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Characterization of prejunctional 5-HT_1 receptors that mediate the inhibition of pressor effects elicited by sympathetic stimulation in the pithed rat

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1 A study was made of the effects of 5-carboxamidotryptamine (5-CT) on pressor responses induced *in vivo* by electrical stimulation of the sympathetic outflow from the spinal cord of pithed rats. All animals had been pretreated with atropine. Sympathetic stimulation (0.1, 0.5, 1 and 5 Hz) resulted in frequency-dependent increases in blood pressure. Intravenous infusion of 5-CT at doses of 0.01, 0.1 and 1 μg kg^{-1} min^{-1} reduced the pressor effects obtained by electrical stimulation. The inhibitory effect of 5-CT was significantly more pronounced at lower frequencies of stimulation. In the present study we characterized the pharmacological profile of the receptors mediating the above inhibitory effect of 5-CT.

2 The inhibition induced by 0.01 μg kg^{-1} min^{-1} of 5-CT on sympathetically-induced pressor responses was partially blocked after i.v. treatment with methiothepin (10 μg kg^{-1}), WAY-100,635 (100 μg kg^{-1}) or GR127935T (250 μg kg^{-1}), but was not affected by cyanopindolol (100 μg kg^{-1}).

3 The selective 5-HT_{1A} receptor agonist 8-OH-DPAT and the selective 5-$HT_{1B/1D}$ receptor agonists sumatriptan and L-694,247 inhibited the pressor response, whereas the 5-HT_{1B} receptor agonists CGS-12066B and CP-93,129 and the 5-HT_{2C} receptor agonist *m*-CPP did not modify the pressor symapthetic responses.

4 The selective 5-HT_{1A} receptor antagonist WAY-100,635 (100 μg kg^{-1}) blocked the inhibition induced by 8-OH-DPAT and the selective 5-$HT_{1B/1D}$ receptor antagonist GR127935T (250 μg kg^{-1}) abolished the inhibition induced either by L-694,247 or sumatriptan.

5 None of the 5-HT receptor agonists used in our experiments modified the pressor responses induced by exogenous noradrenaline (NA).

6 These results suggest that the presynaptic inhibitory action of 5-CT on the electrically-induced pressor response is mediated by both r-5-HT_{1D} and 5-HT_{1A} receptors.

Keywords: 5-Hydroxytryptamine; 5-CT; 5-HT_{1A} receptors; r-5-HT_{1D} receptors; 5-HT_1-like receptors; prejunctional inhibition

Introduction

The existence of regulatory 5-hydroxytryptamine (5-HT) receptors located on postganglionic sympathetic nerve terminals has been demonstrated in recent years, *in vitro*, for several tissues of different species. With regard to this, several studies have shown that noradrenaline release and contractile responses in different blood vessels are inhibited by activation of prejunctional 5-HT_1-like receptors, including 5-HT_{1B} and 5-HT_{1D} receptors. Among others, these blood vessels include the rat vena cava (Molderings *et al*., 1987) and kidney (Charlton *et al*., 1986) and the canine (Humphrey *et al*., 1988) and human (Göthert *et al*., 1990; Molderings *et al*., 1990) saphenous vein.

Working in *in vivo* conditions to produce preganglionic stimulation of sympathetic vasopressor outflow, we have previously shown that 5-HT does indeed inhibit sympathetic transmission in the systemic vascular system (Morán *et al*., 1994). In this study, electrically-induced neurotransmitter release was estimated indirectly by measurement of the evoked vasopressor response, and 5-carboxamidotryptamine (5-CT), a 5-HT receptor agonist, was found to inhibit the vasopressor responses induced by sympathetic stimulation, but not those produced by exogenous noradrenaline (NA). According to our previous results (Morán *et al*., 1994) and others (Villalón *et al*., 1994; 1995a,b), the inhibitory 5-hydroxytryptaminergic activity of sympathetic neurotransmission is mainly mediated by prejunctional activation of 5-HT_1 receptors. In this respect, Shepheard *et al*. (1996) proposed that the reduction in noradrenaline release from postganglionic sympathetic neurones in the rat was mediated through activation of r-5-HT_{1D} receptors, while Jones *et al*. (1995) demonstrated that 5-HT inhibits sympathetic ganglionic transmission by activation of 5-$HT_{1B/1D}$ receptors in the cat.

In view of the above, the present study was carried out to characterize the pharmacological profile of 5-HT_1 receptor subtypes mediating the inhibition of pressor responses induced by stimulation of sympathetic vasopressor outflow in atropine-treated, pithed rats. Thus, we investigated whether the inhibitory effects of 5-HT could be mimicked by different selective 5-HT_1 subtype receptor agonists: 5-HT_{1A}, 8-OH-DPAT, (Middlemis & Fozard, 1983), 5-HT_{1B}, CGS-12066B, (Neale *et al*., 1987) and CP-93,129 (Macor *et al*., 1990), and 5-$HT_{1B/1D}$, L-694,247 (Beer *et al*., 1993) and sumatriptan (Schoeffter & Hoyer, 1989), and by a selective 5-HT_{2C} receptor agonist, *m*-CPP (see Humphrey *et al*., 1993).

Methods

General

A total of 225 male Wistar rats weighing 275–325 g were used in our experiments. The animals were kept and supplied by the Animalarium of the Faculty of Pharmacy of University of Salamanca. After anaesthesia with pentobarbitone (60 mg

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kg^{-1}, i.p.) and cannulation of the trachea, the rats were pithed by inserting a stainless steel rod through the orbit and foramen magnun (Gillespie & Muir, 1967) and artificially respirated with room air by a Harvard respiratory pump (1 ml air 100 g^{-1}, 50 strokes min^{-1}).

The right and left jugular veins were cannulated for the infusions of agonist and for the administration of antagonist respectively, and the left carotid artery connected to a PRS 205 amplifier, displaying the recordings on one channel of a Letica Polygraph 4000, for recording of blood pressure. Heart rate was measured by analysis of the blood pressure data by a Car 1000 tachograph connected to the same PRS 205 amplifier.

The entire sympathetic outflow from the spinal cord was stimulated by the use of a Cibertec Stimulator CS-9. Two electrodes were employed: one was connected to the pithing rod (the stimulating electrode), while the other electrode (the indifferent electrode) was inserted subcutaneously into a leg.

Before electrical stimulation, the animals were treated with heparin (100 u kg^{-1}) and then received (+)-tubocurarine (2 mg kg^{-1}, i.v.) to avoid electrically-induced muscular twitching and atropine (1 m kg^{-1}, i.v.) to prevent cholinergic effects.

Experimental protocols

After a stable haemodynamic condition for at least 10 min, baseline values of mean blood pressure (MBP) were determined. Then, sympathetic vasopressor outflow was stimulated by applying trains of 25 s, consisting of monophasic pulses of 1 ms duration and 15$\pm$3 V at increasing frequencies (0.1, 0.5, 1 and 5 Hz). Thus, the control stimulation-response curve (S-R curve E0) was completed in about 20 min. At this point, the animals were divided into four groups and each group into different subgroups, taking into account that each animal was used to evaluate only a respective dose of agonist or antagonist, and each dose was repeated five times up to a total of $n=5$ experiments: (1) The first group ($n=105$) received a continuous infusion of physiological saline (1 ml h^{-1}, $n=5$, control group for all agonist treatments), 5-CT (0.01, 0.1 and 1 μg kg^{-1} min^{-1}, $n=15$), 8-OH-DPAT (10, 20 and 40 μg kg^{-1} min^{-1}, $n=15$), CGS-12066B (10, 20 and 40 μg kg^{-1} min^{-1}, $n=15$), CP-93,129 (20, 50 and 100 μg kg^{-1} min^{-1}, $n=15$), sumatriptan (10 and 20 μg kg^{-1} min^{-1}, $n=10$), L-694,247 (0.3, 1 and 5 μg kg^{-1} min^{-1}, $n=15$) or *m*-CPP (10, 20, and 40 μg kg^{-1} min^{-1}, $n=15$) through a Harvard model 122 pump. After 5 min, three new S-R curves (E1, E2 and E3) were completed as described above. Each infusion was maintained for 1 h.

(2) The second group ($n=60$) was run in parallel with the above group in order to investigate during the continuous infusions of physiological saline (1 m h^{-1}), the effects of saline (1 ml kg^{-1}, $n=5$), HCl (0.01 M, 1 ml kg^{-1}, $n=5$) (the vehicle used for *m*-CPP and CP-93,129), methiothepin (10 μg kg^{-1}, $n=5$), WAY=100,635 (100, 500 and 1000 μg kg^{-1}, $n=15$), cyanopindolol (100, 300 and 1000 μg kg^{-1}, $n=15$) or GR127935T (250, 500 and 1000 μg kg^{-1} $n=15$) on the electrically-induced pressor responses *per se*.

(3) The third group was used to analyse the 5-HT$_1$ receptor subtype involved in the effects of 5-CT, 8-OH-DPAT and L-694,247 agonists on the S-R curves (E1, E2 and E3). These animals ($n=35$) were subdivided into four treatment groups; methiothepin (10 μg kg^{-1}) 5 min before the infusion of 5-CT (0.01 μg kg^{-1}, $n=5$); WAY-100,635 (100 μg kg^{-1}) 5 min before the infusion of 5-CT (0.1 μg kg^{-1} min^{-1}, $n=5$) or 8-OH-DPAT (20 μg kg^{-1} min^{-1}, $n=5$), cyanopindolol (100 μg kg^{-1}) 5 min before the infusion of 5-CT (0.01 μg kg^{-1} min^{-1},

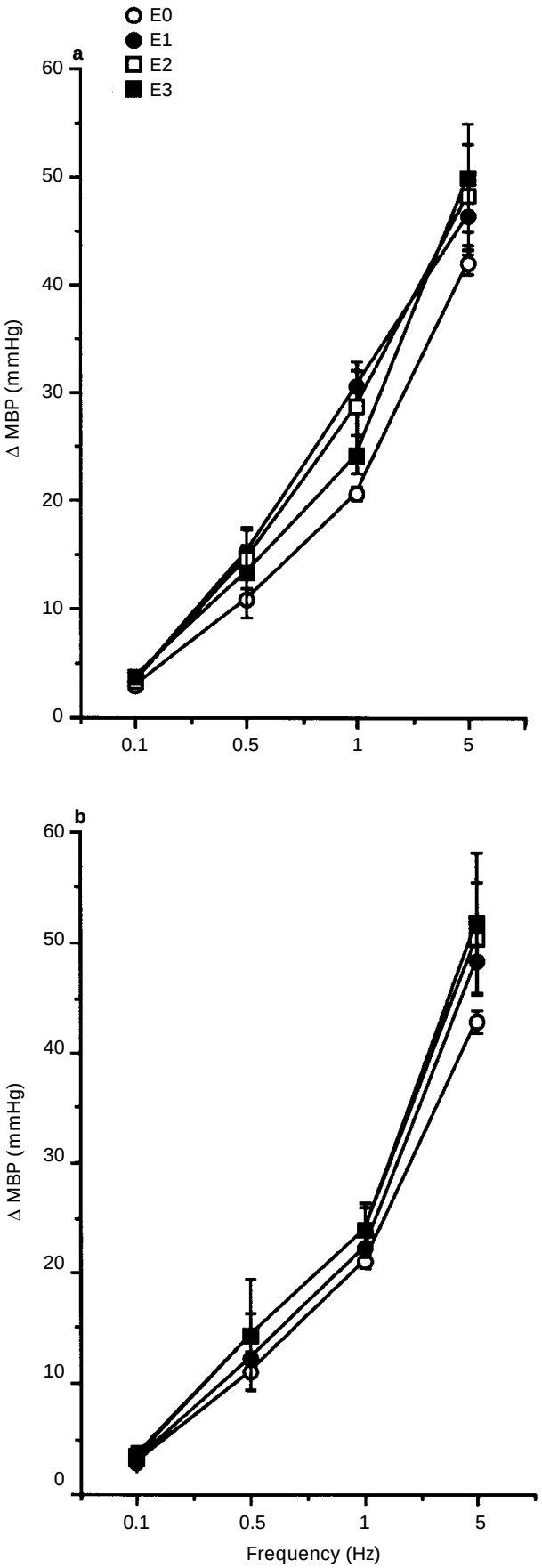

Figure 1 Effect of (a) saline (1 ml kg^{-1}, i.v.) and (b) HCl 0.01 M (1 ml kg^{-1}, i.v.) on increases in mean blood pressure (Δ MBP) induced by electrical stimulation in pithed rats infused with saline (1 ml h^{-1}). E0 control; E1, first; E2, second; E3, third stimulation-response curve. Vertical lines show s.e.mean.

$n=5$) and GR127935T (250 μg kg^{-1}) 5 min before the infusion of 5-CT (0.01 μg kg^{-1} min^{-1}, $n=5$), sumatriptan (10 μg kg^{-1} min^{-1}, $n=5$) or L-694,247 (1 μg kg^{-1} min^{-1}, $n=5$).

(4) Finally, in the last group ($n=25$) blood pressure dose-response curves for NA (0.01, 0.05, 0.1 and 0.5 μg kg^{-1}) were obtained before (E'0) and during (E'1, E'2 and E'3) the continuous infusions of either physiological saline (1 ml h^{-1}, $n=5$), 5-CT (0.01 μg kg^{-1} min^{-1}, $n=5$), 8-OH-DPAT (20 μg kg^{-1} min^{-1}, $n=5$), sumatriptan (10 μg kg^{-1} min^{-1}, $n=5$) or L-694,247 (1 μg kg^{-1} min^{-1}, $n=5$), respectively. Accordingly, four dose-response curves for NA were determined per animal. The infusions were started 5 min before the first response (E'1) was elicited and these were continued over 1 h.

Also, changes in heart rate, as well as increases in blood pressure were induced by electrical stimulation. However, in our experiments these changes were not significant. This observation coincides with previous results found by us (Morán *et al*., 1994) and others (Gillespie & Muir, 1967; Zwkowska-Grojec *et al*., 1983; Borkowski & Quinn, 1983; Roquebert *et al*., 1990).

Drugs employed

Apart from the anaesthetic (pentobarbitone sodium, Sigma Chemical Company, St Louis, MO, U.S.A.), the drugs used in the present study (obtained from the resources indicated) were the following: heparin sodium (Roche, Madrid, Spain), noradrenaline bitartrate and (+)-tubocurarine hydrochloride (Sigma Chemical Company, St Louis, MO, U.S.A.); atropine sulphate (Scharlau, Barcelona, Spain); methiothepin mesylate, 1-(3-chlorophenyl)-piperazine dihydrochloride (*m*-CPP), 5-carboxamidotryptamine maleate (5-CT), 8-hydroxydipropylaminotretalin hydrobromide (8-OH-DPAT), CGS-12066B maleate (7-trifluoromethyl-4(4-methyl-1-piperazinyl)-pyrrolo[3,2-a]quinoxaline maleate) and L-694,247 (2-[5-[3-(4-methylsulphonylamino)benzyl-1,2,4-oxadiazol-5-yl]-1H-indole-3-yl]ethylamine) (Research Biochemicals International, Natick, MA, U.S.A.); GR127935T (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) and sumatriptan succinate (Glaxo Group Research, Ware, U.K.); [3-(1,2,5,6-tetrahydropyrid-4-yl)pyrrolo[3,2-b]pyrid-5-one] (CP-93,129) (Pfizer Inc., Groton, U.S.A.); N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridinyl)cyclohexanecarboxamide trihydrochloride (WAY-100,635, Pharmacia, Milan, Italy) and (−)-4-(3-t-butylamino-2-hydroxypropoxy)indol-2-carbonitrile hemifumarate, (−)-cyanopindolol hemifumarate (Biomol Research Laboratories Inc., PA, U.S.A.).

All drugs used were dissolved in distilled water at the time of the experiments with the exception of *m*-CPP and CP-93,129 which were dissolved in 0.01 M HCl.

Table 1 Inhibitory effect induced by 5-CT in stimulation-response (S-R) curve E2

	Dose (μg kg^{-1} min^{-1})		
	0.01	*0.1*	*1*
Frequency (Hz)			
0.1	83±0.3*	100±0.1*	100±0.1*
0.5	53±0.7*	66±0.1*	86±1*
1	55±2*	64±1*	76±2*
5	49±6*	45±3*	61±5*

Results are presented as % of inhibition with respect to the increases in mean blood pressure (MBP) obtained for S-R curve E0. *$P<0.05$.

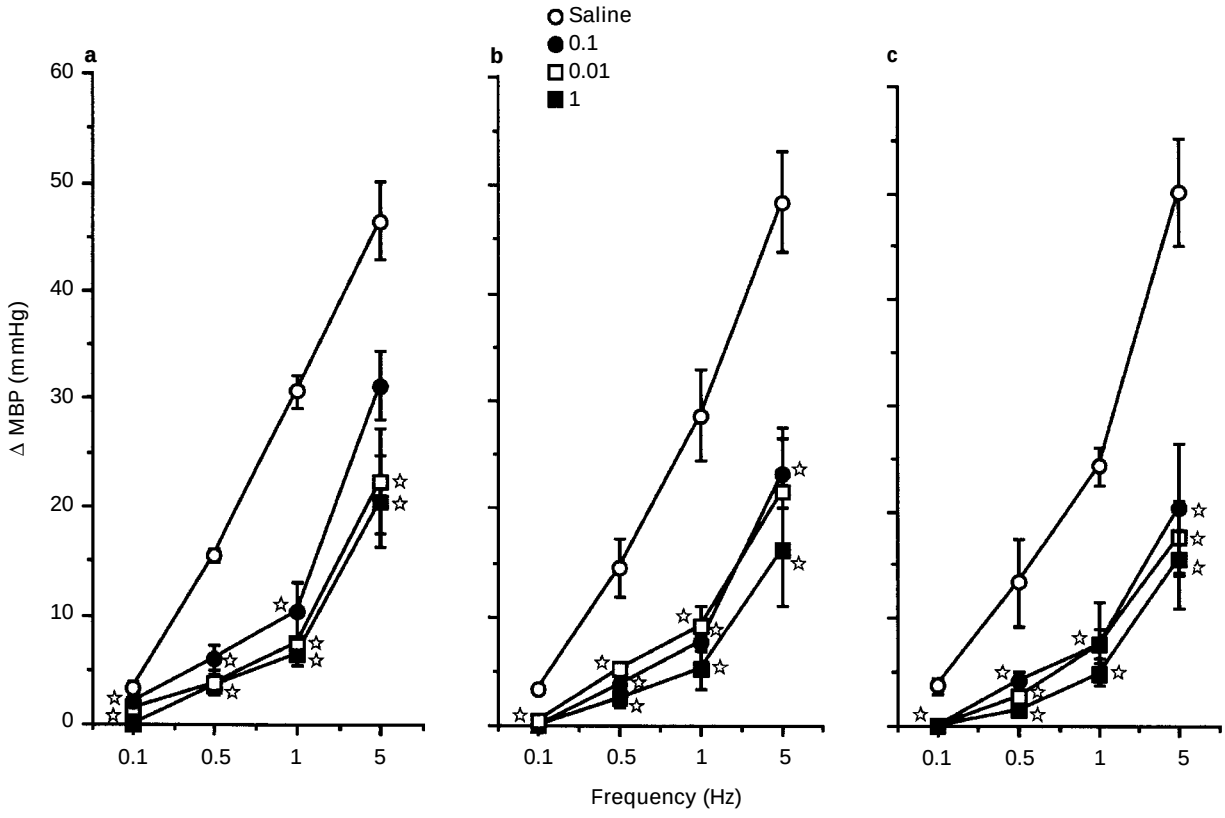

Figure 2 Effect of an i.v. infusion of saline (1 ml h^{-1}) or 5-CT (μg kg^{-1} min^{-1}) on electrically-induced pressor responses in pithed rats. (a) E1, (b) E2, (c) E3, first, second and third stimulation-response curves. *$P<0.05$ versus saline.

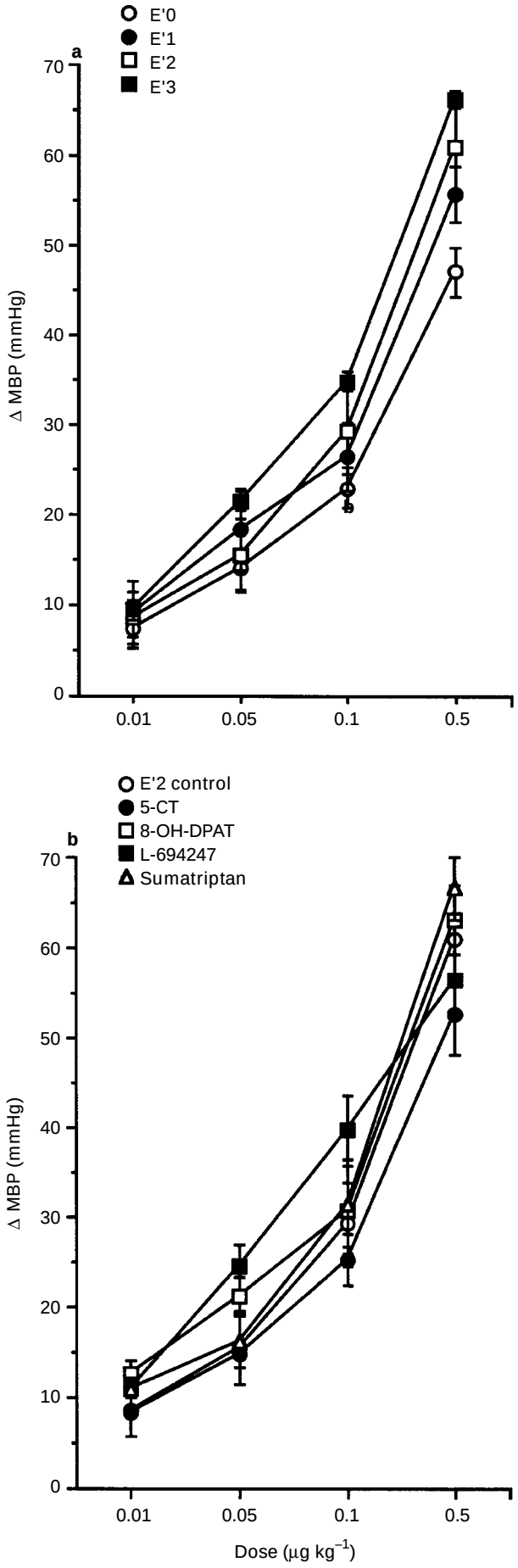

Figure 3 (a) Effect of saline (1 ml kg^{-1}, i.v.) on increases in mean blood pressure (Δ MBP) induced by exogenous i.v. administration of noradrenaline in pithed rats infused with saline (1 ml h^{-1}). E′0 control; E′1, first; E′2, second; E′3, third dose-response curve. (b) Effect of continuous infusion of 5-CT (0.01 μg kg^{-1} min^{-1}), 8-OH-DPAT (20 μg kg^{-1} min^{-1}), L-694,247 (1 μg kg^{-1} min^{-1}) and sumatriptan (10 μg kg^{-1} min^{-1}) on increases in mean blood pressure (Δ MBP) induced by exogenous i.v. administration of noradrenaline in E′2 dose-response curve.

Expression and analysis of results

Modifications in mean blood pressure were expressed as mmHg above the mean control blood pressure, measured both before electrical stimulation and as the stabilized maximum post-stimulation.

All data are expressed as mean values ± s.e.mean of at least five experiments. The comparison of the results from the various experimental groups and their corresponding controls was carried out by one way analysis of variance (ANOVA) followed by the Newman-Keuls multiple comparison test. The differences were considered significant when $P<0.05$. Since the data obtained for S-R curves E1, E2 and E3 were essentially the same, for simplicity only the S-R curve corresponding to E2 stimulation or E'2 NA-administration is shown.

Results

Systemic haemodynamic variables

The mean resting blood pressure and heart rate in these studies were 48 ± 1 mmHg and 295 ± 3 beats min^{-1} ($n=225$), respectively. These values were not significantly altered by the intravenous infusion of saline, HCl (0.01 M), the 5-hydroxytryptamine receptor agonists (8-OH-DPAT, CGS-12066B, CP-93,129, L-694,247, *m*-CPP and sumatriptan) or the 5-HT receptor antagonists (methiothepin, WAY-100,635, cyanopindolol and GR127935T). Only infusion of the 5-hydroxytryptamine receptor agonist 5-CT resulted in dose-dependent decreases in MBP. The highest two doses caused significant falls in blood pressure, reaching a maximum by 5 min and this was maintained over the duration of the infusion. The maximum decreases in basal mean blood pressure (mmHg) after infusion of 0.01, 0.1 and 1 μg kg^{-1} min^{-1} were 4, 12 and 18 mmHg, respectively. In contrast, heart rate remained unchanged before and during all infusions of agonists and antagonists.

Effects of physiological saline or 5-CT on the electrically- or noradrenaline-induced increases in mean blood pressure

Electrical stimulation of the preganglionic sympathetic outflow from the spinal cord in pithed rats resulted in frequency-dependent increases in MBP (Figure 1). At the frequencies used, the increases in MBP in S-R curve E0 were 3 ± 0.1, 11 ± 0.4; 21 ± 1 and 42 ± 1 mmHg. These rises in MBP remained stable in S-R curves E1, E2 and E3 in control animals receiving a saline infusion of 1 ml h^{-1}, the vehicle used for the agonist or antagonist agents (saline or HCl) (Figure 1) and the 5-HT receptor antagonists WAY-100,635 (100, 500 and 1000 μg kg^{-1}), cyanopindolol (100, 500 and 1000 μg kg^{-1}) and GR127935T (100, 500 and 1000 μg kg^{-1}) (Figure 6a,b and c, respectively).

Continuous infusion of 5-CT (0.01, 0.1 and 1 μg kg^{-1} min^{-1}) inhibited the sympathetic-induced pressor responses (Figure 2). The inhibition was more pronounced at lower stimulation frequencies (Table 1).

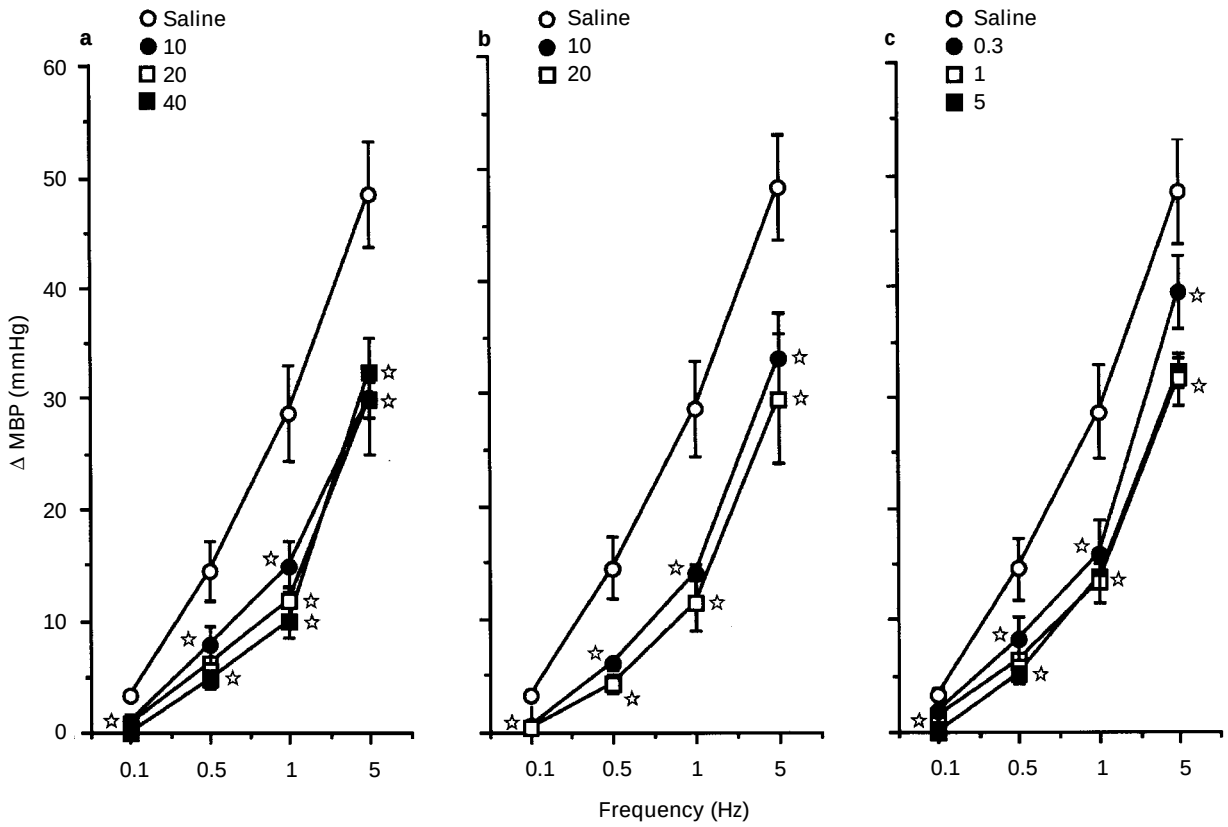

Figure 4 Effect of i.v. infusion (μg kg^{-1} min^{-1}) of (a) 8-OH-DPAT, (b) sumatriptan and (c) L-694,247 on electrically-induced pressor responses in pithed rats (S-R E2). Saline (1 ml kg^{-1} min^{-1}). *$P<0.05$ versus saline.

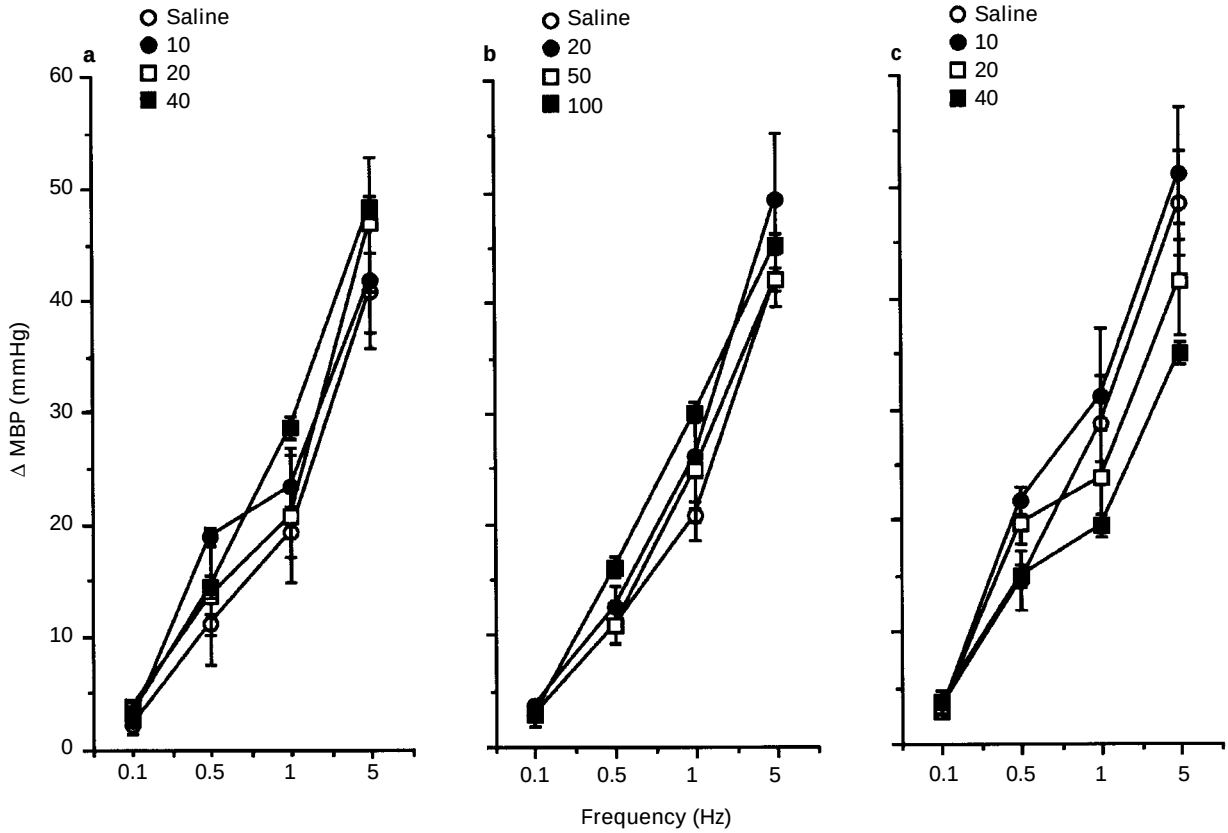

Figure 5 Effect of i.v. infusion (μg kg^{-1} min^{-1}) of (a) CGS-12066B, (b) CP-93,129 and (c) *m*-CPP on E2 electrically-induced pressor responses in pithed rats. Saline (1 ml kg^{-1} min^{-1}).

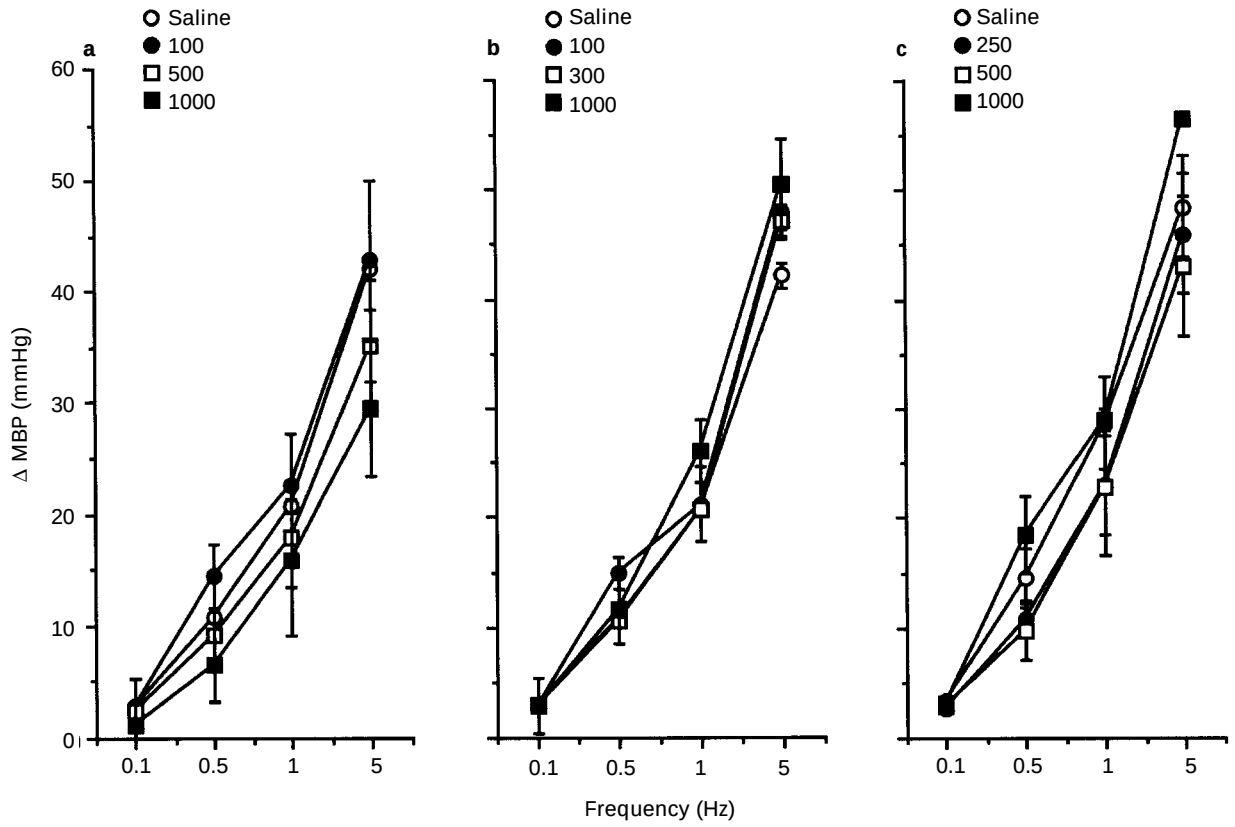

Figure 6 Effect of i.v. administration (in μg kg^{-1}) of (a) WAY-100,635 (100, 500 and 1000), (b) cyanopindolol (100, 300 and 1000) and (c) GR127935T (250, 500 and 1000) on E2 electrically-induced pressor responses in pithed rats. Saline (1 ml kg^{-1}).

The increases in mean arterial blood pressure caused by i.v. noradrenaline (0.01 to 0.5 μg kg^{-1}) were similar before and after each stimulus response curve (Figure 3a) and during infusions of 5-CT (0.01 μg kg^{-1} min^{-1}) or saline (Figure 3b).

Effect of intravenous infusions of 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1B/1D}$ and 5-HT$_2$ receptor agonists on electrically- or noradrenaline-induced increases in mean blood pressure

Intravenous infusions of a 5-HT$_{1A}$ receptor agonist, 8-OH-DPAT, 10, 20 and 40 μg kg^{-1} min^{-1} (Figure 4a), and two 5-HT$_{1B/1D}$ receptor agonists, sumatriptan 10 and 20 μg kg^{-1} min^{-1} (Figure 4b), and L-694,247, 0.3, 1 and 5 μg kg^{-1} min^{-1} (Figure 4c), respectively, inhibited the sympathetically-induced pressor response in the three S-R curves E1, E2 and E3.

Unlike the above agonists, infusions of two 5-HT$_{1B}$ receptor agonists, CGS-12066B, 10, 20 and 40 μg kg^{-1} min^{-1} (Figure 5a), and CP-93,129, 20, 50 and 100 μg kg^{-1} min^{-1} (Figure 5b), or the 5-HT$_{2C}$ receptor agonist, *m*-CPP, 10, 20 and 40 μg kg^{-1} min^{-1} (Figure 5c), had no effect on the pressor responses evoked by sympathetic stimulation.

8-OH-DPAT, sumatriptan and L-694,247 failed to inhibit the pressor responses obtained to exogenous administration of noradrenaline (0.01, 0.05, 0.1 and 0.5 μg kg^{-1}) (Figure 3b).

Effects of 5-HT$_1$, 5-HT$_{1A}$, 5-HT$_{1B}$ and 5-HT$_{1B/1D}$ receptor antagonists on 5-hydroxytryptamine receptor agonist-induced inhibition of electrically-induced pressor responses

Pretreatment with a non-selective 5-HT$_1$ receptor antagonist, methiothepin 10 μg kg^{-1}, produced an attenuation *per se* of the pressor response in S-R curve E0 (Figure 7a) and partially antagonized the inhibitory effect of 5-CT (0.01 μg kg^{-1} min^{-1}) (Figure 7a).

The inhibitory effects of 5-CT (0.01 μg kg^{-1} min^{-1}), sumatriptan (10 μg kg^{-1} min^{-1}) and L-694,247 (1 μg kg^{-1} min^{-1}) were significantly attenuated by 250 μg kg^{-1} GR127935T (Figure 7b, 8b and 8c, respectively), a selective 5-HT$_{1B/1D}$ receptor antagonist which by itself did not modify the pressor responses obtained to electrical stimulation (Figure 6c). GR127935T partially inhibited the effect of 5-CT and the inhibition was only statistically significant at the highest stimulation frequencies (1 and 5 Hz). GR127935T likewise only partially inhibited the effects of sumatriptan and this partial inhibition was similar over the range of stimulus frequencies. However, GR127935T completely blocked the effects of L-694,247.

Pretreatment with WAY-100,635 (100 μg kg^{-1}) did not modify the pressor response (Figure 6a) but completely abolished the inhibitory effect of 8-OH-DPAT and partially blocked the effect on the 5-CT-induced inhibition (Figure 8a and 7b). Pretreatment with cyanopindolol (100 μg kg^{-1}), although failing to inhibit the pressor response (Figure 6b), did not inhibit the inhibitory effect of 5-CT (Figure 7b).

Discussion

The results presented here are consistent with those from previous studies (Morán *et al*., 1994; Villalón *et al*., 1995a,b, Shepheard *et al*., 1996) and confirm that the prejunctional 5-HT receptors mediating the inhibition of pressor effects obtained by stimulation of sympathetic outflow in pithed rats are mainly 5-HT$_1$ in nature.

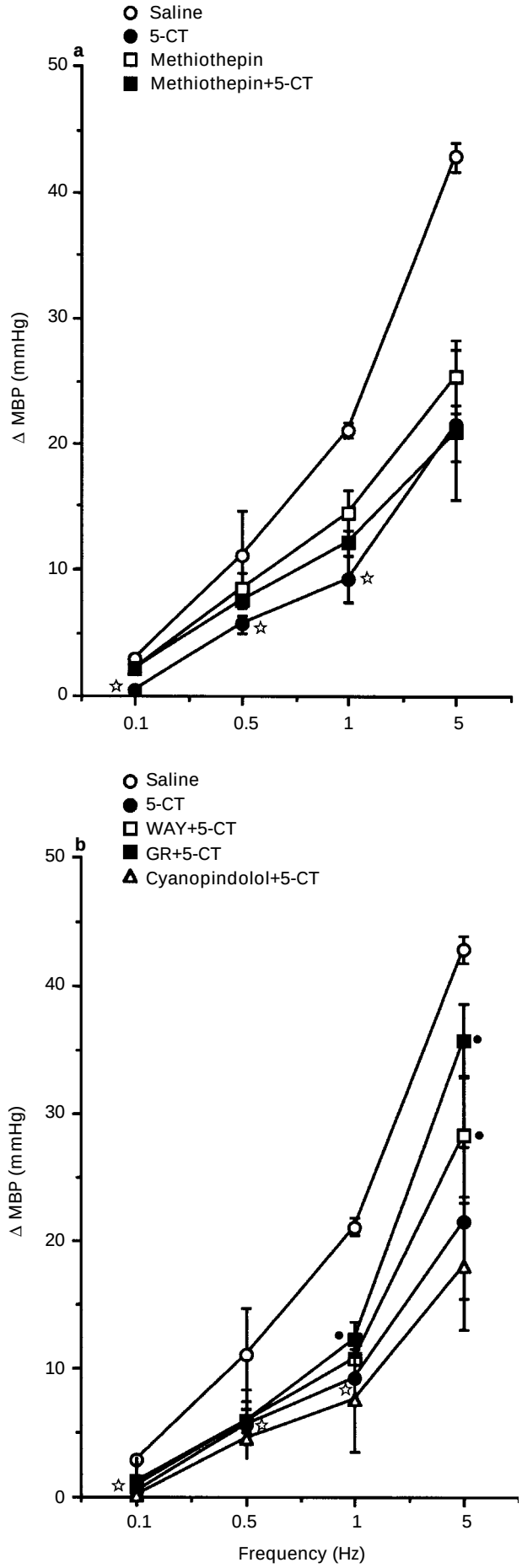

Figure 7 Effect of i.v. administration of (a) methiothepin (10 μg kg^{-1}) and (b) WAY-100,635 (100 μg kg^{-1}), GR127935T (250 μg kg^{-1}) or cyanopindolol (100 μg kg^{-1}) on the inhibitory effect of 5-CT (0.01 μg kg^{-1} min^{-1}). *$P<0.05$ versus saline and •$P<0.05$ versus 5-CT.

In the present work, the inhibitory effect of 5-hydroxytryptamine previously demonstrated by us (Morán *et al*., 1994) was mimicked by 5-CT, a potent 5-HT$_1$ receptor agonist (see Hoyer *et al*., 1994), sumatriptan, a selective 5-HT$_{1B/D}$ and also 5-HT$_1$-like agonist (Schoeffter & Hoyer, 1989; Hoyer *et al*., 1994), and two selective 5-HT$_{1A}$ and 5-HT$_{1B/1D}$ receptor agonists: 8-OH-DPAT (Middlemis & Fozard, 1983) and L-694,247 (Beer *et al*., 1993), respectively. These initial results are in partial agreement with those obtained by Villalón *et al*., (1995b), who proposed that the mechanisms involved in this inhibitory 5-HT effect are 5-HT$_1$-like, with a pharmacological profile similar to other prejunctional 5-HT$_1$-like receptors mediating vascular responses at different levels.

The higher degree of inhibition obtained with 5-CT in this work with respect to that observed with 5-HT (Morán *et al*., 1994), together with the absence of activity with *m*-CPP, a selective 5-HT$_{2C}$ receptor agonist (see Humphrey *et al*., 1993) that also shows affinity for 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors (Shoeffter & Hoyer, 1989) confirm that the main 5-HT receptor subtypes involved in his inhibitory activity are 5-HT$_1$. Another 5-HT$_2$ receptor agonist, DOI, that shows affinity for 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors (see Hoyer *et al*., 1994) was considered for use in our experiments. However, the activation of 5-HT$_{2A}$ receptors by this agonist increases basal blood pressure and obstructs the increases induced by electrical stimulation. Accordingly, in order to establish the 5-HT$_1$ subtype receptor responsible for this action, different antagonists and selective 5-HT$_1$ agonists were used in our experiments.

A non-selective 5-HT$_1$ antagonist, methiothepin (see Hoyer *et al*., 1994), only partially blocked the inhibitory effect of 5-CT. This partial inhibition could be explained by the possible participation of various 5-HT$_1$ receptor subtypes in this inhibitory effect and by the low dose of methiothepin used in our experiments. Such a low dose is justified because this antagonist blocks the electrically-induced pressor response *per se*, in concurrence with previous findings (Villalón *et al*., 1995b) and this may be accounted for by the affinity of this antagonist for α_1-adrenoceptors (see Leysen, 1985).

8-OH-DPAT, a 5-HT$_{1A}$ selective agonist which displays a similar agonist potency to that of 5-CT for this receptor subtype (pEC$_{50}$ values of 8.2 and 8.6, respectively; see Hoyer *et al*., 1994), inhibited the pressor effect obtained by electrical stimulation, apparently with a lower potency than that of 5-CT, since a higher degree of inhibition was obtained at lower doses of this agonist with respect to 8-OH-DPAT. Possible activation of α_2-presynaptic receptors by this agonist would explain this inhibition. However, the total reversibility of the 8-OH-DPAT-induced inhibitory effect following the administration of WAY-100,635 (a selective 5-HT$_{1A}$ antagonist Fletcher *et al*., 1994) with no reported α-adrenoceptor antagonist activity of its own and which, at the dose used by us, does not inhibit the pressor response, together with the partial blockade obtained with this antagonist on the inhibitory effect of 5-CT confirm the participation of the 5-HT$_{1A}$ receptor subtype in this inhibitory activity and do not exclude the possible participation of other receptor subtypes in this action. These initial results suggest that 5-HT$_{1A}$ receptor subtypes are only partially responsible for the inhibitory activity of 5-CT and that other 5-HT$_1$ receptor subtypes are also involved.

The absence of activity observed with two selective 5-HT$_{1B}$ receptor agonists, CGS-12066B (Neale *et al*., 1987) and CP-

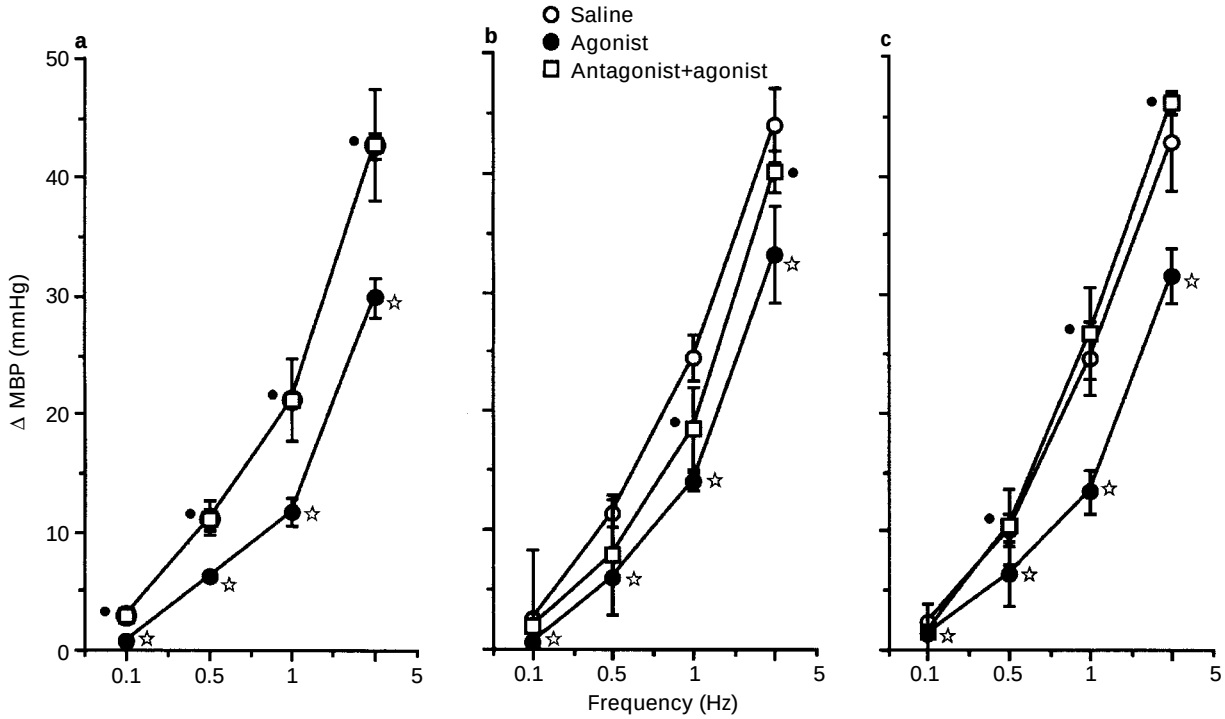

Figure 8 Effect of i.v. administration of: (a) WAY-100,635 (100 μg kg^{-1}) on the inhibition induced by 8-OH-DPAT (20 μg kg^{-1} min^{-1}), (b) GR127935T (250 μg kg^{-1}) on the inhibitory effect of sumatriptan (10 μg kg^{-1} min^{-1}) and (c) GR127935T (250 μg kg^{-1}) on the inhibitory effect of L-694247 (1 μg kg^{-1} min^{-1}). *$P<0.05$ versus saline and •$P<0.05$ versus the corresponding agonist.

93,129 (Macor *et al*., 1990), suggests that this receptor subtype does not participate in 5-hydroxytryptamine-induced inhibitory sympathetic activity. These results contrast with the recognized role assigned to this receptor subtype from rodent species as a presynaptic modulator receptor, not only at autoreceptors on 5-hydroxytryptaminergic neurones (Middlemiss, 1984; 1985; 1986) but also at terminal heteroreceptors to control the release of other neurotransmitters, such as acetylcholine and glutamate (Engel *et al*., 1986; Middlemiss, 1986; Limberger *et al*., 1991). Moreover, the inhibition of noradrenaline release appears to be mediated by 5-HT$_{1B}$ receptors in the rat cava (Göthert *et al*., 1986).

Sumatriptan is also a potent 5-HT receptor agonist that inhibits pressor responses. The degree of inhibition is only slightly lower than that shown by the agonist 5-CT and is also partially blocked by methiothepin. It has previously been found that this agonist inhibits sympathetic vasopressor outflow in the pithed rat (Villalón *et al*., 1995c; Shepheard *et al*., 1996). Initially, Villalón *et al*., (1995c) postulated a 5-HT$_1$-like mechanism to explain the inhibitory 5-hydroxytryptaminergic activity. However, Shepheard *et al*., (1996) proposed a 5-HT$_{1D}$ ganglionic mechanism to account for sumatriptan-induced inhibition.

In order to confirm the nature of 5-HT receptors participating in the inhibitory activity and to establish the possible participation of 5-HT$_{1D}$ receptors in this inhibition *in vivo*, previously observed in several preparations *in vitro* from non-rodent species (Molderings *et al*., 1987; 1990; Charlton *et al*., 1986; Humphrey *et al*., 1988; Göthert *et al*., 1990) and even *in vivo* from pithed rat (Shepheard *et al*., 1996), we used the selective 5-HT$_{1B/1D}$ receptor agonist, L-694,247. Administration of this potent and selective 5-HT$_{1B/1D}$ agonist (pEC$_{50}$ value of 9.4; see Hoyer *et al*., 1994) mimicked the inhibitory effect obtained with 5-CT and sumatriptan. The inhibitory potency was higher than that obtained with the 5-HT$_{1A}$ agonist, 8-OH-DPAT. These combined results show that mainly prejunctional 5-HT$_{1D}$ receptors are involved in the inhibition of pressor stimulation-induced responses in the pithed rat. The participation of the 5-HT$_{1D}$ receptor subtype was confirmed with GR127935T, a selective 5-HT$_{1B/1D}$ receptor antagonist (Skingle *et al*., 1993; 1996) which completely blocked the inhibitory response of L-694,247 and partially blocked that induced by 5-CT or sumatriptan. The different profile of inhibition found between the antagonist GR127935T and these agonists can be explained in terms of the different affinities and intrinsic activities of 5-CT and sumatriptan at 5-HT$_{1A}$, 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors (respective pEC$_{50}$ values: 8.7, 7.9 and 8.1 for 5-CT and 5.6, 6.0 and 7 for sumatriptan) (see Hoyer *et al*., 1994) and the greater selectivity of L-694,247 for 5-HT$_{1D}$ receptors (corresponding pEC$_{50}$ value: 9.4).

All the agonists used in our experiments are more potent at lower stimulation frequencies. These results coincide with those previously obtained by Villalón *et al*., (1995a,b), following the pattern of other prejunctional modulators of noradrenaline release from sympathetic nerves. As previously found by us and other authors, the agonists used by us failed to inhibit the pressor response evoked by i.v. noradrenaline administration. We thus confirm the mainly prejunctional nature of this inhibition, although our results do not exclude, as has been demonstrated in anaesthetized cats (Jones *et al*., 1995) a ganglionic location for these receptors. In conclusion, we suggest that the 5-HT-induced inhibition of sympathetic pressor responses in the pithed rat is mainly mediated by prejunctional 5-HT$_{1D}$ but also modulated by 5-HT$_{1A}$ receptors.

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