635, the responses to 5-HT and indorenate, but not to 8-OH-DPAT, CP93129 and sumatriptan, were blocked. After cyanopindolol, the responses to 5-HT, indorenate and CP93129 were abolished, whilst those to 8-OH-DPAT and sumatriptan (except at the lowest frequency of stimulation) remained unaltered. In contrast, after GR127935, the responses to 5-HT, CP93129 and sumatriptan, but not to 8-OH-DPAT and indorenate, were abolished. In additional experiments, the inhibition induced by 5-HT was not modified after 5-HT_7 receptor blocking doses of mesulergine. The results suggested that the 5-HT_1-like receptors, which inhibit the sympathetic vasopressor outflow in pithed rats, display the pharmacological profile of the 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D}, but not that of 5-HT_7, receptors.

15.2 Samenvatting in het Nederlands

In **Hoofdstuk 1** wordt een overzicht gegeven van de 5-HT receptor nomenclatuur en classificatie. Begonnen is met een beschrijving van de historische aspecten van de ontdekking van 5-HT en de responsen geïnduceerd door dit ligand. Dit heeft uiteindelijk geleid tot de classificatie volgens Bradley c.s. in 1986. Op dat moment waren de 5-HT receptoren ingedeeld in drie klassen, namelijk 5-HT_1-achtige, 5-HT_2 en 5-HT_3 receptoren, hoofdzakelijk gebaseerd op operationele verschillen. Nadat structurele en transductionele karakteristieken werden geaccepteerd als additionele criteria in 1994, heeft de NC-IUPHAR de 5-HT receptoren opnieuw geclassificeerd in 5-HT_1 (5-HT_1-achtig, 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-ht_{1E} en 5-ht_{1F}), 5-HT_2 (5-HT_{2A}, 5-HT_{2B} en 5-HT_{2C}), 5-HT_3, 5-HT_4, recombinante (5-$ht_{5A/5B}$, 5-ht_6, 5-ht_7) en "orphan" receptoren. Gebaseerd op deze classificatie schema's, wordt iedere receptor afzonderlijk bediscussieerd en waarnodig geactualiseerd met de meest recente informatie. De nadruk is gelegd op de evolutie van de zogenaamde 5-HT_1-achtige receptor, die uiteindelijk identiek is gebleken aan tenminste drie verschillende receptoren, namelijk de 5-HT_{1B}, 5-HT_{1D} en 5-HT_7 receptor.

In **Hoofdstuk 2** worden verschillende aspecten van migraine behandeld. De epidemiologie en klinische verschijnselen van migraine zijn kort beschreven. Verder is getracht de pathofysiologie van migraine te verklaren, voor zover dit mogelijk is met de huidige kennis over de aandoening. Vervolgens worden de farmacokinetische en farmacodynamische karakteristieken bediscussieerd van het acuut werkende antimigraine geneesmiddel sumatriptan en de nieuwe, tweede generatie triptannen. Tenslotte worden de farmacologie en de relevantie beschreven van enkele experimentele modellen, waarvan aangenomen wordt dat ze van voorspellende waarde zijn voor therapeutisch potentieel.

In **Hoofdstuk 3** is de selectiviteit van GR127935 onderzocht ten opzichte van 5-HT_1-achtige, 5-HT_2, 5-HT_3, 5-HT_4 of 5-HT_7 receptoren in verschillende proefdiermodellen. GR127935 is recent geïntroduceerd als een selectieve 5-$HT_{1B/1D}$ receptor antagonist en vertoont inderdaad een zeer hoge affiniteit en selectiviteit voor 5-$HT_{1B/1D}$ bindingsplaatsen. De door de 5-HT_1-achtige agonist sumatriptan geïnduceerde vermindering in halsslagader bloeddoorstroming en bloeddrukdaling in het konijn werden potent geantagoneerd door GR127935. GR127935 had geen effect

op de 5-HT geïnduceerde: (i) tachycardie in het varken (5-HT_4 receptor gemedieerd) en de kat (5-HT_7 receptor gemedieerd); (ii) bloeddrukverlagende effecten in de rat en de kat (5-HT_7 receptor gemedieerd); (iii) von Bezold-Jarisch reflex in de rat of de eerste fase van de urineblaascontractie in de kat (beiden 5-HT_3 receptor gemedieerd). Hoge doseringen van GR127935 onderdrukten de 5-HT geïnduceerde bloeddrukstijgingen in de rat en kat, de tweede fase van de urineblaascontractie in de kat en de DOI geïnduceerde bloeddrukstijging in de rat; deze responsen worden allen gemedieerd door 5-HT_{2A} receptoren. Voorts veroorzaakte GR127935 in het varken een methiothepine-resistente vasoconstrictie van arterioveneuze anastomoses in het vaatbed van de halsslagader. Geconcludeerd wordt dat GR127935 een potente en selectieve 5-$HT_{1B/1D}$ receptor antagonist is, zonder interacties te vertonen met 5-HT_3, 5-HT_4 en 5-HT_7 receptoren. Echter, GR127935 bezit een matig 5-HT_{2A} receptor blokkerende eigenschap, welke overeenkomstig is met het bindingsprofiel. Verder lijkt GR127935 intrinsieke vasoconstrictieve activiteit te vertonen op craniële arterioveneuze anastomoses van het varken.

In **Hoofdstuk 4** is geanalyseerd of GR127935 in staat is de sumatriptan geïnduceerde vasculaire effecten in het vaatbed van de carotis arterie van het varken te blokkeren. Eerder is aangetoond dat het antimigraine geneesmiddel sumatriptan een verlaging bewerkstelligt van de bloedstroom in de halsslagader, gemedieerd door 5-HT_1-achtige receptoren. In met fysiologisch zout voorbehandelde dieren, veroorzaakte sumatriptan een dosis-afhankelijke verlaging van de totale carotis doorbloeding en de bloedstroom door de craniële arterioveneuze anastomoses. Anderzijds werden bloedstroomstijgingen geconstateerd in de huid, oren en vet, hoewel de totale weefselfractie niet significant veranderde. Deze effecten werden dosis-afhankelijk geantagoneerd door GR127935. Op grond van deze resultaten kan geconcludeerd worden dat sumatriptan een vasoconstrictie van craniële arterioveneuze anastomoses en mogelijk ook een vaatverwijding van huid, oor en vet vasculatuur induceert via 5-$HT_{1B/1D}$ receptoren. De vasculaire 5-HT_1-achtige receptoren in het halsslagader vaatbed van het varken lijken dus identiek te zijn aan 5-$HT_{1B/1D}$ receptoren.

Hoofdstuk 5 is gewijd aan de bestudering van de bijdrage van 5-$HT_{1B/1D}$ receptoren aan de vasoconstrictie van craniële arterioveneuze anastomoses door 5-HT (in de aanwezigheid van 5-HT_2 receptor blokkade), ergotamine en dihydroergotamine

(DHE) in genarcotiseerde varkens. 5-HT, ergotamine en DHE verminderden de doorbloeding en vasculaire conductantie van craniële arterioveneuze anastomoses en verhoogden de capillaire bloedstroom en vasculaire conductantie. Het vaatverwijdende effect van 5-HT, met name geobserveerd in de huid en het oor, was veel uitgesprokener dan dat van de ergot alkaloïden. De effecten van de ergot derivaten werden gedeeltelijk geantagoneerd door GR127935, maar deze antagonist blokkeerde slechts een klein deel van de 5-HT geïnduceerde respons. Deze resultaten suggereren dat 5-HT de arterioveneuze anastomoses voornamelijk contraheert via een andere receptor en/of mechanisme dan sumatriptan. De ergot alkaloïden produceren arterioveneuze anastomose constrictie gedeeltelijk via 5-$HT_{1B/1D}$ receptoren, maar stimuleren ook receptoren, die op dit moment nog niet gekarakteriseerd zijn. Deze non-5-$HT_{1B/1D}$ receptoren kunnen mogelijkheden bieden voor de ontwikkeling van nieuwe antimigraine geneesmiddelen.

In **Hoofstuk 6** wordt het vasconstrictieve effect van enkele tweede generatie antimigraine farmaca op de craniële arterioveneuze anastomoses in het varken beschreven. Het effect in dit proefdiermodel wordt beschouwd van voorspellende waarde te zijn voor antimigraine effectiviteit. Intraveneuze toediening van BMS181885, avitriptan, eletriptan, GMC2021 en alniditan leidde tot een vermindering van de totale halsslagader bloeddoorstroming, veroorzaakt door een selectieve vasoconstrictie van de arterioveneuze anastomoses. De rangorde van potentie was: alniditan > sumatriptan > avitriptan > eletriptan > GMC2021, terwijl BMS181885 een lagere intrinsieke activiteit leek te vertonen. De arterioveneuze anastomose constrictie door alniditan, eletriptan en GMC2021 werd potent geantagoneerd door GR127935. Na toediening van een enkele dosis, welke na 30 minuten een kwantitatief gelijke vermindering in arterioveneuze anastomose bloeddoorstroming teweegbracht, hield het effect van BMS181885 langer aan dan de respons na sumatriptan. Geconcludeerd wordt dat de nieuwe triptannen een selectieve constrictie van de craniële arterioveneuze anastomoses veroorzaken, voornamelijk gemedieerd door 5-$HT_{1B/1D}$ receptoren. Deze stoffen zouden dus effectief moeten zijn in de behandeling van migraine. Initiële klinische studies in migraine patiënten bevestigen dit. Bovendien, zou het lang aanhoudende vasoconstrictieve effect van BMS181885 de snelheid van opnieuw optreden van een migraine aanval kunnen verminderen.

In **Hoofdstuk 7** wordt beschreven dat direct in de halsslagader van de hond toegediende doses van de antimigraine geneesmiddelen methysergide, ergotamine en DHE een selectieve vasoconstrictie in het vaatbed van de externe halsslagader veroorzaken, zonder de bloeddruk of hartslag te veranderen. Daar deze geneesmiddelen affiniteit vertonen voor meerdere bindingsplaatsen, zoals α-adrenoceptoren, 5-HT_1 en 5-HT_2 receptor subtypen, zijn de exacte mechanismen onderzocht die verantwoordelijk zijn voor bovenstaande responsen. In honden, voorbehandeld met GR127935, werd de vermindering in bloeddoorstroming in de externe halsslagader geïnduceerd door methysergide niet alleen compleet geblokkeerd, maar werd zelfs een verhoging van de bloedstroom geobserveerd. De verlaging in halsslagader bloedstroom geïnduceerd door ergotamine en DHE bleef onveranderd na toediening van prazosine, maar werden gedeeltelijk geblokkeerd na GR127935 of yohimbine. De combinatie van GR127935 en yohimbine blokkeerde de effecten van zowel ergotamine als DHE compleet. De doseringen van de gebruikte antagonisten vertoonden een selectief blokkerend effect. Deze resultaten tonen aan dat de responsen in het externe halsslagader vaatbed van de hond door methysergide voornamelijk gemedieerd zijn door 5-$HT_{1B/1D}$ receptoren, terwijl zowel 5-$HT_{1B/1D}$ receptoren als α_2-adrenoceptoren de effecten van ergotamine en DHE mediëren.

In **Hoofdstuk 8** worden de resultaten beschreven van experimenten met de recent ontwikkelde, selectieve 5-HT_{1B} (SB224289) of 5-HT_{1D} (BRL15572) receptor antagonisten. Direct in de halsslagader toegediende doses van 5-HT en sumatriptan verlaagden dosis-afhankelijk de bloeddoorstroming van de externe halsslagader van de hond, zonder een effect te hebben op bloeddruk en hartslag. Dit effect werd dosis afhankelijk geblokkeerd door SB224289, maar niet door BRL15572. De conclusie van deze studie is dat de 5-HT en sumatriptan geïnduceerde vasoconstrictie in het vaatbed van de externe halsslagader van de hond voornamelijk gemedieerd wordt door 5-HT_{1B}, maar niet door 5-HT_{1D} receptoren.

In **Hoofdstuk 9** worden de effecten beschreven van de selectieve 5-HT_{1B} (SB224289) en 5-HT_{1D} (BRL15572) receptor antagonisten op de sumatriptan geïnduceerde constrictie van craniële arterioveneuze anastomoses in het varken. Aangezien deze effecten werden geantagoneerd door SB224289, maar niet door BRL15572, is

geconcludeerd dat dit effect gemedieerd wordt door 5-HT_{1B}, maar niet door 5-HT_{1D} receptoren.

In **Hoofdstuk 10** wordt het farmacologisch profiel bestudeerd van de receptoren die verantwoordelijk zijn voor de 5-HT geïnduceerde late hypotensieve respons in de rat. Eerder onderzoek heeft aangetoond dat deze respons gemedieerd wordt door 5-HT_1-achtige receptoren, aangezien dit effect werd geïmiteerd door 5-CT, niet werd geblokkeerd door cyproheptadine, ketanserin of MDL72222, maar wel door methysergide. Bolus injecties van 5-CT, 5-HT en 5-MeO-T veroorzaakten dosis-afhankelijke hypotensieve responsen, met een rangorde van potentie van: 5-CT >> 5-HT ≥ 5-MeO-T, terwijl sumatriptan geen activiteit vertoonde. De bloeddrukverlagingen door 5-HT en 5-CT werden niet veranderd door GR127935 of gelijke volumes fysiologisch zout, maar werden wel dosis-afhankelijk geantagoneerd door lisuride, methiothepin, mesulergine, metergoline en clozapine. De rangorde van sterkte van antagonisme was: lisuride > methiothepin ≥ mesulergine > clozapine; metergoline vertoonde variabele potenties. Gebaseerd op de rangorde van agonist potentie, de blokkade door een serie van farmaca met hoge affiniteit voor de recombinante 5-ht_7 receptor en de afwezigheid van antagonisme door GR127935, wordt geconcludeerd dat de 5-HT receptor die de late hypotensieve respons medieert, operationeel gelijk is aan de 5-ht_7 receptor. Aangezien er nu verschillende functionele responsen toegeschreven kunnen worden aan de recombinante 5-ht_7 receptor, kan de 5-HT_7 receptor met hoofdletters geschreven worden.

In **Hoofdstuk 11**, wordt onderzocht welke vaatbedden verantwoordelijk zijn voor de 5-HT geïnduceerde late hypotensieve respons in de rat. In hoofdstuk 10 is beschreven dat dit effect voornamelijk gemedieerd wordt door de 5-HT_7 receptor. 5-HT produceerde een dosis-afhankelijke bloeddrukdaling in de genarcotiseerde rat, die vergezeld ging van een verhoging van de systemische vasculaire conductantie (SVC), terwijl het hartminuutvolume onveranderd bleef. De stijging van de SVC was exclusief gelokaliseerd in skeletspieren, karkas, mesenterium/pancreas en de bijnieren. Toediening van R(+)lisuride blokkeerde alle 5-HT geïnduceerde systemische en regionale haemodynamische effecten, terwijl toediening van S(-)lisuride of GR127935 geen antagonisme vertoonden. De resultaten suggereren dat de 5-HT_7 receptor gemedieerde hypotensie veroorzaakt wordt door een vasodilatatie van de systemische

vasculatuur, welke zich beperkt tot de vaatbedden van skeletspieren, karkas, mesenterium/pancreas en bijnieren. Tenslotte blijkt dat het 5-HT_7 receptor antagonistisch effect van lisuride stereoselectief te zijn.

In **Hoofdstuk 12**, wordt nader ingegaan op de verdere karakterisering van de receptoren die de 5-HT geïnduceerde tachycardia in de kat veroorzaken. Eerder werd gedemonstreerd dat 5-HT_1-achtige receptoren dit effect mediëren. De rangorde van agonist potentie (5-CT >> 5-HT > 5-MeO-T >> clozapine), de afwezigheid van hartslagverhogend effect door sumatriptan, indorenate of cisapride en de blokkade door stoffen met hoge 5-HT_7 receptor affiniteit, wijzen erop de 5-HT geïnduceerde tachycardie in de kat gemedieerd wordt door 5-HT_7 receptoren. Dit experimentele model, dat niet gecompliceerd wordt door de aanwezigheid van andere receptoren, is geschikt voor de karakterisering en ontwikkeling van nieuwe geneesmiddelen met potentieel agonistische en antagonistische eigenschappen op 5-HT_7 receptoren.

In **Hoofdstuk 13** wordt onderzocht welke 5-HT_1 receptor subtypen een rol spelen bij de inhibitie van bloeddrukstijgingen geïnduceerd door stimulatie van sympathische zenuwen in centraal gedenerveerde ratten. Toediening van 5-HT en de 5-HT_1 agonisten, 8-OH-DPAT (5-HT_{1A}), indorenate (5-HT_{1A}), CP93129 (5-HT_{1B}) en sumatriptan (5-$HT_{1B/1D}$), resulteerde in een dosis-afhankelijke inhibitie van sympathisch geïnduceerde bloeddrukstijgingen. Deze responsen werden tevens bestudeerd na infusies van de 5-HT receptor antagonisten, WAY100635 (5-HT_{1A}), cyanopindolol (5-$HT_{1A/1B}$) of GR127935 (5-$HT_{1B/1D}$). WAY100635 blokkeerde de 5-HT en indorenate geïnduceerde effecten, maar niet die van 8-OH-DPAT, CP93129 en sumatriptan. Cyanopindolol antagoneerde de responsen opgewekt door 5-HT, indorenate en CP93129, maar had geen invloed op de responsen na 8-OH-DPAT en sumatriptan. De responsen na 5-HT, CP93129 en sumatriptan, maar niet na 8-OH-DPAT en indorenate, werden compleet geblokkeerd door GR127935. Uit additionele experimenten bleek verder dat mesulergine niet in staat was de remming door 5-HT te antagoneren. Deze resultaten tonen aan dat the 5-HT_1-achtige receptoren, die de sympathische vasopressor activiteit remmen, het farmacologisch profiel vertonen van zowel 5-HT_{1A}, 5-HT_{1B} als 5-HT_{1D} receptoren, maar niet die van 5-HT_7 receptoren.

Chapter 16
Appendix

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16.2 About the author

Peter de Vries was born in Alphen aan den Rijn on 23 November 1972. After attending "Het Christelijk Lyceum" in Alphen aan den Rijn for secondary schooling (1984-1990), he studied medicine at the Erasmus University Rotterdam. In 1995, he obtained his Master's degree in medicine. Thereafter, he initiated his research career with a project entitled "Cardiovascular pharmacology of 5-HT receptors" under the supervision of Prof. Dr. P.R. Saxena. During this period, he also had an opportunity to work in the laboratories of Dr. A. Gulati (Department of Pharmaceutics and Pharmacodynamics, University of Illinois at Chicago: The effects of DCLHb on systemic and regional haemodynamics in anaesthetised non-human primates) and Prof. Dr. C.M. Villalón (Departamento de Farmacología y Toxicología, México D.F.:

5-HT receptors mediating canine external carotid vascular effects). In January 2000, he will resume studies for completing the Medical degree.

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16.4 List of abbreviations

5-CT	:	5-carboxamidotryptamine
5-HT	:	5-hydroxytryptamine; serotonin
5-MeO-T	:	5-methoxytryptamine
8-OH-DPAT	:	8-hydroxy-2-(di-n-propylamino) tetralin
BIMU8	:	endo-N-(8-methyl-8-azabicyclo [3.2.1]oct-3-yl)-2,3-dihydro-(1-methyl) ethyl-2-oxo-1H-benzimidazole-1-carboxamide
BMS181885	:	3-[3-[4-(5-methoxy-4-pyrimidyl)-1-piperazinyl]propyl]-5-(1,2-dioxo-4-methyl-3-cyclobuten-3-yl) amino-1H-indole trihydrochloride
BRL15572	:	1-(3-chlorophenyl)-4-[3,3-diphenyl (2-(S,R) hydroxypropanyl) piperazine] hydrochloride
BQ788	:	N-cis-2,6-dimethylpiperidinocarbonyl-L-γ-methylleucyl-D-1-methoxy-carbonyltryptophanyl-D-norleucin
BW723C86	:	1-[5-(thienylmethoxy)-1H-3-indoyl] propan-2-amine
CGRP	:	calcitonin gene-related peptide
CP93129	:	3-(1,2,5,6-tetrahydropyrid-4-yl) pyrrolo [3,2-b] pyrid-5-one
CP99994	:	(2S,3S)-3-(2-methoxybenzylamino)-2-phenylpiperidine
CP122288	:	5-methylaminosulphonylmethyl-3-(N-methoxy-pyrrolidin-2R-yl-methyl)-1H-indole
DAG	:	diacylglycerol
DOI	:	(±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride
F11356	:	4-[4-(2-[3-(2-aminoethyl)-1H-indol-5-yloxy]-acetyl]-piperazin-1-yl)-benzonitrile hydrochloride
GMC2021	:	3-[2-(dimethylamino)ethyl]-5-[(trifluoromethyl) sulfonyl] oxy] [1H] indole oxalate
GR56764	:	3-(2-methylaminoethyl)-1H-indole-5-yl-1H-[1,2,4] triazol-3-yl)-methanol
GR82334	:	[D-Pro^{9} [Spiro-γ-Lactam] Leu^{10}, Trp^{11}] physalaemin(1-11)
GR113808	:	[1-[2-(methylsulphonyl)amino]ethyl]-4-piperidinyl]methyl-1-methyl-1H-indole-3-carboxylate
GR127935	:	N-[methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2'-methyl-4'-(5-methyl-1,2,4-oxadiazol-3-yl) [1,1-biphenyl]-4-carboxamide hydrochloride
GR205171	:	[(2S, 3S)-2-methoxy-5-tetrazol-1-yl-benzyl]-(2-phenyl-piperidin-3-yl)-amine dihydrochloride
IP_3	:	inositol triphosphate
IS159	:	3-(2-aminoethyl)-5-[acetamidyl-3-(4-hydroxyphenyl)-propionamidyl-acetamidyl-oxy]-indole
L775606	:	1-(3-[5-(1,2,4-triazol-4-yl)-1H-indol-3-yl] propyl)-4-(2-(3-fluorophenyl) ethyl) piperazine
L-NAME	:	N^{G}-nitro-L-arginine methyl ester
LY334370	:	4-fluoro-N-[3-(1-methyl-4-piperidinyl)-1H-indol-5-yl]-benzamide
LY344864	:	N-[3-(dimethylamino)-2,3,4,9-tetrahydro-1H-carbazol-6-yl]-4-fluorobenzamide
MDL72222	:	1αH,3α,5αH-tropan-3-yl-3,5-dichlorobezoate

MDL100907	:	R(+)-α-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenylethyl)]-4-piperidine-methanol
*m*RNA	:	messenger ribonucleic acid
NO	:	nitric oxide
PIP_2	:	phosphatidyl-inositol 4,5-biphosphate
PNU109291	:	(s)-3,4-dihydro-1-[2-[4-(4-methoxyphenyl)-1-piperazinyl] ethyl]-N-methyl-1H-2-benzopyran-6-carboximide
PNU142633	:	(s)-3,4-dihydro-1-[2-[4-[4-aminocarbonyl) phenyl]-1-piperazinyl] ethyl]-N-methyl-1H-2-benzopyran-6-carboximide
RO046790	:	4-amino-N-(2,6 bis- methylamino-pyrimidin-4-yl)-benzene sulphonamide
RO600175	:	(S)-2-(6-chloro-5-fluoroindol-1-yl)-1-methylethylamine
RO630563	:	4-amino-N-(2,6 bis-methylamino-pyridin-4-yl)-benzene sulphonamide
RP67580	:	2-[1-amino-2-(2-methoxy phenyl) ethyl]-7,7 diphenyl-4 perhydro-isoindolone-(3aR,7aR)
RPR100893	:	(3aS,4S,7aS)-7,7-diphenyl-4-(2-methoxyphenyl)-2-[(S)-2-(2-methoxyphenyl) proprionyl] perhydroisoindol-4-ol
RS102221	:	8-[5-(5-amino 2,4-dimethoxyphenyl) 5-oxopentyl]-1,3,8-triazaspiro[4,5]decane-2,4-dione
RU24969	:	5-methoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl-1H-indole-succinate
SB204070	:	1-butyl-4-piperidinylmethyl-8-amino-7-chloro-1-4-benzoioxan-5-carboxylate
SB204741	:	1-(1-methylindol-5-yl)-3-(3-methylisothiazol-5-yl)-urea
SB206553	:	5-methyl-1-(3-pyridylcarbamoyl)-1,2,3,5- tetrahydropyrrolo[2,3-f] indole
SB220453	:	(-)-cis-6-acetyl-4S-(3-chloro-4-fluoro-benzoylamino)-3,4-dihydro-2,2-dimethyl-2H-benzo [b] pyran-3S-ol
SB224289	:	2,3,6,7-tetrahydro-1'-methyl-5-[2'-methyl-4'(5-methyl-1,2,4-oxadiazol-3-yl)biphenyl-4-carbonyl] furo [2,3-f] indole-3-spiro-4`-piperidine hydrochloride
SB242084	:	6-chloro-5-methyl-1-[2-(2-methylpyridyl-3-oxy)-pyrid-5-yl carbamoyl] indoline
SB258719	:	R-3,N-dimethyl-N-[1-methyl-3-(4-methyl-piperidin-1-yl)propyl] benzenesulfonamide
SDZ21009	:	4(3-terbutylamino-2-hydroxypropoxy)indol-2-carbonic acid-isopropylester
SKF-99101H	:	3-(2-dimethylaminoethyl)-4-chloro-5-propoxyindole hemifumarate
SR57227	:	4-amino-(6-chloro-2-pyridyl)-1-piperidine hydrochloride
VIP	:	vasoactive intestinal peptide
WAY100635	:	N-(2-[4-(2-methoxy-phenyl)-1-piperazinyl]ethyl)-N-(2-pyridinyl) cyclohexane carboxamide trihydrochloride

16.5 References

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Stellingen behorende bij het proefschrift

5-Hydroxytryptamine Receptors Mediating Carotid and Systemic Haemodynamic Effects: the Relation to Acute Antimigraine Therapy

Rotterdam, 27 oktober 1999

Peter de Vries

1. Hoewel eerder toegeschreven aan achtereenvolgens de 5-HT_1-like (1986), 5-HT_{1X} (1990), 5-HT_1-like ongerelateerd aan 5-$HT_{1A/1B/1C/1D}$ (1991), 5-HT_{1D} (1996), 5-$HT_{1D\alpha/1D\beta}$ (1996) en 5-$HT_{1B/1D}$ (1997) receptor, is het nu duidelijk dat de 5-HT_{1B} receptor de sumatriptan-geïnduceerde craniële vasoconstrictie medieert (dit proefschrift).

2. In tegenstelling tot sumatriptan, contraheert 5-HT arterioveneuze anastomosen in het halsslagader vaatbed van het varken via een op dit moment niet te classificeren receptor (dit proefschrift).

3. De verbeterde therapeutische effectiviteit van orale doseringen van tweede generatie triptannen ten opzichte van sumatriptan wordt verklaard door het verbeterde farmacokinetische profiel.

4. Affiniteit voor de 5-HT_{1F} receptor is niet noodzakelijk voor succesvolle antimigraine therapie.

5. There is nothing more horrible than the murder of a beautiful theory by a brutal gang of facts (François la Rochefoucauld, 1747-1827).

6. Het is jammer dat Goadsby *et al.* (*Ann. Neurol.*, 1990, **28**, 183-187), naast de plasma concentratie van neuropeptiden in het jugulair veneus bloed tijdens de migraine aanval, niet gelijktijdig de zuurstof saturatie hebben gemeten.

7. Migraine zit tussen de oren.

8. Personen die hoofdpijn zien als reden voor het afzien van gemeenschap, zullen nooit ontdekken dat de liefde bedrijven hoofdpijn kan laten verdwijnen.

9. Als ze er niet is, weet een man pas wat hij mist (De Dijk, 1994).

10. Het definitief gestopt zijn met roken wordt meestal bekend gemaakt middels een advertentie.

11. Slim en succesvol gaan lang niet altijd samen; vaak moet je niet nadenken om succesvol te zijn.

12. De recente bevinding van het Centrum voor Verslavingsonderzoek Utrecht dat studenten meer alcohol nuttigen dan hun niet-studerende leeftijdsgenoten is net zo vernieuwend als de conclusie dat pindakaas geen effect heeft op het draaien van de aarde.

13. 640K ought to be enough for anybody (Bill Gates, 1981).

14. Waar zit die "any" key dan? (FAQ na het vastlopen van de computer).

15. I am confident that the Republican Party will pick a nominee that will beat Bill Clinton (Dan Quayle, 1998).