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Characterization of prejunctional 5-HT receptors mediating inhibition of sympathetic vasopressor responses in the pithed rat

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1 It has recently been shown that continuous infusions of 5-hydroxytryptamine (5-HT) are able to inhibit, in a dose-dependent manner, the pressor responses induced by preganglionic (T_7-T_9) sympathetic stimulation in pithed rats pretreated with desipramine (50 $\mu g\ kg^{-1}$, i.v.). This inhibitory effect, besides being significantly more pronounced at lower frequencies of stimulation (0.03-1 Hz) and devoid of tachyphylaxis, is reversible after interrupting the infusions of 5-HT (up to 5.6 $\mu g\ kg^{-1}\ min^{-1}$). In the present study we have characterized the pharmacological profile of the receptors mediating the above inhibitory effect of 5-HT.

2 The inhibition induced by 5.6 $\mu g\ kg^{-1}\ min^{-1}$ of 5-HT on sympathetically-induced pressor responses was not blocked after i.v. treatment with physiological saline (1 ml kg^{-1}), ritanserin (0.1 mg kg^{-1}), MDL 72222 (0.15 mg kg^{-1}) or tropisetron (3 mg kg^{-1}), which did not modify the sympathetically-induced pressor responses *per se*, but was significantly antagonized by the 5-HT_1-like and 5-HT_2 receptor antagonist, methysergide (0.3 mg kg^{-1}), which also produced a slight attenuation of the pressor responses to 0.03 and 0.1 Hz *per se*.

3 Unexpectedly and contrasting with methysergide, the 5-HT_1-like and 5-HT_2 receptor antagonists, methiothepin (0.01, 0.03 and 0.1 mg kg^{-1}) and metergoline (1 and 3 mg kg^{-1}), apparently failed to block the above 5-HT-induced inhibition. Nevertheless, it is noteworthy that these antagonists also blocked the electrically-induced pressor responses *per se*, presumably by blockade of vascular α_1-adrenoceptors and, indeed, this property might have masked their potential antagonism at the inhibitory 5-HT_1-like receptors.

4 Consistent with the above findings, 5-carboxamidotryptamine (5-CT, a potent 5-HT_1-like receptor agonist), metergoline and methysergide mimicked the inhibitory action of 5-HT with the following rank order of agonist potency: 5CT > > 5-HT > metergoline ≥ methysergide.

5 Taken together, the above results suggest that the inhibitory action of 5-HT on the electrically-induced pressor responses is primarily mediated by an action on inhibitory prejunctional 5-HT_1-like receptors leading to a decrease in the sympathetic nerve discharge. Interestingly, 5-HT-induced excitatory mechanisms could be made manifest once the inhibitory action of 5-HT had been antagonized.

Keywords: 5-Hydroxytryptamine; 5-HT_1-like receptors; prejunctional inhibition; sympathetic outflow

Introduction

Of the complex cardiovascular effects of 5-hydroxytryptamine (5-HT), its vasodilator and/or hypotensive effects may involve, at least, four distinct mechanisms, namely: central vasomotor inhibition, direct vascular smooth muscle relaxation, release of endothelium-derived relaxing factor (EDRF) and/or inhibition of noradrenaline release from postganglionic sympathetic neurones (for references see Martin, 1994; Saxena & Villalón, 1990a,b; 1991).

With respect to the latter, several lines of pharmacological evidence have shown that 5-HT inhibits the contractile responses to adrenergic nerve stimulation in blood vessels by activation of prejunctional 5-HT_1-like receptors; these blood vessels include, amongst others, the canine (Humphrey *et al.*, 1988) and human (Göthert *et al.*, 1990) saphenous veins; the canine common carotid (Mylecharane & Phillips, 1989), external carotid (Terrón *et al.*, 1994; Villalón *et al.*, 1993) and femoral (Feniuk *et al.*, 1981) arterial beds; the rat kidney (Charlton *et al.*, 1986) and vena cava (Molderings *et al.*, 1987); and the porcine coronary artery (Molderings *et al.*, 1989).

It is worth noting that all of the above studies have been carried out *in vitro* or in an individual arterial bed in the whole animal, but not in the systemic vasculature. In this respect, we have recently shown by producing preganglionic (T_7-T_9) stimulation of the sympathetic vasopressor outflow, that 5-HT does indeed inhibit the sympathetic transmission in the systemic vasculature (Villalón *et al.*, 1995a). In this study, the electrically-induced neurotransmitter release could be estimated indirectly by measurement of the evoked vasopressor response, so that 5-HT inhibited the vasopressor responses induced by sympathetic stimulation, but not those by exogenous noradrenaline. Nevertheless, little is known about the nature of the receptors involved in the above inhibitory effect of 5-HT under such expereimental conditions.

On this basis, the present study was carried out to characterize the pharmacological profile of the 5-HT receptors mediating the inhibition of pressor responses induced by stimulation of the sympathetic vasopressor outflow in vagotomized pithed rats, with particular emphasis on verifying if these receptors belong to the 5-HT_1-like type. Therefore, we investigated if the inhibitory effects of the endogenous ligand, 5-HT, could be mimicked by the 5-HT_1-like receptor agonist, 5-carboxamidotryptamine (5-CT; Hoyer *et al.*, 1994), or me-

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thysergide and metergoline, which are partial agonists at 5-HT$_1$-like receptors (Saxena & Villalón, 1990a; Martin, 1994). Furthermore, methiothepin, metergoline, methysergide, ritanserin, MDL 72222 and tropisetron were used as potential antagonists of the response to 5-HT. Preliminary results of this investigation were communicated to the Third IUPHAR Satellite Meeting on Serotonin (Villalón *et al.*, 1994) and to the British Pharmacological Society (Villalón *et al.*, 1995b).

Methods

General

Experiments were carried out in a total of 88 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, and down the vertebral foramen (Shippley & Tilden, 1947) and artificially respired with room air by 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 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.). Prior to electrical stimulation, the animals received gallamine (25 mg kg^{-1}, i.v.) to avoid electrically-induced muscular twitching.

Since the inhibitory effects of 5-HT on sympathetic transmission are particularly more pronounced at lower frequencies of stimulation, all animals were systematically pretreated with 50 μg kg^{-1} (i.v.) of desipramine before each stimulus-response curve (S-R curve). Under these conditions, the resulting pressor responses to lower frequencies of stimulation were greater in magnitude when compared to those elicited in rats without desipramine (Villalón *et al.*, 1995a).

Experimental protocol

After a stable haemodynamic condition for at least 30 min, baseline values of diastolic blood pressure and heart rate were determined. Then, 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). Thus, the S-R curve was completed in about 30 min; at this point, the animals were divided into three groups.

The first group ($n=35$) received a continuous infusion of 5-HT (5.6 μg kg^{-1} min^{-1}) by a Harvard model 901 pump (Harvard Apparatus Co. Inc., Millis, MA, U.S.A.); after 10 min, a S-R curve was completed as described above and the motor-driven syringe was stopped. These animals were then subdivided into 7 treatment groups ($n=5$ each) comprising i.v. bolus injections of: physiological saline (1 ml kg^{-1}), metergoline (1 and 3 mg kg^{-1}), methiothepin (0.01, 0.03 and 0.1 mg kg^{-1}), methysergide (0.3 mg kg^{-1}), ritanserin (0.1 mg kg^{-1}), MDL 72222 (0.15 mg kg^{-1}) and tropisetron (3 mg kg^{-1}); 10 min after each treatment the infusion of 5-HT was started and a S-R curve was elicited again.

The second group ($n=35$ and subdivided into 7 treatment groups; $n=5$ each) was run in parallel with the above group in order to investigate, during a continuous infusion of physiological saline (0.01 ml min^{-1}), the effects of the same doses of saline, metergoline, methiothepin, methysergide, ritanserin, MDL 72222 or tropisetron on the electrically-induced pressor responses *per se*.

Finally, the third group ($n=18$) was used to analyze the effect of 5-HT$_1$-like receptor agonists on the S-R curves. For this purpose, the animals were subdivided into 3 subgroups ($n=6$ each); following the procedure previously described for the infusion of 5-HT, each subgroup received a continuous infusion of 5-CT (0.01, 0.03 and 0.1 μg kg^{-1} min^{-1}), metergoline (3, 10 and 30 μg kg^{-1} min^{-1}) or methysergide (10, 30 and 100 μg kg^{-1} min^{-1}) and a S-R curve was elicited during the infusion of each dose of the corresponding agonist.

Each dose of 5-HT, 5-CT, metergoline or methysergide was administered at a rate of 0.01 ml min^{-1} during a period of about 30 min, at which time the corresponding S-R curve was completed. The doses of 5-HT, 5-CT, metergoline and methysergide 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 (see Villalón *et al.*, 1995a). The dosing with all drugs used was sequential.

Drugs

Apart from the anaesthetic (diethyl ether), the drugs used in the present study (obtained from the sources indicated) were the following: 5-hydroxytryptamine creatinine sulphate and gallamine triethiodide (Sigma Chemical Co., St. Louis, MO, U.S.A.); 5-carboxamidotryptamine maleate (gift: Dr P.P.A. Humphrey, Glaxo Group Research, Ware, U.K.); tropisetron (ICS 205-930: (3α-tropanyl)-1H-indole-3-carboxylic acid ester) and methysergide maleate (gift: Sandoz A.G., Basel, Switzerland); methiothepin maleate (gift: Hoffman-La Roche Ltd., Basel, Switzerland); metergoline (gift: Farmitalia, Milan, Italy); ritanserin (gift: Dr J.M. Van Neuten, Janssen Pharmaceutica, Beerse, Belgium); 1αH, 3α,5αH-tropan-3yl-3,5-dichlorobenzoate (MDL 72222) and desipramine hydrochloride (Research Biochemicals Int., Natick, MA, U.S.A.); all compounds were dissolved in distilled water. When needed, 1% (w/v) ascorbic acid (ritanserin and metergoline) or 5% (v/v) propylene glycol (methiothepin) was added; these vehicles 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 and 5-CT, where they refer to the free base.

Data presentation and statistical analysis

All data in the text and figures are presented as 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 Newman-Keuls' test, once an analysis of variance (randomized 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.

Results

Systemic haemodynamic variables

Baseline values of diastolic blood pressure and heart rate in desipramine-pretreated rats were, respectively, 74 ± 7 mmHg and 210 ± 8 beats min^{-1} ($n=88$); these values were not significantly modified (not shown) by the continuous infusion of either physiological saline, the agonists (5-HT, 5-CT, metergoline and methysergide) or the 5-HT receptor antagonists.

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 1–4); these pressor 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; Villalón *et al.*, 1995a).

Effect of physiological saline or 5-HT$_1$-like, 5-HT$_2$, 5-HT$_3$ and 5-HT$_4$ receptor antagonists on the 5-HT-induced inhibition of electrically-induced pressor responses

We have previously shown (Villalón *et al.*, 1995a) that the inhibition of electrically-induced pressor responses by a continuous infusion of 5.6 μg kg^{-1} min^{-1} of 5-HT is highly reproducible. Consistent with these findings, Figure 1 shows that both the electrically-induced pressor responses (Figure 1a) and the 5-HT-induced inhibition of the electrically-induced pressor responses (Figure 1b) were not inhibited in control animals receiving saline (1 ml kg^{-1}, i.v.); similar results were obtained, as shown in the upper and lower panels of Figure 2, respectively, after ritanserin (0.1 mg kg^{-1}; Figure 2b), MDL 72222 (0.15 mg kg^{-1}; Figure 2c) or tropisetron (3 mg kg^{-1}; Figure 2d). Unlike the above antagonists, methysergide (0.3 mg kg^{-1}), at a dose that did not block the pressor responses to 0.3, 1 and 3 Hz *per se* (Figure 2a, upper panel), did antagonize the inhibitory effect of 5-HT (Figure 2a, lower panel). Interestingly, methysergide produced a slight–though significant–attenuation of the pressor responses to 0.03 and 0.1 Hz *per se* (Figure 2a, upper panel), and apparently–though not significantly–potentiated the pressor response to 3 Hz during the infusion of 5-HT (Figure 2a, lower panel).

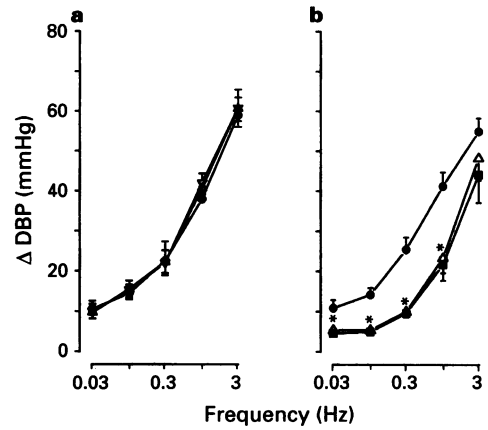

Figure 1 The effects of i.v. bolus injections of physiological saline (△, 1 ml kg^{-1}; $n=5$ each) on: (a) the increases in diastolic blood pressure (Δ DBP) by electrical stimulation (●); and (b) the corresponding inhibition by 5.6 μg kg^{-1} min^{-1} of 5-hydroxytryptamine (5-HT, ■) of electrically-induced pressor responses (●). Note that a further 1 ml kg^{-1} of saline (▽, $n=5$) did not affect (a). *$P<0.05$ vs. control.

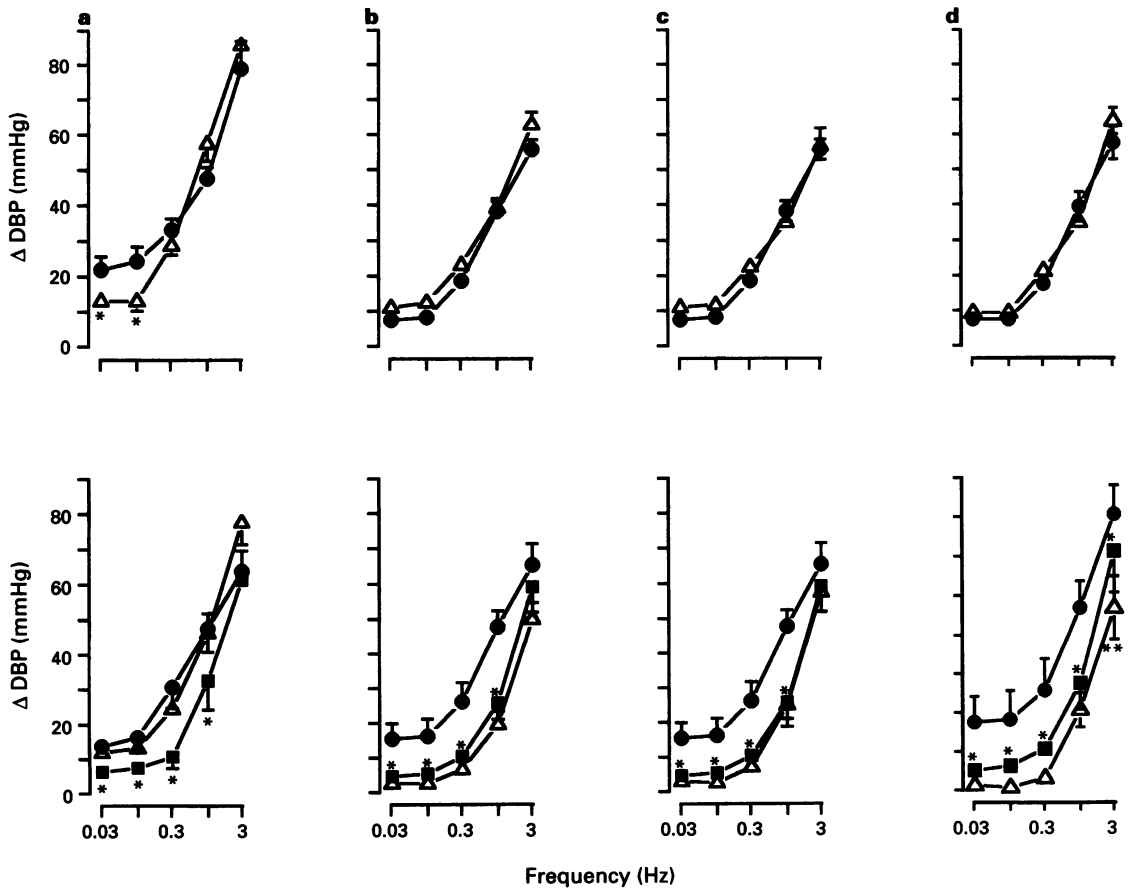

Figure 2 The effects of (a) methysergide (0.3 mg kg^{-1}), (b) ritanserin (0.1 mg kg^{-1}), (c) MDL 72222 (0.15 mg kg^{-1}) and (d) tropisetron (3 mg kg^{-1}) (all represented by △) on either the increases in diastolic blood pressure (Δ DBP) by electrical stimulation (●) (upper panels) or the corresponding inhibition by 5.6 μg kg^{-1} min^{-1} of 5-hydroxytryptamine (5-HT) (■) of electrically-induced pressor responses (●) (lower panels). *$P<0.05$ vs. control.

In contrast to methysergide, Figure 3 shows that both methiothepin (0.01, 0.03 and 0.1 mg kg^{-1}, i.v.) and metergoline (1 and 3 mg kg^{-1}) produced a dose-dependent blockade of the electrically-induced pressor responses *per se* (see upper panels of Figure 3a and b, respectively), with the former being more potent than the latter. Furthermore, it is noteworthy that both antagonists, at the doses used, did apparently fail to antagonize the inhibitory effects of 5-HT (lower panels of Figures 3a and 3b, respectively).

Agonist action of 5-CT, methysergide and metergoline

In keeping with the above findings, Figure 4 shows that the infusions of 5-CT (0.01, 0.03 and 0.1 μg kg^{-1} min^{-1}), methysergide (10, 30 and 100 μg kg^{-1} min^{-1}) or metergoline (3, 10 and 30 μg kg^{-1} min^{-1}) mimicked 5-HT producing a dose-dependent inhibition of the electrically-induced pressor responses (Figure 4a, b and c, respectively). 5-CT was about 2 log units more potent than 5-HT (Villalón *et al.*, 1995a), which was itself more potent than metergoline or methysergide.

Discussion

The aim of this study was to characterize pharmacologically the 5-HT receptors mediating the inhibition of sympathetic vasopressor responses in pithed rats. Thus, it is shown that, apart from the possible disclosure of excitatory mechanisms, the receptors mediating the inhibition by 5-HT: (i) can be stimulated by 5-CT, metergoline and methysergide; (ii) do not resemble either 5-HT$_2$, 5-HT$_3$ or 5-HT$_4$ receptors; and (iii) resemble the inhibitory 5-HT$_1$-like receptors present on the sympathetic nerves of other vascular preparations carried out *in vivo* (see for example Feniuk *et al.*, 1981; Charlton *et al.*, 1986; Mylecharane & Phillips, 1989; Villalón *et al.*, 1993; Terrón *et al.*, 1994).

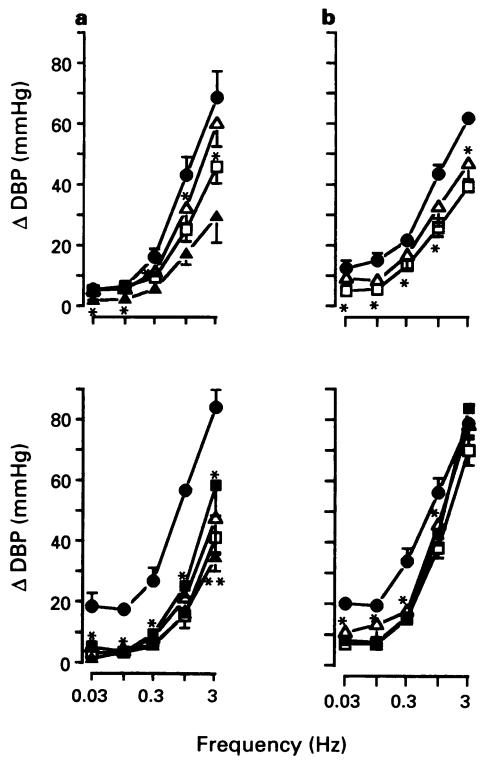

Figure 3 The effects of (a) methiothepin (△, 0.01 mg kg^{-1}; □ 0.03 mg kg^{-1}; and ▲, 0.1 mg kg^{-1}; $n=5$ each) and (b) metergoline (△, 1 mg kg^{-1} and □, 3 mg kg^{-1}; $n=5$ each) on either the increases in diastolic blood pressure (Δ DBP) by electrical stimulation (●) (upper panels) or the corresponding inhibition by 5.6 μg kg^{-1} min^{-1} of 5-hydroxytryptamine (5-HT) (■) of electrically-induced pressor responses (●) (lower panels). *$P<0.05$ vs. control.

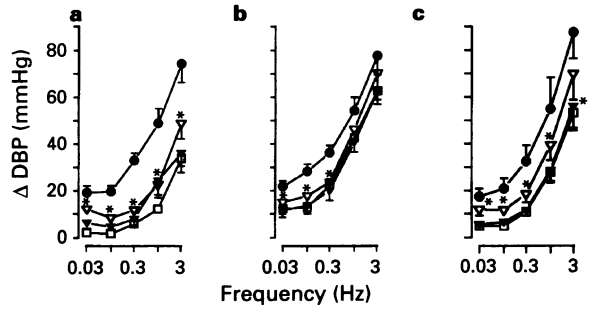

Figure 4 The increases in diastolic blood pressure (Δ DBP) by electrical stimultion before (control, ●) and during the continuous infusion of: (a) 5-carboxamidotryptamine (▽, 0.01; ▼ 0.03; and □, 0.1 μg kg^{-1} min^{-1}); (b) methysergide (▽, 10; ▼ 30; and □, 100 μg kg^{-1} min^{-1}); and (c) metergoline (▽, 3; ▼ 10; and □, 30 μg kg^{-1} min^{-1}). *$P<0.05$ vs. control.

With respect to our experimental model, we should consider two important features. On the one hand, 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); notwithstanding, we deliberately avoided using it since the β-blocker has affinity for some 5-HT$_1$ binding sites and, indeed, blocks some 5-HT$_1$-like receptor-mediated functional responses in the rat (Martin, 1994). On the other hand, the possible influences arising from the central nervous system via 5-HT mechanisms can be ruled out, since pithed rats were used. However, we cannot exclude an action of the agonists at sympathetic ganglia, which have modulatory 5-HT receptors (Fozard, 1984).

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 (for comparison see Flavahan *et al.*, 1985; Bulloch & McGrath, 1988; Villalón *et al.*, 1995a) have relevance for the purpose of the present study, since the prejunctional inhibitory effects of 5-HT (and of any other 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 may be due to tachyphylaxis of the sympathetic vasopressor responses. However, this seems unlikely since such responses remained essentially unchanged when the animals received a subsequent i.v. bolus injection of physiological saline (Figure 1).

Pharmacological profile of the rat inhibitory prejunctional 5-HT receptors

Our findings support the notion that the prejunctional 5-HT receptors mediating inhibition of the sympathetic vasopressor outflow in the rat could resemble the 5-HT$_1$-like receptor type, since the inhibitory effects of 5-HT were: (i) resistant to ritanserin, MDL 72222 and tropisetron at doses that are sufficient to antagonize, respectively, 5-HT$_2$, 5-HT$_3$ and 5-HT$_3$/5-HT$_4$ receptors (Villalón *et al.*, 1990; 1991; 1993); (ii) amenable to blockade by methysergide; and (iii) mimicked by 5-CT, metergoline and methysergide with a rank order of agonist potency of 5-CT > > 5-HT (see Villalón *et al.*, 1995a) > metergoline ≥ methysergide. Consistent with the above notion, preliminary experiments show that sumatriptan is as potent as methysergide at inhibiting the sympathetic vasopressor outflow in the rat (Villalón *et al.*, 1995b); apart from the im-

plications of this finding, it is important to emphasize that the 5-HT$_1$-like receptor and related 5-HT$_1$ subtypes are, by definition, negatively coupled to adenylate cyclase (Hoyer *et al.*, 1994), and this is a signal transduction system usually associated with a decrease in the release of noradrenaline from sympathetic neurones (Langer, 1980; Rand *et al.*, 1987).

Despite the above considerations, it could still be argued that 5-ht$_7$ receptors might be involved (Plassat *et al.*, 1993). However, this seems unlikely since 5-ht$_7$ receptors are pharmacologically characterized by: (i) the rank order of agonist potency of 5-CT = 5-HT = 5-methoxytryptamine > bufotenine > 8-hydroxy-2-(di-N-propylamino)tetralin, with sumatriptan inactive at 10 μM; and (ii) the rank order of antagonist potency of methiothepin = methysergide = mesulergine = metergoline = butaclamol = clozapine = ergotamine > spiperone. Significantly, and contrasting with 5-HT$_1$-like receptors, the 5-ht$_7$ receptors are postively coupled to adenylate cyclase (Plassat *et al.*, 1993), and this is a signal transduction system associated with an increase (not a decrease) in the release of noradrenaline from sympathetic neurones (Langer, 1980; Rand *et al.*, 1987).

No attempt was made in the present study to relate the inhibitory prejunctional 5-HT$_1$-like receptor in terms of the 5-HT$_{1B}$ or 5-HT$_{1D\alpha}$ subtypes, which are the most likely candidates in rodents (Hoyer *et al.*, 1994); in this connection, although 5-HT$_{1B}$ receptor-mediated vasoconstrictor effects have been demonstrated in rats (Craig & Martin, 1993), there are no formally recognized functional roles for the 5-HT$_{1D\alpha}$ subtypes. Hence, CP 93,129/cyanopindolol and sumatriptan/GR 127935, which are agonists/antagonists at, respectively, 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors (Hoyer *et al.*, 1994), become crucial probes to explore whether or not neuronal effects in the rat are mediated by the 5-HT$_{1D\alpha}$ subtype.

Nevertheless, the failure of methiothepin and metergoline to block the inhibition to 5-HT deserves further comment, particularly in terms of their selectivity and considering that the rat sympathetic vasopressor responses involve, to an important extent, activation of vascular α_1-adrenoceptors (Flavahan *et al.*, 1985; Bulloch & McGrath, 1988).

Differential effects of methysergide, methiothepin and metergoline

The attenuation of pressor responses by methysergide (Figure 2a, upper panel) seems to be due to its partial agonist properties at inhibitory 5-HT$_1$-like receptors on sympathetic nerves (Martin, 1994; see Figure 4b) and not to α_1-adrenoceptor blockade, since the drug: (i) has a low affinity for α_1-adrenoceptor binding sites (K_i = 2300 nM versus ^{3}H-WB 4101 binding, which is negligible to that of prazosin: K_i = 0.58 nM; Leysen, 1985); and (ii) does not alter the pressor responses to 0.3, 1 and 3 μg kg^{-1} of phenylephrine in pithed rats (20±3, 40±8 and 53±8 mmHg before; and 20±2, 32±5 and 50±13 mmHg after 1 mg kg^{-1} of methysergide, n = 3; unpublished).

In contrast, the block by methiothepin of the S-R curves (Figure 3a, upper panel) suggests a blockade of α_1-adrenoceptors, since the drug (i) displays an affinity for α_1-adrenoceptor binding sites (K_i = 0.47 nM; Leysen, 1985) which is even higher than that of prazosin; and (ii) blocks the pressor responses to 0.3, 1 and 3 μg kg^{-1} of phenylephrine in pithed rats (20±2, 38±4 and 61±10 mmHg before; and 7±1*, 12±2* and 18±3* mmHg (P < 0.05) after 0.1 mg kg^{-1} of methiothepin, n = 3; unpublished). Consequently, the failure of methiothepin to block the inhibition by 5-HT does not categorically rule out the involvement of 5-HT$_1$-like receptors, but rather implies that the α_1-adrenoceptor blocking properties of the drug might have masked its potential antagonism at the inhibitory 5-HT$_1$-like receptors; this could explain the further blockade of the S-R curves obtained during the infusion of 5-HT by methiothepin (Figure 3a, lower panel).

Similarly, neither can the failure of metergoline to antagonize the inhibition by 5-HT unequivocally exclude a possible correlation between the prejunctional 5-HT$_1$-like receptor and the 5-HT$_{1D}$ receptor subtype (for which metergoline displays the highest affinity; Waeber *et al.*, 1988), particularly when considering that the pressor response to 3 Hz during the infusion of 5-HT was apparently potentiated (Figure 3b, lower panel), as observed with methysergide (Figure 2a, lower panel). These findings may suggest the possible disclosure of excitatory mechanisms once the inhibitory effect of 5-HT has been blocked (see below).

It is noteworthy that metergoline displays a moderate affinity for α_1-adrenoceptor binding sites (K_i = 38 nM versus [^{3}H]-WB 4101 binding; Leysen, 1985), a finding that is compatible with the moderate blockade of pressor responses induced by 0.3, 1 and 3 μg kg^{-1} of phenylephrine (21±1, 37±1 and 58±2 mmHg before; and 18±1, 28±2* and 43±2* mmHg (P < 0.05) after 3 mg kg^{-1} of metergoline, respectively, n = 6; unpublished). Although these findings could partly explain the metergoline-induced blockade of sympathetically-induced pressor responses (Figure 3b, upper panel), we cannot exclude a possible 'methysergide-like action'; indeed, the agonist potency of metergoline at 5-HT$_{1A}$, 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors (with pEC$_{50}$ values of 7.6, 7.2 and 7.5, respectively) is higher than that of methysergide (pEC$_{50}$ values of 6.4, 6.7 and 7.0, respectively; Hoyer *et al.*, 1994).

Thus, the simplest interpretation suggests that the α_1-adrenoceptor blocking properties of methiothepin and metergoline, in addition to other properties overlooked here (see Leysen, 1985; Waeber *et al.*, 1988) make the use of these drugs inappropriate in the characterization of prejunctional autonomic 5-HT receptors. Obviously, the identification of the specific subtype of 5-HT$_1$-like receptor involved in the inhibition by 5-HT in this study will require, as previously discussed, further experiments with selective agonists and antagonists of the various 5-HT$_1$ receptor subclasses (Hoyer *et al.*, 1994).

In contrast to the results of the present study, both methiothepin and metergoline have proven adequate antagonists if the inhibition to 5-HT is measured as a response unrelated to stimulation of α_1-adrenoceptors, for example, the inhibition of electrically-evoked release of [^{3}H]-noradrenaline (Göthert *et al.*, 1990; Molderings *et al.*, 1990) or the vasodilatation of the canine external carotid bed resulting from inhibition of the sympathetic discharge (Villalón & Terrón, 1994).

Possible involvement of 5-HT-induced excitatory mechanisms

It is interesting to note the apparent potentiation of some of the pressor responses after methysergide and metergoline during the infusion of 5-HT (lower panels of Figures 2a and 3b, respectively). Hence, it would seem reasonable to suggest that only when the inhibitory action of 5-HT has been blocked (by methysergide and, conceivably, by metergoline), are its excitatory components unmasked; these excitatory components may include, amongst others: (1) A prejunctional mechanism (although the effect was observed only at 3 Hz) which is apparently unrelated to stimulation of 5-HT$_2$ and 5-HT$_3$ receptors, since no effect was noticed after ritanserin or MDL 72222 (see lower panel of Figures 2b and 2c, respectively). However, the possible role of excitatory prejunctional 5-HT$_4$ receptors cannot be categorically excluded since tropisetron, at a dose that antagonizes functional 5-HT$_4$ receptors (Villalón *et al.*, 1990; 1991) did potentiate (significantly at 3 Hz) the inhibitory effect of 5-HT (Figure 2d, lower panel); certainly, the 5-HT$_4$ receptor is, by definition, positively coupled to adenylate cyclase (Hoyer *et al.*, 1994) and, as previously discussed, this is a signal transduction system associated with an increase in noradrenaline release from sympathetic neurones. (2) A postjunctional synergy between α_1-adrenoceptors, which could be activated by neuronally released noradrenaline, and 5-HT$_{1(D)}$-like receptors at which methysergide and metergoline are partial agonists (Figures 4b and 4c, respectively; Saxena & Villalón, 1990a; Hoyer *et al.*, 1994). (3) An inhibition of prejunctional autoreceptors (α_2-adrenoceptors), preventing

feed-back inhibition of noradrenaline release with a resulting increase in the α_1-adrenoceptor-mediated pressor response. Indeed, this resulting effect could have been partly masked by the mild α_1-adrenoceptor blocking properties of metergoline (Leysen, 1985; see above).

Further considerations on the agonist action of 5-HT, 5-CT, methysergide and metergoline

The inhibition by 5-HT, 5-CT, methysergide and metergoline, being more pronounced at lower frequencies of stimulation, resembles the effect of other prejunctional modulators on noradrenaline release from sympathetic neurones (Langer, 1980). However, we could not reach the maximum effects with these agonists, as higher doses, particularly of 5-CT, produced a marked hypotension (not shown), which is mediated by postjunctional 5-HT$_1$-like receptors (Saxena & Villalón 1990a). In addition to recognizing the possible interference by pharmacokinetic factors in our experimental model, it is noteworthy that a fall in diastolic blood pressure of 10 mmHg evoked by an infusion of nitroprusside (which acts directly on vascular smooth muscle to cause relaxation independently of 5-HT receptors), inhibits the sympathetically-induced pressor responses *per se* (Grant & McGrath, 1988).

The finding that 5-CT and, to a lesser extent, methysergide and metergoline, mimicked 5-HT is consistent with the inhibitory action of these drugs on the sympathetic nerves of the human saphenous vein (Molderings *et al.*, 1990) or both the canine femoral (Feniuk *et al.*, 1981) and external carotid (Terrón *et al.*, 1994) circulations, responses that involve the activation of 5-HT$_1$-like receptors. This observation, coupled to the blockade by methysergide of the inhibition to 5-HT, raises the possibility that methysergide and 5-HT are acting on a common site of action, as previously considered (Martin, 1994).

The physiological relevance of a prejunctional inhibitory 5-HT$_1$-like receptor in the systemic vasculature of the rat is, to the best of our knowledge, unknown; one possibility exists, however. 5-HT has been shown to be taken-up into and released from sympathetic nerves (Saxena & Villalón, 1990a), and may act, therefore, as a modulator of the neuroeffector transmission; thus, feed-back inhibition of the sympathetic discharge by 5-HT may be involved, as described for other substances (Rand *et al.*, 1987).

In conclusion, we suggest that the 5-HT-induced inhibition of sympathetic vasopressor responses in the pithed rat is primarily mediated by prejunctional 5-HT$_1$-like receptors leading to a decrease in the sympathetic nerve discharge. The pharmacological profile of these receptors is similar (sympathetic nerves of the rat kidney, human saphenous vein and canine external carotid artery) to other prejunctional 5-HT$_1$-like receptors mediating vascular responses. We also acknowledge the possible involvement of 5-HT-induced excitatory mechanisms which could be made manifest once the inhibitory action of 5-HT has been blocked.

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