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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

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1 It has been suggested that the inhibition of sympathetically-induced vasopressor responses produced by 5-hydroxytryptamine (5-HT) in pithed rats is mediated by 5-HT_1-like receptors. The present study has re-analysed this suggestion with regard to the classification schemes recently proposed by the NC-IUPHAR subcommittee on 5-HT receptors.

2 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}), CP 93,129 (5-HT_{1B}) and sumatriptan (5-$HT_{1B/1D}$), resulted in a dose-dependent inhibition of sympathetically-induced vasopressor responses.

3 The sympatho-inhibitory responses induced by 5-HT, 8-OH-DPAT, indorenate, CP 93,129 or sumatriptan were analysed before and after i.v. treatment with blocking doses of the putative 5-HT receptor antagonists, WAY 100635 (5-HT_{1A}), cyanopindolol (5-$HT_{1A/1B}$) or GR 127935 (5-$HT_{1B/1D}$). Thus, after WAY 100635, the responses to 5-HT and indorenate, but not to 8-OH-DPAT, CP 93,129 and sumatriptan, were blocked. After cyanopindolol, the responses to 5-HT, indorenate and CP 93,129 were abolished, whilst those to 8-OH-DPAT and sumatriptan (except at the lowest frequency of stimulation) remained unaltered. In contrast, after GR 127935, the responses to 5-HT, CP 93,129 and sumatriptan, but not to 8-OH-DPAT and indorenate, were abolished.

4 In additional experiments, the inhibition induced by 5-HT was not modified after 5-HT_7 receptor blocking doses of mesulergine.

5 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.

Keywords: 5-Hydroxytryptamine; 5-HT_1 receptors; cyanopindolol; GR 127935; prejunctional inhibition; sympathetic outflow; WAY 100635

Introduction

The complexity of cardiovascular effects produced by 5-hydroxytryptamine (5-HT), including bradycardia or tachycardia, hypotension or hypertension and vasodilatation or vasoconstriction, has been explained by the ability 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 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*., 1998).

With respect to the ability 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, 1994) 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 (previously called 5-$HT_{1D\beta/1D_2}$; GR 127935-sensitive) in the case of non-rodent species (e.g. Molderings *et al*., 1990). However, these studies 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 mediated by exogenous noradrenaline (Villalón *et al*., 1995a). Since this sympatho-inhibitory response to 5-HT, being potently mimicked by 5-carboxamidotryptamine (5-CT), is not modified by ritanserin, MDL 72222 or tropisetron, but it is blocked by methysergide, we suggested the involvement of sympatho-inhibitory '5-HT_1-like' receptors (Villalón *et al*., 1995b).

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 GR 127935, but not by mesulergine) and 5-HT_7 receptors (potently stimulated by 5-CT, but not by sumatriptan; blocked by mesulergine, but not by GR 127935) (see

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Eglen *et al*., 1997; Saxena *et al*., 1998). On this basis, the present study was carried out to re-analyse 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.

Methods

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, U.S.A.) 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, U.S.A.). Before electrical stimulation, the animals received gallamine (25 m 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 shown (Villalón *et al*., 1995a,b): (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.

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 s, 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, CP 93,129 and sumatriptan (10, 30 and 100 μg kg^{-1} min^{-1} each) by a Harvard model 901 pump (Harvard Apparatus Co. Inc., Millis, MA, U.S.A.). Twenty minutes 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 1) which received continuous i.v. infusions of

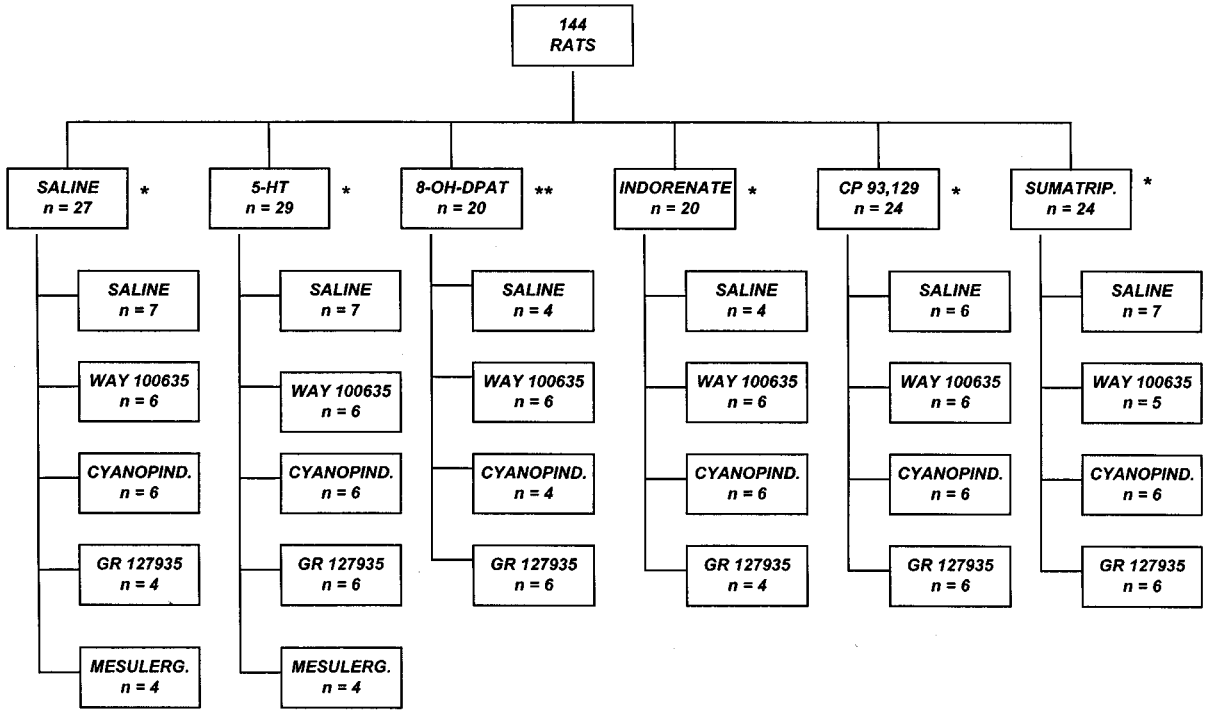

Figure 1 Experimental protocols showing the number of animals used in the 6 main groups (receiving infusions of physiological saline or agonist infusions) and the different subgroups (receiving i.v. bolus injections of physiological saline or antagonists). *Continuous infusion during a total period of 50 min. **Continuous infusion until the end of the experiment. SUMATRIP: sumatriptan; CYANOPIND: cyanopindolol; MESULERG: mesulergine.

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}; $n=20$), CP 93,129 (100 μg kg^{-1} min^{-1}; $n=24$) or sumatriptan (100 μg kg^{-1} min^{-1}; $n=24$) using the aforementioned pump. Twenty minutes 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 1).

The first group, infused with saline, was subdivided into 5 treatment groups (see Figure 1) comprising i.v. bolus injections of: saline (0.3, 1, 3 and 10 ml kg^{-1}; $n=7$), WAY 100635 (3, 10, 30 and 100 μg kg^{-1}; $n=6$), cyanopindolol (100, 300 and 1000 μg kg^{-1}; $n=6$), GR 127935 (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 minutes 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 1) comprising i.v. bolus injections of: saline (0.3, 1 and 3 ml kg^{-1}; $n=7$), WAY 100635 (10 μg kg^{-1}; $n=6$), cyanopindolol (100 μg kg^{-1}; $n=6$), GR 127935 (100 μg kg^{-1}; $n=6$) or mesulergine (300 μg kg^{-1}; $n=4$); ten minutes after each treatment, a S-R curve was elicited again. As shown in Figure 1, each of the other four groups, infused with 8-OH-DPAT, indorenate, CP 93,129 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}), WAY 100635 (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 GR 127935 (100 μg kg^{-1}). Ten minutes after each treatment a S-R curve was elicited again (for the number of experiments, see Figure 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, CP 93,129 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) it was 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, CP 93,129 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,b). The dosing with all compounds used was sequential.

Drugs

Apart from the anaesthetic (diethyl ether), the drugs used in the study (obtained from the sources indicated) were: 5-hydroxytryptamine creatinine sulphate and gallamine triethiodide (Sigma Chemical Co., St. Louis, MO, U.S.A.); indorenate (5-methoxytryptamine-β-methylcarboxylate hydrochloride or TR3369; gift: Prof. Enrique Hong, CINVESTAV-IPN, Mexico City, Mexico); CP 93,129 ([3-(1,2,5,6-tetrahydropyrid-4-yl)pyrrolo[3,2-b]pyrid-5-one]) (gift: Pfizer Inc., Groton, U.S.A.); 8-OH-DPAT (8-hydroxy-2-(di-N-propylamino)tetralin), WAY 100635 (N-{2-[4-(2-methoxy-phenyl)-1-piperazinyl]ethyl}-N-(2-pyridinyl)cyclohexanecarboxamide trihydrochloride) and desipramine hydrochloride (Research Biochemicals Int., Natick, MA, U.S.A.); GR 127935 (N-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)[1,1,-biphenyl]-4-carboxamide hydrochloride monohydrate) and sumatriptan succinate (gift: Dr Simon Lister and Dr Helen Connor, GlaxoWellcome, Stevenage, Hertfordshire, UK); cyanopindolol and mesulergine hydrochloride (gift: Sandoz AG, Basel, Switzerland). All compounds were dissolved in distilled water. When needed, 1% (w/v) ascorbic acid (CP 93,129) 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, CP 93,129 and sumatriptan, where they refer to the free base.

Data presentation and statistical analysis

All data in the text and figures are presented as mean$\pm$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 (randomized block design) had revealed that the samples represented different populations (see Steel & Torrie, 1980). A P value of 0.05 or less (two-tailed) was considered statistically significant.

Results

Systemic haemodynamic variables

The baseline values of diastolic blood pressure and heart rate in the 164 rats were, respectively, 59.9$\pm$0.3 mmHg and 290$\pm$3.4 beats min^{-1}. Following the first i.v. bolus injection of desipramine (50 μg kg^{-1}) these values were transiently increased, but after 8 min they did not significantly differ from the baseline values (i.e. 61.2$\pm$0.6 mmHg and 292$\pm$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, CP 93,129 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}), WAY 100635 (3, 10, 30 and 100 μg kg^{-1}), GR 127935 (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$\pm$7, +34$\pm$8 and −2$\pm$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 (CP 93,129) and 22±8 (sumatriptan) beats min^{-1}.

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 2–5). These vasopressor responses are due to selective stimulation of the systemic vasculature since only negligible (if any) changes in heart rate were observed, as shown by other authors (e.g. Gillespie *et al*., 1970; Flavahan *et al*., 1985; Grant & McGrath, 1988).

Effect of 5-HT and the 5-HT$_1$ receptor agonists, 8-OH-DPAT, indorenate, CP 93,129 or sumatriptan, on the sympathetically-induced vasopressor responses

Figure 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 of sympathetically-induced vasopressor responses, as previously observed (Villalón *et al*., 1995a). This sympatho-inhibitory response to 5-HT was dose-dependently mimicked by continuous infusions of the 5-HT$_1$ receptor agonists, 8-OH-DPAT, indorenate, CP 93,129 and sumatriptan (10, 30 and 100 μg kg^{-1} min^{-1} each; see Figure 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.

Effect of physiological saline, WAY 100635, cyanopindolol, GR 127935 or mesulergine on the sympathetically-induced vasopressor responses per se *and on the inhibition of sympathetically-induced vasopressor responses produced by 5-HT*

Figure 3a 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., for 50 min). After the infusion of saline had been stopped, 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 WAY 100635 (3, 10 and 30 μg kg^{-1}), cyanopindolol (100 and 300 μg kg^{-1}) or all doses tested of GR 127935 (100, 300 and 1000 μg kg^{-1}) and mesulergine (300 μg kg^{-1}). In contrast, the highest doses of WAY 100635 (100 μg kg^{-1}) and cyanopindolol (1000 μg kg^{-1}) produced a significant inhibition of the S-R curves which was, coincidentally, more pronounced at lower frequencies of stimulation (Figure 3a).

Figure 3b shows the effects produced by i.v. bolus injections of physiological saline (0.3, 1 and 3 ml kg^{-1}), WAY 100635 (10 μg kg^{-1}), cyanopindolol (100 μg kg^{-1}), GR 127935 (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., for 50 min). Under these experimental conditions, the sympatho-inhibition induced by the infusion of 5-HT was reproducible since even after the infusion had ceased, the corresponding S-R curves remained without significant changes when the animals received three subsequent bolus injections of physiological

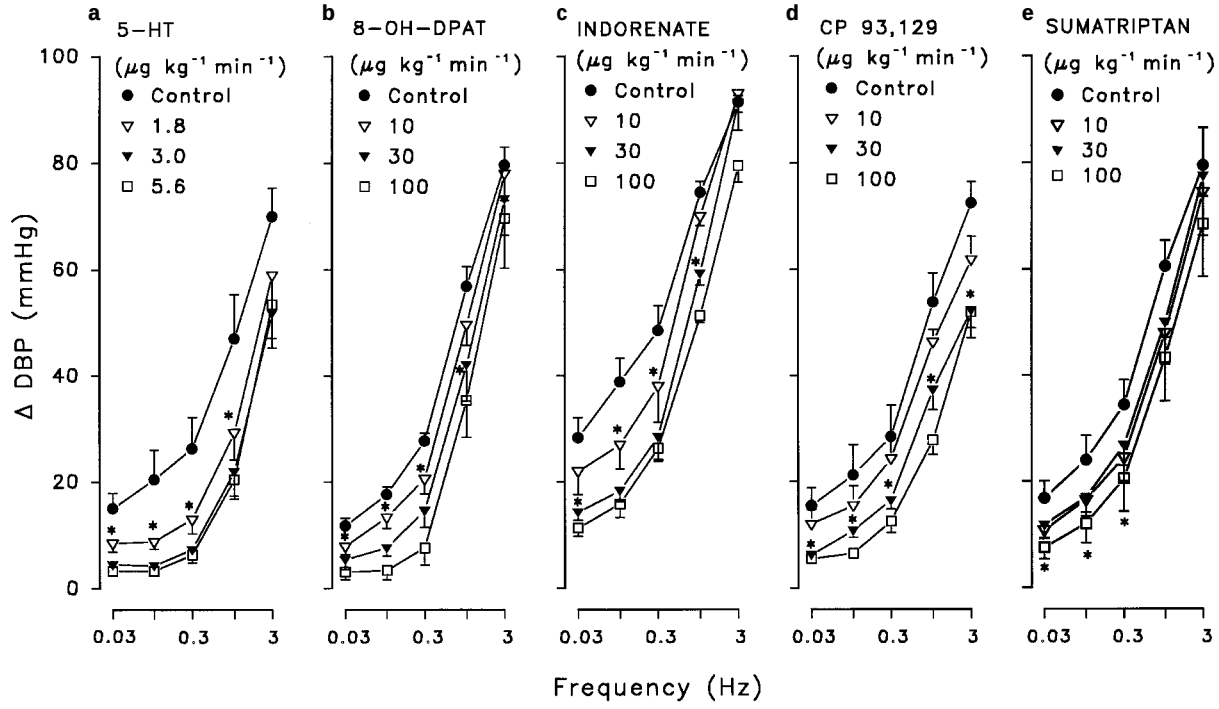

Figure 2 Increases in diastolic blood pressure (Δ DBP) produced by electrical stimulation of the sympathetic vasopressor outflow in pithed rats before (control response) and during the i.v. continuous infusions of: (a) 5-HT; (b) 8-OH-DPAT; (c) indorenate; (d) CP 93,129; and (e) sumatriptan. *$P<0.05$ *vs* the control response. All the other graphs after the starred (*) graph are also significantly different from the control response.

saline (Figure 3b). In contrast, when the effects of antagonists were analysed, the response to 5-HT was found to be practically abolished after administration of WAY 100635, cyanopindolol or GR 127935, but was unaffected by mesulergine (Figure 3b).

Effect of physiological saline, WAY 100635, cyanopindolol or GR 127935 on the inhibition of sympathetically-induced vasopressor responses produced by some 5-HT$_1$ receptor agonists

Figures 4 and 5 show the effects of physiological saline (0.3, 1 and 3 ml kg^{-1}), WAY 100635 (10 and/or 30 μg kg^{-1}), cyanopindolol (100 μg kg^{-1}) or GR 127935 (100 μg kg^{-1}) on the inhibition of sympathetically-induced vasopressor responses induced by 8-OH-DPAT (30 μg kg^{-1} min^{-1}; Figure 4a), indorenate (30 μg kg^{-1} min^{-1}; Figure 4b), CP 93,129 (100 μg kg^{-1} min^{-1}; Figure 5a) and sumatriptan (100 μg kg^{-1} min^{-1}; Figure 5b). As previously observed with 5-HT, the sympatho-inhibition produced by the continuous infusion (during 50 min) of indorenate, CP 93,129 and sumatriptan was reproducible since even after the infusion had ceased, 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 4a), 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 the infusion was interrupted. 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

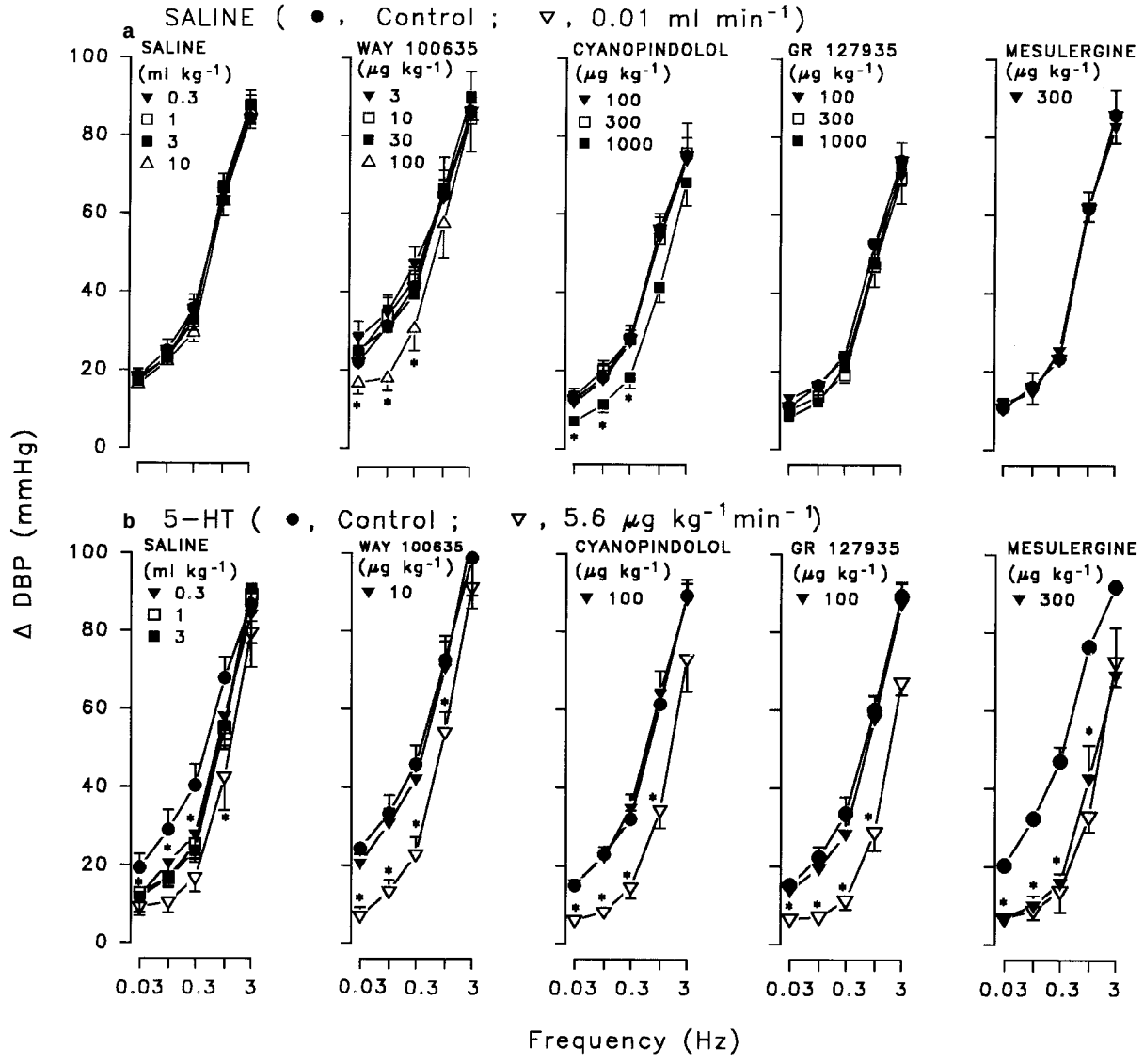

Figure 3 Effects of i.v. bolus injections of saline, WAY 100635, cyanopindolol, GR 127935 or mesulergine on either: (a) the increases in diastolic blood pressure (Δ DBP) by stimulation of the sympathetic vasopressor outflow in animals infused with saline (0.01 ml min^{-1}, i.v., for 50 min) or (b) the corresponding inhibition by the infusion of 5-HT (5.6 μg kg^{-1} min^{-1}, i.v. during 50 min) of sympathetically-induced vasopressor responses. The above doses of antagonists (or saline) were injected after the infusion of saline (a) or 5-HT (b) had been stopped and the S-R curves were elicited after each dose. *$P<0.05$ *vs* the control response (produced before the continuous infusion of any compound). All the other graphs after the starred (*) graph are also significantly different from control.

received three subsequent bolus injections of physiological saline (Figure 4a).

When the effects of the antagonists were analysed, differential effects were observed on the sympatho-inhibition produced by each agonist. Thus, Figure 4a shows that the sympatho-inhibition produced by 8-OH-DPAT was practically resistant to blockade by WAY 100635 (10 and 30 μg kg^{-1}), cyanopindolol (100 μg kg^{-1}) and GR 127935 (100 μg kg^{-1}). Similarly, in additional experiments (not presented 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 WAY 100635 (30 μg kg^{-1}, i.v., $n=4$).

In contrast, the response to indorenate (Figure 4b) was virtually abolished by WAY 100635 (10 μg kg^{-1}) and cyanopindolol (100 μg kg^{-1}), but not by GR 127935 (100 μg kg^{-1}). With respect to the sympatho-inhibition produced by CP 93,129 and sumatriptan, it is evident that the responses to CP 93,129 (Figure 5a) and sumatriptan (Figure 5b) were blocked by GR 127935 (100 μg kg^{-1}), but remained unchanged after WAY 100635 (10 and 30 μg kg^{-1}). Furthermore, after cyanopindolol, the responses to CP 93,129 were abolished (Figure 5a) and those to sumatriptan were blocked only at 0.03 Hz (Figure 5b), but remained unaffected at the other frequencies studied (0.1 – 3 Hz).

Discussion

The aim of this study was to re-analyse the pharmacological profile of the '5-HT$_1$-like' receptors mediating the inhibition of

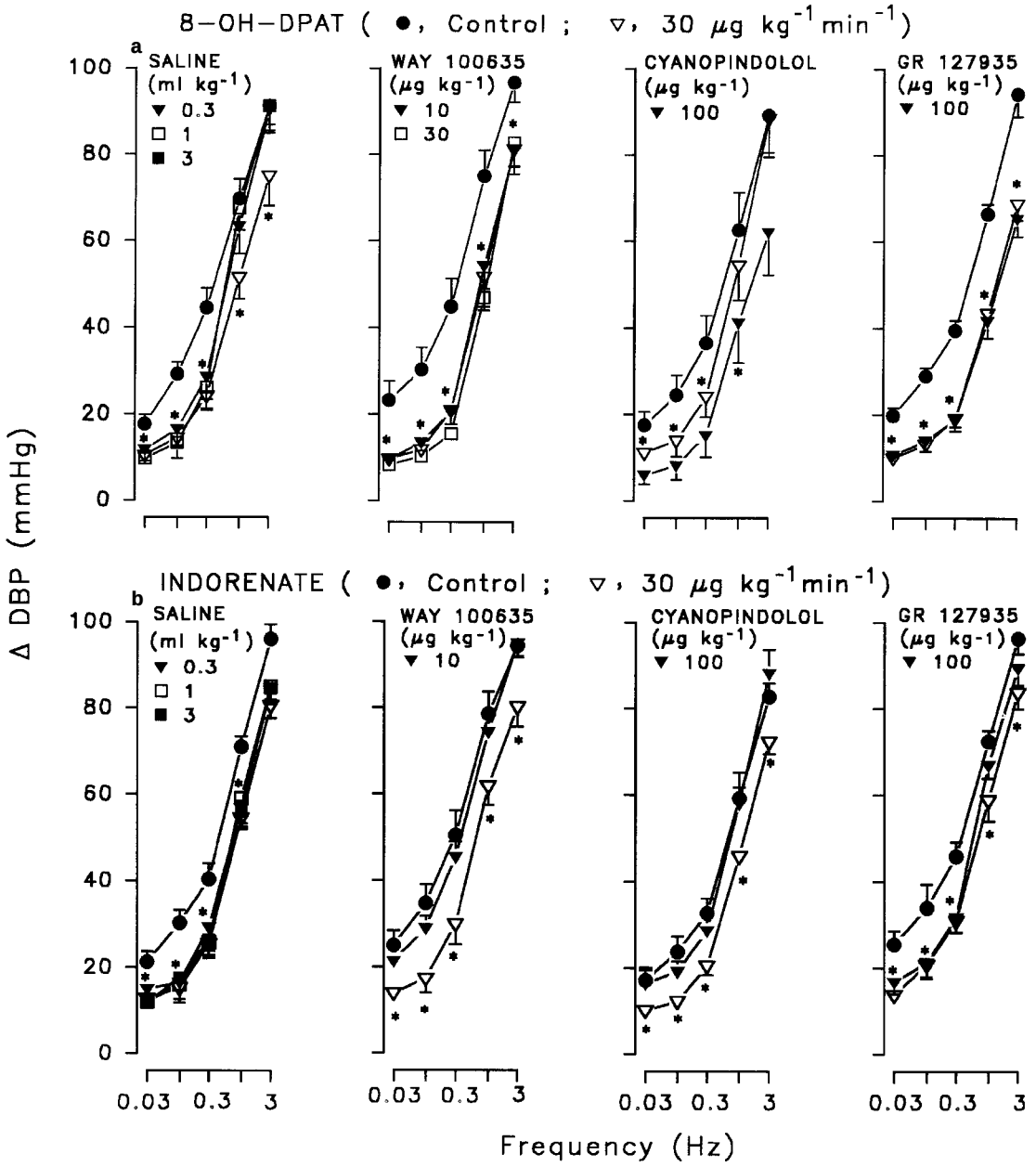

Figure 4 Effects of i.v. bolus injections of saline, WAY 100635, cyanopindolol or GR 127935 on the inhibition of sympathetically-induced vasopressor responses induced by: (a) 8-OH-DPAT (30 μg kg^{-1} min^{-1}, i.v., infused uninterruptedly); and (b) indorenate (30 μg kg^{-1} min^{-1}, i.v., infused for 50 min). The above doses of antagonists (or saline) were injected either after concluding each S-R during the infusion of 8-OH-DPAT (a) or after stopping the infusion of indorenate (b); the S-R curves were elicited after each dose. *$P<0.05$ *vs* the control response (produced before the continuous infusion of any compound). All the other graphs after the starred (*) graph are also significantly different from control.

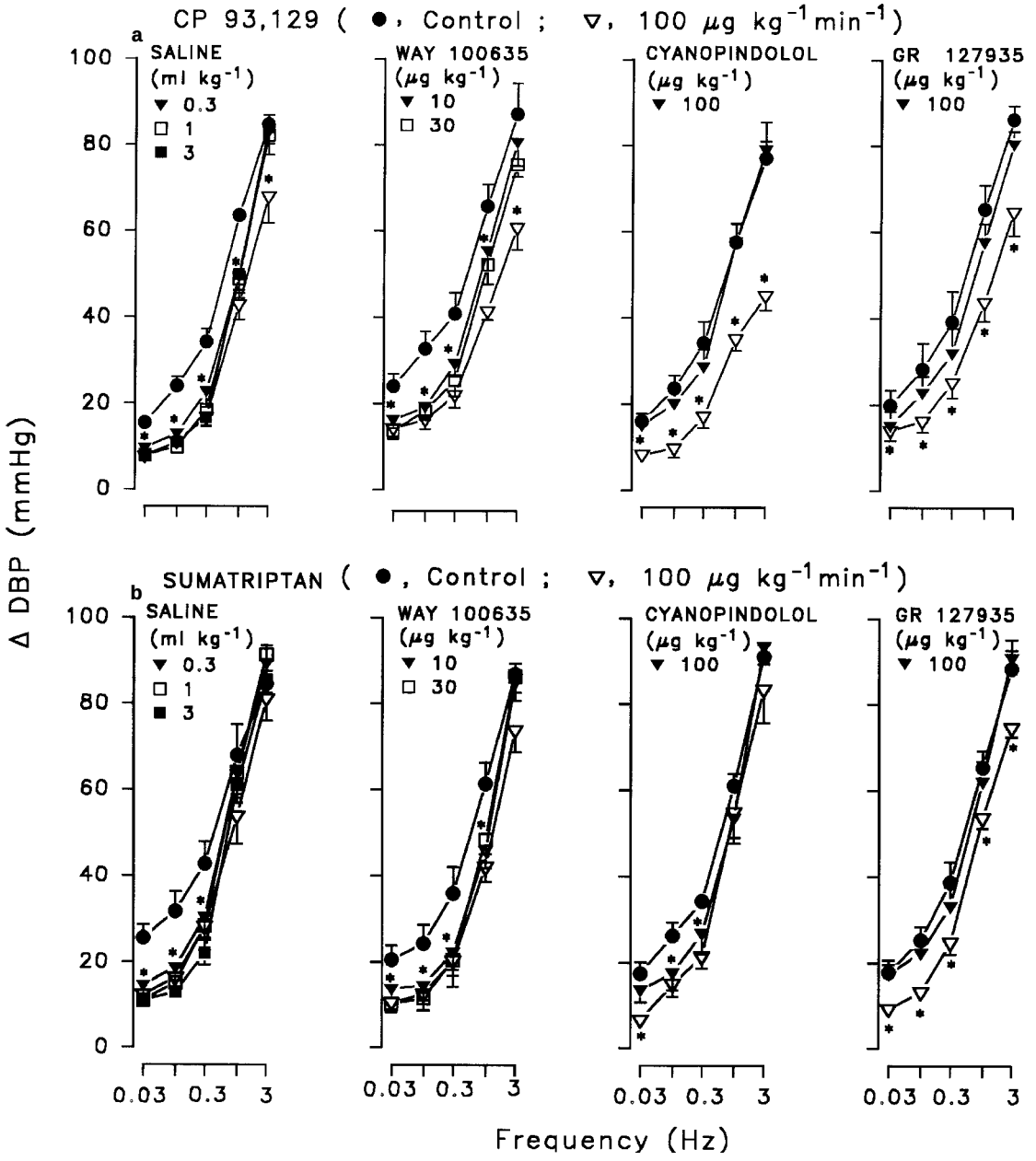

Figure 5 Effects of i.v. bolus injections of saline, WAY 100635, cyanopindolol or GR 127935 on the inhibition of sympathetically-induced vasopressor responses induced by the i.v. continuous infusion (during 50 min) of 100 μg kg^{-1} min^{-1} of: (a) CP 93,129; and (b) sumatriptan. The above doses of antagonists (or saline) were injected after stopping the infusion of CP 93,129 (a) or sumatriptan (b), and the S-R curves were elicited after each dose. *$P<0.05$ *vs* the control response (produced before the continuous infusion of any compound). All the other graphs after the starred (*) graph are also significantly different from control.

sympathetically-induced vasopressor responses in pithed rats, with regard to 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*., 1998). Within this framework, it has to be admitted that the 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*., 1995a).

Thus, the present study shows that the inhibition of sympathetically-induced vasopressor responses induced by 5-HT: (i) can be mimicked by agonists at 5-HT$_{1A}$ (indorenate and 8-OH-DPAT; see Dompert *et al*., 1985; Hoyer *et al*., 1994); 5-HT$_{1B}$ (CP 93,129; Macor *et al*., 1990) and 5-HT$_{1B/1D}$ (sumatriptan; see Hoyer *et al*., 1994) receptors; and (ii) can be blocked by antagonists at 5-HT$_{1A}$ (WAY 100635), 5-HT$_{1A/1B}$ (cyanopindolol) or 5-HT$_{1B/1D}$ (GR 127935) receptors, but not by antagonists at cardiovascular 5-HT$_7$ (mesulergine) receptors (see 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 the experimental model, some features deserve further consideration. Thus, though selective stimulation of the sympathetic vasopressor outflow was produced, 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 (see Hoyer, 1988) and, indeed, blocks some '5-HT$_1$-like' receptor-mediated functional responses in the rat (see Martin, 1994), as we have shown with another β-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.

Systemic haemodynamic changes

With regard to 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,b) 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 (see Langer, 1980; Göthert *et al*., 1990).

Hence, it could be alternatively argued that the marked inhibitory effects of 5-HT (Figure 3b), 8-OH-DPAT (Figure 4a), indorenate (Figure 4b), CP 93,129 (Figure 5a) and sumatriptan (Figure 5b) 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 3, 4 and 5), as previously found for 5-HT (Villalón *et al*., 1995a,b).

On the other hand, it is interesting to note that higher doses of WAY 100635 (100 μg kg^{-1}) and cyanopindolol (1000 μg kg^{-1}) produced a significant inhibition on the S-R curves *per se* (Figure 3a). Since this inhibitory effect was, coincidentally, more pronounced at lower frequencies of stimulation, as previously found for 5-HT (Villalón *et al*., 1995a,b) and other 5-HT$_1$ receptor agonists (see Figure 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.

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 WAY 100635, a highly potent and selective 5-HT$_{1A}$ receptor antagonist (Fletcher *et al*., 1996) and cyanopindolol, a putative 5-HT$_{1A/1B}$ receptor antagonist (see Hoyer *et al*., 1994). At doses devoid of effects on sympathetically-induced vasopressor responses *per se* (Figure 3a), these compounds were capable of blocking the (sympatho)-inhibition induced by both 5-HT (the endogenous ligand; Figure 3b) and indorenate (a 5-HT$_{1A}$ receptor agonist; Dompert *et al*., 1985; Figure 4b). Accordingly, the (sympatho)-inhibition produced by indorenate being resistant to blockade by GR 127935, an antagonist at 5-HT$_{1B/1D}$ receptors (previously described as, respectively, the 5-HT$_{1D\beta/1D\alpha}$ subtypes of the 5-HT$_{1D}$ receptor; see Hartig *et al*., 1996) may be explained in terms of its low affinity for 5-HT$_{1A}$ receptors (see Skingle *et al*., 1996). It must be emphasized that the doses of the 5-HT receptor antagonists, WAY 100635, cyanopindolol and GR 127935 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 (see Hoyer *et al*., 1994), presumably also produced (sympatho)-inhibition in the pithed rat (Figure 4a), but this response, interestingly, was not blocked by WAY 100635, cyanopindolol or GR 127935. 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 WAY 100635, to antagonize the inhibition by 8-OH-DPAT (see Results section) do not support this hypothesis.

In view of the fact 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 the infusion was interrupted (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.

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

Evidence for the involvement of 5-HT$_{1B}$ receptors stems from the ability of cyanopindolol (a putative 5-HT$_{1A/1B}$ receptor antagonist; see Hoyer *et al*., 1994) to block the (sympatho)-inhibition induced by 5-HT (Figure 3b) and by the selective rodent 5-HT$_{1B}$ receptor agonist, CP 93,129 (Figure 5a; Macor *et al*., 1990). Thus, the (sympatho)-inhibition by CP 93,129, being resistant to blockade by WAY 100635 (at doses even higher than those required to block the (sympatho)-inhibition by 5-HT and indorenate; Figure 5a), confirms the high selectivity of the latter as a 5-HT$_{1A}$ receptor antagonist (see Fletcher *et al*., 1996). A relevant finding was also the blockade of CP 93,129-induced (sympatho)-inhibition by GR 127935 (Figure 5a), since this compound is now apparently considered to be an antagonist at non-rodent 5-HT$_{1B/1D}$ receptors (see Hartig *et al*., 1996; Villalón *et al*., 1997b; see above). Notwithstanding, binding data show that GR 127935 has similar affinities for 5-HT$_{1D\alpha}$ (pK$_i$: 8.9; non-rodent 5-HT$_{1D}$), 5-HT$_{1D\beta}$ (pK$_i$: 9.9; non-rodent 5-HT$_{1B}$) and 5-HT$_{1B}$ (pK$_i$: 8.5; rodent 5-HT$_{1B}$) receptors (see Skingle *et al*., 1996). Thus, the simplest interpretation suggests that the blockade of CP 93,129-induced (sympatho)-inhibition by GR 127935 may be

due to the high affinity of the latter for rodent 5-HT$_{1B}$ receptors.

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 (see 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*. (1997). In keeping with this suggestion, the (sympatho)-inhibition produced by sumatriptan (see Figure 5b) was resistant to blockade by WAY 100635 (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, GR 127935, 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 (see 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 the 5-HT$_{1B}$ receptors, this was cyanopindolol-sensitive only at 0.03 Hz (Figure 5b).

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 WAY 100635, cyanopindolol and GR 127935 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 (Villalón *et al*., 1995a,b; present results). 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).

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, CP 93,129 and sumatriptan in our experiments involves inhibition of adenylate cyclase, it is important to emphasize that all 5-HT$_1$ receptor subtypes are, by definition, negatively coupled to adenylyl cyclase (see Hoyer *et al*., 1994), and this is a signal transduction system usually associated with the decrease in noradrenaline release from sympathetic neurones (see 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 ability of methysergide, an antagonist at cardiovascular 5-HT$_1$ and 5-HT$_7$ receptors (see Villalón *et al*., 1997b), to block the (sympatho)-inhibition induced by 5-HT (Villalón *et al*., 1995b), 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*., 1997; Villalón *et al*., 1997a,c). However, this is unlikely since mesulergine, an ergoline derivative devoid of interactions with the 5-HT$_1$ receptor family (see Hoyer *et al*., 1994), at doses that are high enough to block cardiovascular 5-HT$_7$ receptors (De Vries *et al*., 1997; Villalón *et al*., 1997a,c), failed to antagonize the inhibition by 5-HT (Figure 3b). Consistent with this finding, the cardiovascular 5-HT$_7$ receptors, unlike 5-HT$_{1A/1B/1D}$ receptors are: (i) resistant to blockade by GR 127935 and cyanopindolol and to the agonist action of indorenate, CP 93129 and sumatriptan (De Vries *et al*., 1997; Villalón *et al*., 1997a,c); 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 (see Langer, 1980; Rand *et al*., 1987).

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

Lastly, one might 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 (see Fozard, 1984) and postganglionic sympathetic neurones (see 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 hyperpolarization) 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 WAY 100635; 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. CP 93129 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*., 1998). 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) (see 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 neurones 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-mentioned 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 neurones. Hence, subtype-selective 5-HT$_{1B}$ and 5-HT$_{1D}$ receptor ligands (see Saxena *et al*., 1998) become crucial probes to explore whether or not sympathetic neuronal effects in the rat are mediated by the 5-HT$_{1D}$ subtype.

In conclusion, these experiments 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.

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