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Role of 5-ht$_7$ receptors in the long-lasting hypotensive response induced by 5-hydroxytryptamine in the rat

José A. Terrón

Sección de Terapéutica Experimental, Departamento de Farmacología y Toxicología, CINVESTAV, I.P.N., Apdo. Postal 22026, 14000 México D.F., México

1 The receptor mediating the long-lasting hypotensive effect of intravenous (i.v.) 5-hydroxytryptamine (5-HT) in the rat was originally classified as 5-HT$_1$-like. Since some pharmacological properties of this receptor are closely similar to those for the cloned 5-ht$_7$ receptor, the present study investigated the effects of several 5-HT receptor agonists and antagonists showing high affinity for the cloned 5-ht$_7$ receptor in pithed rats with artificially raised blood pressure.

2 I.v. bolus administration of 5-HT, 5-carboxamidotryptamine (5-CT), 5-methoxytryptamine, lisuride and sumatriptan to bilaterally vagotomized pithed rats pretreated with ketanserin (0.18 μmol kg^{-1}, i.v.), the diastolic blood pressure of which had been raised by a continuous i.v. infusion of methoxamine (60–80 nmol kg^{-1} min^{-1}), produced dose-dependent hypotensive responses; only 5-HT and 5-CT displayed similar maximum effects. In addition to mimicking the hypotensive action of 5-HT with a lower maximum effect, lisuride strongly antagonized the 5-CT-induced hypotensive responses thus suggesting a partial agonist effect. The rank order of hypotensive agonist potency was 5-CT >> 5-HT $\geqslant$ 5-methoxytryptamine $\geqslant$ lisuride >> sumatriptan.

3 In experiments with antagonists, i.v. treatment with metergoline (2.48 μmol kg^{-1}), mesulergine (2.76 μmol kg^{-1}), methysergide (2.13 μmol kg^{-1}), lisuride (0.22 μmol kg^{-1}), methiothepin (0.68 μmol kg^{-1}), mianserin (10.6 μmol kg^{-1}), or the atypical antipsychotic drugs, clozapine (11.0 μmol kg^{-1}) or risperidone (78.0 nmol kg^{-1}), produced significant rightward displacements of the dose-response curve for 5-CT in methoxamine-infused pithed animals pretreated with ketanserin (0.18 μmol kg^{-1}, i.v.); lisuride, methiothepin and risperidone behaved as non-competitive antagonists as they elicited a significant reduction of the maximum effect to 5-CT. In contrast, blockade of 5-HT$_1$, 5-HT$_3$ and 5-HT$_4$ receptors with i.v. propranolol (3.38 μmol kg^{-1}), MDL-72222 (1.59 μmol kg^{-1}) and GR125487 (1.91 μmol kg^{-1}), respectively, did not alter 5-CT-induced hypotensive responses; ketanserin (0.18 μmol kg^{-1}, i.v.) failed to modify the dose-response curve for 5-CT in saline-pretreated animals. Lastly, inhibition of the prostaglandin-forming cyclo-oxygenase and nitric oxide synthase with indomethacin (14 μmol kg^{-1}, i.v.) and N^G-nitro-L-arginine methyl ester (L-NAME, 120 μmol kg^{-1}, i.v.), respectively, had no significant effects on 5-CT-induced hypotensive effects.

4 Taken together, the present pharmacological data suggest that the long-lasting vasodepressor action of 5-HT in the rat involves activation of receptors closely similar to the cloned 5-ht$_7$ subtype. Since no evidence for an indirect mechanism could be obtained, these receptors may be primarily located in the vascular smooth muscle of the systemic resistance vessels. These findings represent further evidence favouring the functional role of the 5-ht$_7$ receptor.

Keywords: 5-Hydroxytryptamine; hypotension; vascular 5-ht$_7$ receptors

Introduction

The intravenous (i.v.) bolus administration of 5-hydroxytryptamine (5-HT) to either conscious or anaesthetized rats produces a complex triphasic response (Page & McCubbin, 1953; Page, 1957). This response consists of a short-lasting depressor phase (via the von Bezold-Jarisch reflex) that is mediated by 5-HT$_3$ receptors (Fozard, 1984; Kalkman *et al*., 1984; Saxena & Lawang, 1985), a pressor phase mediated by vascular 5-HT$_2$ receptors (Kalkman *et al*., 1984; Saxena & Lawang, 1985), and a long-lasting hypotensive phase which, based upon the correlation of the hypotensive potency and the affinity of some tryptamine derivatives for 5-HT$_1$ binding sites in brain tissues, was characterized as being mediated by vascular 5-HT$_1$-like receptors (Kalkman *et al*., 1983).

As noticed with other 5-HT-induced effects involving vasodilatation (see Hoyer *et al*., 1994; Martin, 1994; Saxena, 1995, for review), the hypotensive effects of i.v. 5-HT in anaesthetized rats are mimicked with higher potency by 5-carboxamidotryptamine (5-CT) and antagonized by methysergide which is suggestive of mediation by 5-HT$_1$-like receptors (Saxena & Lawang, 1985; Martin *et al*., 1987). Interestingly, the 5-HT$_{1D/1F}$ receptor agonist, sumatriptan (Peroutka & McCarthy, 1989; Adham *et al*., 1993), which was originally developed as a selective 5-HT$_1$-like receptor agonist (Humphrey *et al*., 1988), does not produce relaxant and/or vasodilator responses (see Martin, 1994), thereby raising the possibility that the 5-HT receptor involved is not a 5-HT$_1$-like subtype. The pharmacological characteristics of the 5-HT receptor mediating hypotension in the systemic vasculature indeed show close similarities to those found for the cloned 5-ht$_7$ receptor i.e. high affinity for 5-CT, intermediate affinity for methysergide and very low affinity for sumatriptan (Bard *et al*., 1993; Lovenberg *et al*., 1993; Plassat *et al*., 1993; Ruat *et al*., 1993; Shen *et al*., 1993; To *et al*., 1995). On this basis, the present study was designed to investigate further the pharmacological properties of the receptor mediating the long-lasting hypotensive effect of 5-HT in order to determine its possible identity with the cloned 5-ht$_7$ receptor subtype. For this purpose, the effects of several 5-HT receptor agonists and antagonists displaying high affinity for the cloned 5-ht$_7$ receptor, including the atypical antipsychotic drugs, clozapine and risperidone (Roth *et al*., 1994), in methoxamine-infused pithed rats were analysed. The affinities of these drugs for the currently identified 5-HT$_1$ receptor subtypes and the 5-ht$_6$ and 5-ht$_7$ receptors are depicted in Table 1. Since 5-HT and other 5-HT receptor agonists produce vasopressor responses in this preparation through stimulation of vascular 5-HT$_2$ receptors

Table 1 Dissociation constants of 5-HT receptor agonists and antagonists determined by radioligand binding studies

	[$-log$ K$_i$]						
	5-HT$_{1A}$	*5-HT$_{1B}$*	*5-HT$_{1D}$*	*5-ht$_{1E}$*	*5-ht$_{1F}$*	*5-ht$_6$*	*5-ht$_7$*
Agonists							
5-HT	8.38	8.61	8.46	7.96	8.0	7.25	8.74
5-CT	9.53	8.29	8.62	5.1	6.14	6.6	9.48
5-MT	8.04	6.4	8.4	5.5	5.93	7.74	8.75
Lisuride	9.12	6.65	7.51	ND	ND	8.28	9.05
Sumatriptan	6.62	6.81	7.17	5.6	7.64	ND	6.3
Antagonists							
Propranolol	6.8	7.3	5.5	ND	< 5	ND	ND
Ketanserin	5.86	5.72	6.0	< 5	< 5	< 5	< 5.1
Metergoline	8.1	7.39	9.09	6.11	6.47	7.52	7.2
Mesulergine	6.23	4.88	5.2	ND	< 5	5.76	7.68
Methysergide	7.63	5.82	8.42	6.64	7.47	6.43	7.87
Methiothepin	7.1	7.28	6.25	6.71	6.19	8.74	9.42
Mianserin	6.03	5.21	6.37	ND	7.0	7.34	7.17
Clozapine	6.85	6.24	6.41	6.37	6.89	7.7	7.21
Risperidone	6.38	6.85	8.01	5.88	5.91	6.37	8.86

The data for 5-HT$_{1A}$, 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors are from Hoyer (1988), Van Wijngaarden *et al.* (1990) and Schotte *et al.* (1996) for pig, rat and calf brain membranes or human recombinant receptors (h5-HT$_{1D}$ [previously termed 5-HT$_{1D\alpha}$] and h5-HT$_{1B}$ [previously termed 5-HT$_{1D\beta}$] receptors which correspond to 5-HT$_{1D}$ and 5-HT$_{1B}$ receptors, respectively; see Hartig *et al.*, 1996). The data for 5-ht$_{1E}$ and 5-ht$_{1F}$ receptors are from Amlaiky *et al.* (1992), McAllister *et al.* (1992), Zgombick *et al.* (1992) and Adham *et al.* (1993) for human receptors. The data for 5-ht$_6$ receptors are from Monsma *et al.* (1993) and Roth *et al.* (1994) for rat receptors. The data for 5-ht$_7$ receptors are from Ruat *et al.* (1993), Shen *et al.* (1993) and Roth *et al.* (1994) for rat receptors. 5-HT, 5-hydroxytryptamine; 5-CT, 5-carboxamidotryptamine; and 5-MT, 5-methoxytryptamine. ND, not determined.

(Kalkman *et al.*, 1983; Saxena & Lawang, 1985; Martin *et al.*, 1987), the experiments were conducted in the presence of ketanserin (0.18 μmol kg^{-1}, i.v.).

Methods

General

A total of 84 male Wistar normotensive rats (350–450 g) were used for the experiments. The animals were collectively housed and maintained at a 12/12-h light-dark cycle, with light beginning at 07 h 00 min. The rats were kept in a special room at constant temperature (22$\pm$2°C) and humidity (50%), with food and water provided *ad libitum* in their home cages. After anaesthesia with ether and cannulation of the trachea, the rats were pithed as previously described (Shipley & Tilden, 1947). Immediately afterwards, the animals were artificially respired with room air, with an Ideal Palmer pump (56 cycles min^{-1}; volume: 20 ml kg^{-1}), and bilaterally vagotomized; catheters were placed in: the left femoral vein, for the continuous infusion of methoxamine; the right femoral vein, for the administration of 5-HT receptor agonists and antagonists; and the left carotid artery, connected to a TXD-300 pressure transducer (Digi-Med, KY, U.S.A.), for the recording of diastolic blood pressure and heart rate. Both diastolic blood pressure and heart rate were recorded simultaneously by a BPA-190 blood pressure analyser (Digi-Med., KY, U.S.A.) and the body temperature of the animals was kept constant.

Experimental protocols

After the animals had been in a stable haemodynamic condition for at least 15 min, baseline values of diastolic blood pressure and heart rate were determined. After collection of these data, the animals received the 5-HT$_2$ receptor antagonist, ketanserin (0.18 μmol kg^{-1}, i.v.), and 10 min later, they were given a continuous i.v. infusion of the α_1-adrenoceptor agonist, methoxamine (60–80 nmol kg^{-1} min^{-1}, provided in a volume of 0.6–0.8 ml h^{-1}), in order to raise diastolic blood pressure to an approximate value of 110 mmHg. Once the raised blood pressure had become stable (i.e. after 10–15 min), dose-response curves with respect to the decrease in diastolic blood pressure were obtained for 5-HT (0.57 nmol–5.7 μmol kg^{-1}), 5-CT (15.6 pmol–491.8 nmol kg^{-1}), 5-methyoxytryptamine (1.7 nmol–5.26 μmol kg^{-1}), lisuride (2.95 nmol–2.95 μmol kg^{-1}) and sumatriptan (33.9 nmol–3.39 μmol kg^{-1}) by i.v. bolus administration of single doses; each dose was given in a volume of 1 ml kg^{-1}. With the lower doses eliciting less than a 30 mmHg decrease in diastolic blood pressure, full recovery was permitted before the next dose was injected. The depressor effects of the higher doses, producing a decrease in diastolic blood pressure of more than 30 mmHg, were evaluated by the method of stepwise cumulative administration, increasing each dose by approximately 0.5 log units increments, with each successive injection immediately after the preceding dose had reached its maximum effect; this administration protocol was not strictly followed with lisuride as the drug produced short-lasting hypotensive effects. Only one agonist dose-response curve was obtained per animal.

In other groups of animals that had received ketanserin and methoxamine as described above, dose-response curves for 5-CT, the most potent of the agonists tested (see below), were obtained 30 min after the i.v. bolus administration of either vehicle (1 ml kg^{-1}) or one of the following 5-HT receptor antagonists: propranolol (5-HT$_1$; 3.38 μmol kg^{-1}), MDL-72222 (5-HT$_3$; 1.59 μmol kg^{-1}), GR125487 (5-HT$_4$; 1.91 μmol kg^{-1}), metergoline (5-HT$_{1D}$ and 5-ht$_7$; 2.48 μmol kg^{-1}), mesulergine (5-HT$_{2C}$ and 5-ht$_7$; 2.76 μmol kg^{-1}), methysergide (5-HT$_1$ and 5-ht$_7$; 2.13 μmol kg^{-1}), lisuride (5-ht$_6$ and 5-ht$_7$; 0.22 μmol kg^{-1}), methiothepin (5-HT$_1$, 5-ht$_6$ and 5-ht$_7$; 0.68 μmol kg^{-1}), mianserin (5-HT$_2$ and 5-ht$_7$; 10.6 μmol kg^{-1}), clozapine (5-ht$_6$ and 5-ht$_7$; 11.0 μmol kg^{-1}) or risperidone (5-ht$_7$; 78.0 nmol kg^{-1}). Since some of these drugs display moderate (metergoline, mesulergine and methysergide) or even high (clozapine, methiothepin and risperidone) affinity for α_1-adrenoceptors (Leysen, 1985; Cohen & Lipinski, 1986; Leysen *et al.*, 1988; Schotte *et al.*, 1996) leading to blockade of the methoxamine-induced pressor response, the dose of methoxamine employed in these cases was set between 200 and 400 nmol kg^{-1} min^{-1} so that the antagonism of α_1-adrenoceptors could be overcome and diastolic blood pressure raised to the levels obtained in the control experiments (110 mmHg, approximately, see above).

Finally, in an attempt to determine a possible linkage of the 'hypotensive' 5-HT receptor with other vasoactive mediators such as nitric oxide and/or vasodilator prostaglandins, two groups of animals pretreated with vehicle received either N^G-nitro-L-arginine methyl ester (L-NAME, 120 μmol kg^{-1}, i.v.) or indomethacin (14 μmol kg^{-1}, i.v.) followed by L-NAME

(120 μmol kg^{-1}, i.v.) in doses high enough to inhibit their respective mechanisms in the rat (Pucci *et al*., 1992; Wang *et al*., 1993). Since in preliminary experiments it was observed that the pressor effects induced by the above dose of L-NAME significantly decreased after 30–45 min, these animals were also given an infusion of methoxamine (8–20 nmol kg^{-1} · min^{-1}) to support blood pressure as close to 110 mmHg as possible. After blood pressure had remained stable during 10 min, dose-response curves for 5-CT were obtained as described above. The responses induced by 5-CT in indomethacin- and L-NAME-pretreated animals were compared with those produced by the agonist in a saline-pretreated group infused with methoxamine at a rate of 60–80 nmol kg^{-1} · min^{-1} (i.v.).

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 indomethacin (Sigma Chemical Company St. Louis, MO, USA); lisuride hydrogen maleate, 1αH,3α,5αH-tropan-3yl-3,5-dichlorobenzoate (MDL 72222), methoxamine hydrochloride, 5-methoxytryptamine hydrochloride, N^G-nitro-L-arginine methyl ester (L-NAME) and propranolol hydrochloride (Research Biochemicals Int., Natick, MA, U.S.A.); 5-carboxamidotryptamine maleate, [1-[2(methylsulphonyl)amino]ethyl]-4-piperidinyl]methyl-5-fluoro-2-methoxy-1H-indole-3-carboxylate hydrochloride (GR125487) and sumatriptan succinate (gift: Glaxo Group Research, Ware, U.K.); ketanserin tartrate and risperidone (gift: Janssen Pharmaceutica, Beerse, Belgium); methiothepin maleate (gift: Hoffman-La Roche Ltd., Basel, Switzerland); metergoline (gift: Farmitalia Carlo Erba, Milan, Italy); mianserin hydrochloride (gift: Organon de México, Mexico city); and clozapine, mesulergine and methysergide (gift: Sandoz A.G., Basel, Switzerland).

All compounds were dissolved in physiological saline. When needed, 4% ascorbic acid (clozapine and metergoline) or 5% (v/v) dimethylsulphoxide (DMSO) (lisuride, mesulergine, methiothepin, methysergide and risperidone) was added to stock solutions. Fresh solutions were prepared for each experiment and the vehicles had no effect either on the pressor effects induced by the infusion of methoxamine or the hypotensive responses to 5-CT. The doses mentioned in the text refer to the salts of substances except in the case of 5-HT, 5-CT. 5-methoxytryptamine, lisuride and sumatriptan where they refer to the free base; the salt of lisuride was used in the interaction experiments.

Data presentation and statistical analysis

All data in the text, figures and tables are presented as the mean ± s.e.mean, where n represents the number of rats employed in each set of experiments. The agonist-induced hypotensive effects are expressed as percentage of the pressor effect induced by the methoxamine infusion, 100% being represented by the maximum possible fall in blood pressure. Thus, the values of diastolic blood pressure recorded immediately before the injection of an agonist dose were taken as 0%; in the case of cumulative doses, 0% was represented by the value of diastolic blood pressure that was recorded immediately before the administration of the first dose of the sequence. Although all the agonist dose-response curves were always started from similar values of diastolic blood pressure (i.e. around 110 mmHg), the above procedure allowed for normalization of possible variations associated with small differences in the initial basal tone. The hypotensive activity calculated as $-\log ED_{50}$ (the negative logarithm of the dose of agonist producing 50% of the maximum hypotensive response) in mol kg^{-1}, the E_{max} (the maximum response) and the dose-ratios (ED_{50} of the agonist in the presence of the antagonist divided by the ED_{50} of the agonist in vehicle-treated animals), were determined. ED_{50} and E_{max} values were calculated by nonlinear regression analysis by use of the computer programme, pcNONLIN. The differences between the groups were evaluated by, as appropriate, t test or Student 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

The baseline values of diastolic blood pressure and heart rate in the 84 pithed rats used in the present study were, respectively, 33.2 ± 0.6 mmHg and 274.3 ± 2.8 beats min^{-1}. Although these values were modified in some degree by the different antagonist drugs used in the present study, it is to be noted that the use of the methoxamine infusion allowed, in all cases, the start of the dose-response curves to be from very similar values of diastolic blood pressure for the agonists; accordingly, these values in control (108.6 ± 1.5 mmHg; n = 24) and antagonist-treated (107.4 ± 1.2 mmttg; n = 42) animals that had received ketanserin (0.18 μmol kg^{-1}, i.v.) were not significantly different ($P > 0.05$).

Initial effects of 5-HT receptor agonists

The hypotensive activity of several 5-HT receptor agonists, showing from low to high affinity for the cloned 5-ht$_7$ receptor (Table 1), was assessed in methoxamine-infused pithed rats. Thus, in addition to 5-HT, 5-CT, 5-methoxytryptamine and lisuride produced clear hypotensive responses whereas sumatriptan behaved as a very weak agonist. The onset of the above responses was immediate and resulted in dose-dependent decreases in diastolic blood pressure (Figure 1). With the exception of the high doses (more than 500 nmol kg^{-1}) of 5-HT and 5-methoxytryptamine, which also produced notable increases in heart rate, the other agonists failed to alter the baseline values of heart rate obtained under the infusion of methoxamine (not shown). Thus, under the present experimental circumstances, the apparent rank order of agonist potency was 5-CT >> 5-HT ⩾ 5-methoxytryptamine ⩾ lisuride >> sumatriptan. Nonetheless, 5-methoxytryptamine and lisuride elicited similar maximum effects which were lower than those attained by 5-HT and 5-CT; the lower efficacy of lisuride, but not that of 5-methoxytryptamine, could be associated with partial agonism as only the ergoline potently antagonized the hypotensive responses induced by 5-CT (see below).

The rank order of agonist potency for causing hypotension was consistent with that of either a 5-HT$_1$-like or a 5-ht$_7$ receptor (Table 1). Intriguingly, in the presence of 0.18 μmol kg^{-1} (0.1 mg kg^{-1}, i.v.) of ketanserin, both 5-HT and 5-methoxytryptamine, but not 5-CT or lisuride, displayed a biphasic hypotensive pattern (Figure 1) that could not be fitted into the sigmoidal regression analysis. Under these conditions, increases in blood pressure were still observed with 5-HT (7.1 ± 2, 28.7 ± 3, 35.4 ± 5 and 40.1 ± 5 % of the maximum pressor response to methoxamine infusion after 0.18, 0.57, 1.8 and 5.7 μmol kg^{-1}, i.v., respectively; n = 5) and 5-methoxytryptamine (2.7 ± 1, 8.7 ± 3, 15.7 ± 4 and 19.4 ± 4 % of the maximum pressor response to methoxamine infusion after 0.17, 0.53, 1.7 and 5.3 μmol kg^{-1}, i.v., respectively; n = 6) before the hypotensive responses became apparent. Interestingly, increasing the dose of ketanserin up to 3.2 μmol kg^{-1} (1.8 mg kg^{-1}, i.v.) which completely blocks pressor 5-HT$_2$ receptors in the pithed rat (Göthert *et al*., 1986), strongly inhibited the above pressor effects to 5-HT (0, 0.9 ± 0.5, 6.7 ± 1 and 20.8 ± 3 % of the maximum pressor response to methoxamine infusion after 0.18, 0.57, 1.8 and 5.7 μmol kg^{-1}, i.v., respectively; n = 4) and 5-methoxytryptamine (0, 0, 0 and 3 ± 2 % of the maximum pressor response to methoxamine infusion after 0.17, 0.53, 1.7 and 5.3 μmol kg^{-1}, i.v., respectively; n = 3),

and led to monophasic dose-response curves for both agonists (not shown). Under these experimental circumstances, the hypotensive potency for 5-HT and 5-methoxytryptamine, expressed as mol kg^{-1} ($-$log ED$_{50}$), was 7.68$\pm$0.06 and 7.38$\pm$0.08, respectively. These values are closely similar to those previously obtained in anaesthetized rats (7.62 and 7.48 mol kg^{-1} for 5-HT and 5-methoxytryptamine, respectively; Kalkman *et al*., 1983) and they are, in contrast, more than 1.5 log units lower than those obtained in vasopressin-infused pithed rats (5.9 and 5.7 mol kg^{-1} for 5-HT and 5-methoxytryptamine, respectively; Kalkman *et al*., 1983). Since these data clearly indicate a decreased hypotensive potency for 5-HT in pithed rats infused with vasopressin, methoxamine was preferred to support blood pressure in the present experiments.

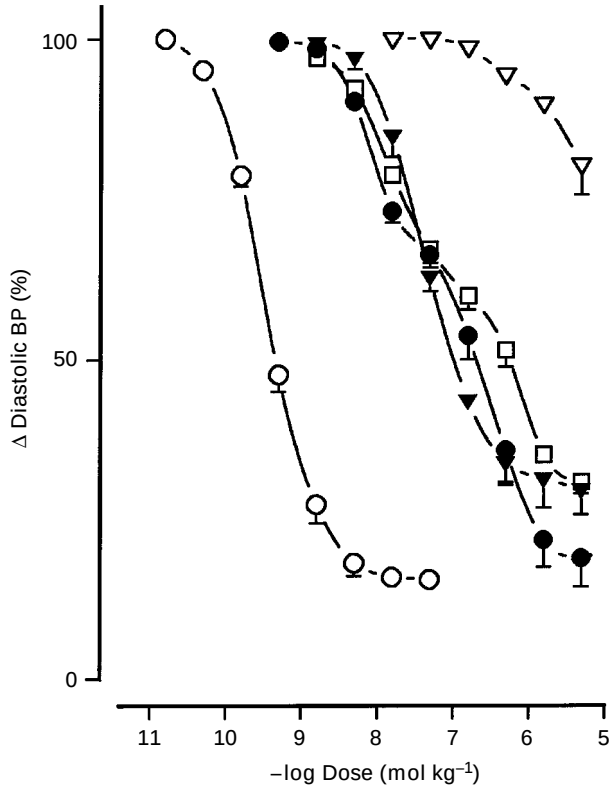

Figure 1 Comparative effects of i.v. bolus injections of 5-HT (●), 5-CT (○), 5-methoxytryptamine (□), lisuride (▼) and sumatriptan (▽) on diastolic blood pressure (BP) of methoxamine-infused pithed rats pretreated with ketanserin (0.18 μmol kg^{-1}, i.v.). The effects are expressed as the percentage (%) of the maximum decrease in diastolic blood pressure. Points represent the mean and vertical lines denote the s.e.mean of 3–6 experiments. With the exception of 5-HT and 5-methoxytryptamine, none of the agonists produced concomitant changes in heart rate (see Results section).

Table 2 The effects of i.v. bolus administration of propranolol (3.38 μmol kg^{-1}), MDL-72222 (1.59 μmol kg^{-1}) and GR125487 (1.91 μmol kg^{-1}) on the potency ($-$log ED$_{50}$) and efficacy (E$_{max}$) estimates for 5-carboxamidotryptamine in methoxamine-infused pithed rats pretreated with ketanserin (0.18 μmol kg^{-1}, i.v.)

Group	n	$-log\ ED_{50}$ (mol kg^{-1})	E_{max} (%)	*Dose-ratio*[a]
Control	6	9.50$\pm$0.05	85$\pm$1	–
Propranolol	3	9.59$\pm$0.02	78$\pm$6	0.8
MDL-72222	3	9.53$\pm$0.15	85$\pm$2	1.0
GR125487	4	9.66$\pm$0.14	86$\pm$1	0.8

Values are the mean$\pm$s.e.mean of *n* experiments. [a] Ratio of ED$_{50}$ with antagonist/ED$_{50}$ of the control group. None of these antagonists modified the hypotensive effects induced by 5-CT.

Effect of 5-HT$_1$, 5-HT$_2$, 5-HT$_3$ and 5-HT$_4$ receptor antagonists on 5-CT-induced hypotensive responses

The blockade of 5-HT$_1$, 5-HT$_3$ and 5-HT$_4$ receptors with propranolol, MDL-72222 and GR125487, respectively, did not significantly modify the hypotensive responses induced by 5-CT in animals pretreated with ketanserin i.e. the ED$_{50}$ values for 5-CT in vehicle- and antagonist-treated animals did not differ significantly (Table 2). Similarly, ketanserin, at the dose of 0.18 μmol kg^{-1} (i.v.) that was systematically employed in most of the experiments, did not produce any change of the dose-response curve for 5-CT (the $-$log ED$_{50}$ and E$_{max}$ values for 5-CT in the absence and the presence of ketanserin were, respectively, 9.51$\pm$0.06 and 9.50$\pm$0.05 mol kg^{-1}, and 85$\pm$0.7 and 84.9$\pm$1.2 % of the maximum hypotensive effect; n=6 each).

Effect of several 5-ht$_7$ receptor ligands on 5-CT-induced hypotensive responses

Figures 2 and 3 show the effect of several 5-HT receptor antagonists, having from moderate to high affinity for the cloned

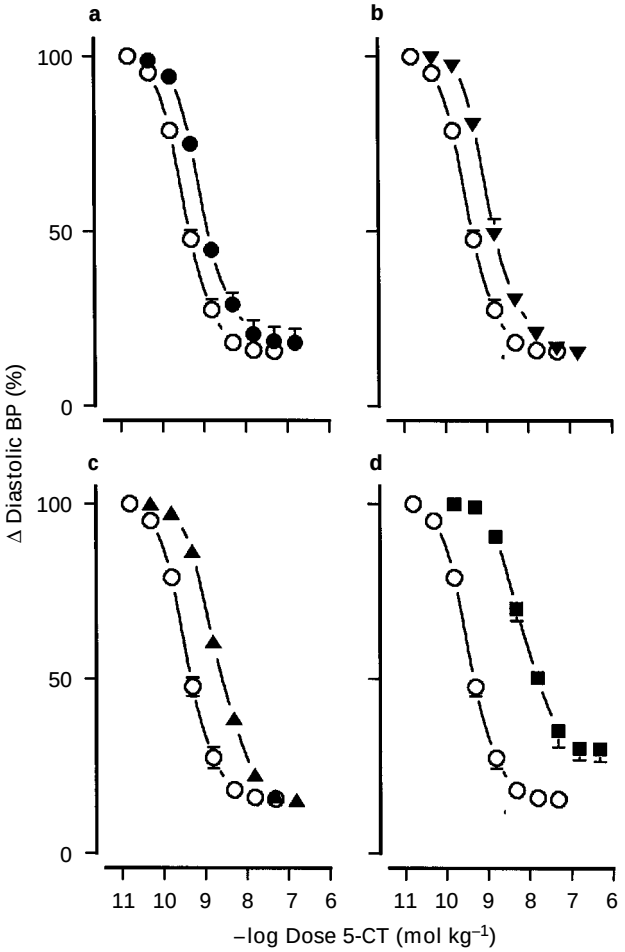

Figure 2 The effects of (a) metergoline (●, 2.48 μmol kg^{-1}); (b) mesulergine (▼, 2.76 μmol kg^{-1}); (c) methysergide (▲, 2.13 μmol kg^{-1}); and (d) lisuride (■, 0.22 μmol kg^{-1}) on the decreases in diastolic blood pressure (BP) induced by 5-CT compared to those produced by 5-CT alone (○) in methoxamine-infused pithed rats pretreated with ketanserin (0.18 μmol kg^{-1}, i.v.). The effects of 5-CT are expressed as a percentage (%) of the maximum decrease in diastolic blood pressure. Points represent the mean and vertical lines denote the s.e.mean of 4 experiments.

Table 3 The antagonist effects of i.v. bolus administration of metergoline, mesulergin, methysergide, lisuride, methiothepin, mianserin, clozapine and risperidone on the potency ($-\log ED_{50}$) and efficacy (E_{max}) estimates for 5-carboxamidotryptamine in methoxamine-infused pithed rats pretreated with ketanserin (0.18 μmol kg^{-1}, i.v.)

Antagonist	*Dose* (μmol kg^{-1})	n	$-log\ ED_{50}$ (mol kg^{-1})	E_{max} (%)	*Dose-ratio*[a]
Control		6	9.50±0.05	85±1	–
Metergoline	2.48	4	9.06±0.04[b]	82±4	2.7
Mesulergine	2.76	4	8.94±0.07[c]	84±2	3.8
Methysergide	2.13	4	8.74±0.04[b]	86±1	5.6
Lisuride	0.22	4	8.20±0.08[b]	75±1[c]	20.6
Methiothepin	0.68	4	8.68±0.02[b]	74±1[b]	6.5
Mianserin	10.60	4	9.00±0.09[c]	89±2	3.3
Clozapine	11.00	3	8.90±0.06[b]	86±1	3.9
Risperidone	0.078	4	8.71±0.07[b]	73±3[d]	6.2

Values are the mean±s.e.mean of n experiments. [a] Ratio of ED_{50} with antagonist/ED_{50} of the control group; [b] $P<0.001$; [c] $P<0.01$; [d] $P<0.05$ vs control values.

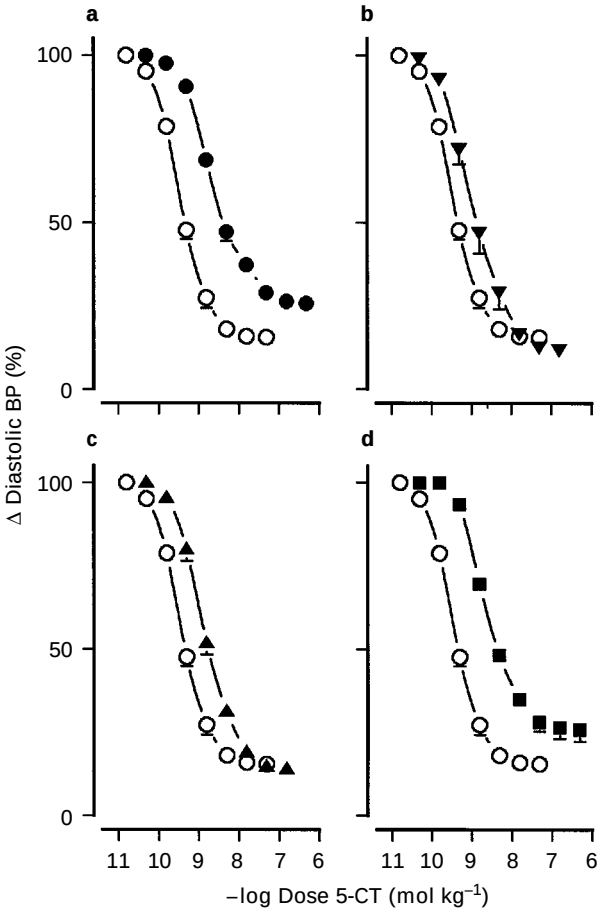

Figure 3 The effects of (a) methiothepin (●, 0.68 μmol kg^{-1}); (b) mianserin (▼, 10.6 μmol kg^{-1}); (c) clozapine (▲, 11.0 μmol kg^{-1}); and (d) risperidone (■, 78 nmol kg^{-1}) on the decreases in diastolic blood pressure (BP) induced by 5-CT compared to those produced by 5-CT alone (○) in methoxamine-infused pithed rats pretreated with ketanserin (0.18 μmol kg^{-1}, i.v.). The effects of 5-CT are expressed as a percentage (%) of the maximum decrease in diastolic blood pressure. Points represent the mean and vertical lines denote the s.e.mean of 3–4 experiments.

5-ht$_7$ receptor (Table 1), on the hypotensive responses induced by 5-CT in methoxamine-infused pithed rats pretreated with ketanserin. Thus, the commonly used ergoline-derivatives, metergoline, mesulergine and methysergide, as well as the dopamine D_2 receptor ligand, lisuride, produced a significant rightward shift of the dose-response curve for 5-CT (Figure 2a, b, c and d, respectively; see also Table 3); in the case of lisuride, a significant reduction in E_{max} was observed. Since in preliminary experiments in methoxamine-infused pithed rats, it was observed that the hypotensive effect induced by lisuride (0.22 μmol kg^{-1}, 0.1 mg kg^{-1} of the salt, i.v.) had completely worn off after 15 min, the inhibition of the 5-CT-induced hypotension by the same dose of the drug (Figure 2d) may be best explained by an interaction with the 'hypotensive' 5-HT receptor, as lisuride was given 30 min before the dose-response curve for 5-CT was constructed.

Similar antagonist effects were produced by methiothepin, mianserin, clozapine and risperidone (Figure 3a, b, c and d, respectively). However, as noticed with lisuride, methiothepin and risperidone produced a significant reduction in E_{max} (Table 3) thus suggesting a non-competitive interaction. Based upon the doses employed and the corresponding dose-ratios, the apparent rank order of potency for the antagonists was lisuride $\geqslant$ risperidone > methiothepin > methysergide > mesulergine > metergoline > clozapine > mianserin; thus, in the presence of these antagonists, the ED_{50} for 5-CT was increased between 3 and 20 fold (see Table 3). As can be observed from Table 1, the above antagonist profile shows a close alignment with the affinity order at the 5-ht$_7$ receptor.

Effect of indomethacin and L-NAME on 5-CT-induced hypotensive responses

The i.v. bolus administration of L-NAME (120 μmol kg^{-1}) to pithed rats pretreated with saline (1 ml kg^{-1}, i.v.; $n=7$) or indomethacin (14 μmol kg^{-1}, i.v.; $n=5$) maximally increased diastolic blood pressure to 105±4 and 118±6 mmHg, respectively. Since the pressor effect of L-NAME in these animals significantly decreased after 30–45 min, an infusion of methoxamine (8–20 nmol kg^{-1} min^{-1}, i.v.) was employed to support blood pressure within the control values (i.e. around 110 mmHg; see Experimental protocols). The hypotensive effects induced by 5-CT were not significantly inhibited by the above treatments (the $-\log ED_{50}$ and E_{max} values for 5-CT in animals pretreated with saline [$n=6$], L-NAME [$n=7$] and indomethacin plus L-NAME [$n=5$] were, respectively, 9.51±0.06, 9.54±0.06 and 9.57±0.05 mol kg^{-1}, and 85±0.7, 85.8±1.2 and 85.5±1.6 % of the maximum hypotensive effect).

Discussion

The major finding of the present study was that the 5-HT receptor mediating the long-lasting hypotension in pithed rats with artificially raised blood pressure is highly sensitive to blockade by several 5-HT receptor antagonists and some psychoactive drugs, all of which display high affinity for the cloned 5-ht$_7$ receptor (Table 1). It should be stressed that a possible association of the 'hypotensive' 5-HT receptor with the cloned 5-ht$_7$ subtype was approached as both receptors share some pharmacological properties (see Introduction).

In the search for the appropriate experimental conditions for studying the long-lasting hypotensive effect of 5-HT, previous studies in rats employed 0.18 μmol kg^{-1} (0.1 mg kg^{-1}, i.v.) of ketanserin to block 5-HT$_2$ receptors mediating vasopressor responses (Kalkman *et al*., 1983; Martin *et al*., 1987). In the light of the present findings showing a biphasic pattern of the dose-response curves for 5-HT and 5-methoxytryptamine (Figure 1) with considerable pressor effects remaining apparent in the presence of ketanserin, it seems that 0.18 μmol kg^{-1} of the antagonist produces only a partial blockade of pressor 5-HT$_2$ receptors which may counteract the vasodilator effects to the agonists. The monophasic dose-response curves for 5-HT and 5-methoxytryptamine along with the blockade of their remaining pressor effects in the presence of a higher dose (3.2 μmol kg^{-1}, 1.8 mg kg^{-1}, i.v.) of ketanserin, which completely antagonizes 5-HT$_2$ receptors in the pithed rat (Göthert *et al*., 1986), support such a possibility. The incomplete blockade of pressor 5-HT$_2$ receptors by 0.18 μmol kg^{-1} ketanserin may indeed explain the lower maximum hypotensive activity of 5-methoxytryptamine, with respect to 5-HT, in anaesthetized rats (Martin *et al*., 1987). In spite of the above observations and taking into consideration that 5-CT displays very low affinity and efficacy at 5-HT$_2$ receptors (see Martin, 1994) and may also produce, at the high doses employed in the experiments with antagonists (i.e. 492 nmol kg^{-1}, i.v.), slight 5-HT$_2$ receptor-mediated pressor effects in the rat (Saxena & Lawang, 1985), the dose of 0.18 μmol kg^{-1} ketanserin was preferred in order to prevent potential 5-CT-induced pressor effects and to avoid any possible interaction of this antagonist with the 5-HT receptor mediating long-lasting hypotension. On the other hand, the above dose of ketanserin, a drug which also displays considerable affinity at α_1-adrenoceptors (Leysen, 1985; Van Wijngaarden *et al*., 1990), is less than that (0.55 μmol kg^{-1}, 0.3 mg kg^{-1}, i.v.) shown hardly to antagonize the pressor responses to methoxamine in pithed rats (Kalkman *et al*., 1982). Lastly, the use of ketanserin counteracted the 5-HT$_2$ receptor-mediated tachycardiac effects that have been demonstrated in pithed rats (Göthert *et al*., 1986).

Agonist profile at the 'hypotensive' 5-HT receptor

The rank order of agonist potency obtained in the present study was closely similar to that obtained in both anaesthetized (Kalkman *et al*., 1983; Martin *et al*., 1987) and vasopressin-infused pithed rats (Kalkman *et al*., 1983) pretreated with ketanserin (0.18 μmol kg^{-1}, i.v.) i.e. 5-CT > 5-HT $\geqslant$ 5-methoxytryptamine. Although the above agonist profile is apparently consistent with the involvement of a 5-HT$_1$-like receptor (Bradley *et al*., 1986), as was indeed suggested by Kalkman *et al*. (1983) and Martin *et al*. (1987), the very low hypotensive activity of sumatriptan (present study), a selective agonist for the 5-HT$_1$-like receptor mediating vascular contraction (Humphrey *et al*., 1988), points toward the involvement of another 5-HT receptor subtype. Indeed, based upon the inability of sumatriptan to mimic the vasodilator effects of 5-HT in several vascular preparations, it was originally proposed that 5-HT$_1$-like receptors should be subdivided into at least two subtypes i.e. one mediating contraction and another mediating relaxation (Humphrey *et al*., 1988; see also Feniuk & Humphrey, 1990).

Since the direct relaxant effects of 5-HT had been, on the other hand, associated with stimulation of the adenylate cyclase system (Trevethick *et al*., 1984; 1986; Sumner *et al*., 1989), which contrasts with the negative linkage of the contractile 5-HT$_1$-like receptor with this transductional pathway (Sumner & Humphrey, 1990; Sumner *et al*., 1992), it was no longer considered that a 5-HT$_1$-like, but an 'orphan' 5-HT receptor, mediates direct smooth muscle relaxation (see Hoyer *et al*., 1994; Martin, 1994; Saxena, 1995, for review).

Interestingly, among the new members of the 5-HT receptor family (i.e. 5-ht$_{5A/5B}$, 5-ht$_6$ and 5-ht$_7$), the 5-ht$_7$ subtype displays pharmacological and transductional properties closely resembling those of the 5-HT receptor mediating direct vasorelaxant responses. That is, in addition to its positive linkage with the adenylate cyclase system, the cloned 5-ht$_7$ receptor exhibits an agonist rank order of affinity of 5-CT > lisuride $\geqslant$ 5-methoxytryptamine $\geqslant$ 5-HT >> sumatriptan (Bard *et al*., 1993; Plassat *et al*., 1993; Ruat *et al*., 1993; Shen *et al*., 1993). Clearly, this order of agonist potency is practically identical to that obtained in the present study, thus suggesting that the 'hypotensive' 5-HT receptor in the rat may represent a functional counterpart of the cloned 5-ht$_7$ receptor. Interestingly, the above order of agonist affinity is also similar to that found in other vascular and nonvascular preparations in which a 5-HT receptor, possibly related to the 5-ht$_7$ subtype, mediates direct relaxant effects; some of these preparations include the rabbit femoral (Martin & Wilson, 1995) and jugular (Leff *et al*., 1987; Martin *et al*., 1987) veins, the cat saphenous vein (Feniuk *et al*., 1983), the pig vena cava (Trevethick *et al*., 1984; 1986; Sumner *et al*., 1989), the dog coronary artery (Houston & Vanhoutte, 1988; Cushing *et al*., 1996; Terrón, 1996a, b) and the guinea-pig ileum (Feniuk *et al*., 1983; Kalkman *et al*., 1986; Carter *et al*., 1995).

Although the above agonist profile displays also some alignment with that at the 5-HT$_{1A}$ receptor (see Table 1), convincing evidence showing the inability of the 5-HT$_{1A}$ receptor agonist, 8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT), to decrease blood pressure in angiotensin II- (Fozard *et al*., 1987) and vasopressin-infused (Gradin *et al*., 1985) pithed rats strongly argues against the involvement of a 5-HT$_{1A}$ receptor. Further arguments against the participation of 5-HT$_{1A}$ receptors were obtained by the use of antagonist drugs (see below).

Effects of antagonists at 5-HT$_1$, 5-HT$_2$, 5-HT$_3$ and 5-HT$_4$ receptors on 5-CT-induced hypotensive responses

In support of the notion that a receptor unrelated to the 5-HT$_1$-like subtype is responsible for the hypotensive effects of 5-HT in the rat, the β-adrenoceptor blocker with relatively high affinity for 5-HT$_1$ receptors, propranolol (Table 1), had no effect on 5-CT-induced responses. Similarly, MDL-72222 and GR125487, in doses high enough to block functional 5-HT$_3$ and 5-HT$_4$ receptors, respectively (Fozard, 1984; Bunce *et al*., 1994), were incapable of modifying the effects of 5-CT. Inasmuch as ketanserin, at a dose (0.18 μmol kg^{-1}, 0.1 mg kg^{-1}, i.v.) previously shown to block pressor 5-HT$_2$ receptors in the rat (Kalkman *et al*., 1983; Martin *et al*., 1987), failed to antagonize significantly the hypotensive effects of 5-CT, a role for 5-HT$_{2A}$ receptors can be ruled out. With regard to the possible involvement of 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors, for which ketanserin displays low or moderate affinity (pK_i values of 5.4 and 7, respectively; Baxter *et al*., 1995), three findings should be considered: (i) a high dose (3.2 μmol kg^{-1}, i.v.) of ketanserin did not antagonize but rather favoured the hypotensive effects of 5-HT and 5-methoxytryptamine (see Results); (ii) the low potency of mianserin in antagonizing the hypotensive response to 5-CT (Table 3) does not appear consistent with its high affinity for 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors (pK_i values of 7.3 and 8.0, respectively; Baxter *et al*., 1995); and (iii) the 5-HT receptor agonist with high affinity and selectivity for 5-HT$_{2B}$ receptors, α-methyl-5-HT (pEC$_{50}$ values of 8.4, 7.3 and 6.1 for 5-HT$_{2B}$, 5-HT$_{2C}$ and 5-HT$_{2A}$ receptors, respectively; Baxter *et al*., 1995), failed to mimic the hypotensive responses induced by 5-HT in anaesthetized rats pretreated with 0.18 μmol kg^{-1} (i.v.) ketanserin (Martin *et al*., 1987). Based upon these observations it seems unlikely that 5-HT$_{2B}$ and/or 5-HT$_{2C}$ receptors play a significant role in the vasodepressor responses induced by 5-HT. On the other hand, the failure of ketanserin to antagonize the hypotensive effects of 5-HT, 5-methoxytryptamine and 5-CT is consistent with its very low affinity for the cloned 5-ht$_7$ receptor (Table 1).

Effect of several 5-ht$_7$ receptor ligands on 5-CT-induced hypotensive responses

In order to provide a further insight into the nature of the 'hypotensive' 5-HT receptor in the rat, several 5-HT receptor antagonists showing from moderate to high affinity for the cloned 5-ht$_7$ receptor (Table 1) were selected as potential antagonists of 5-CT. Moreover, since one distinctive feature of the cloned 5-ht$_7$ (and 5-ht$_6$) receptor is its high affinity for a range of typical and atypical antipsychotic drugs (Shen *et al*., 1993; Roth *et al*., 1994), which have indeed been shown to antagonize a functional 5-ht$_7$ receptor mediating direct relaxation in the canine coronary artery (Terrón, 1996a,b), the present pharmacological analysis was extended by investigating the possible antagonist effects of the atypical antipsychotics, clozapine and risperidone, on the hypotensive responses induced by 5-CT.

Consistent with the involvement of a 5-ht$_7$-like receptor, the ergoline-derivatives, metergoline, mesulergine, methysergide and lisuride, strongly antagonized the hypotensive responses induced by 5-CT. These drugs, in fact, were selected as potential antagonists because they show either high affinity or relative selectivity for the cloned 5-ht$_7$ receptor (Ruat *et al*., 1993; Shen *et al*., 1993; see Table 1). Of special relevance is the fact that mesulergine, a drug displaying an almost 100 fold selectivity for the rat cloned 5-ht$_7$ receptor with respect to the rat cloned 5-ht$_6$ receptor (Table 1), competitively antagonized the hypotensive effects of 5-CT. In addition, though lisuride displays high affinity for both the cloned rat 5-ht$_6$ and 5-ht$_7$ receptors (Table 1), its ability to mimic the hypotensive effects of 5-HT and to cause, at a relatively low dose (220 nmol kg^{-1}, i.v.), a 6 fold increase in the ED_{50} for 5-CT (Table 3) is in keeping with the involvement of a 5-ht$_7$-like subtype. Indeed, the iodinated form of (+)-lysergic acid diethylamide (LSD), a close analogue of lisuride showing a similarly high affinity for the rat ($pK_i = 8$; Ruat *et al*., 1993) and guinea-pig ($pK_i = 7.8$; To *et al*., 1995) cloned 5-ht$_7$ receptor, has previously been shown to label 5-HT binding sites with a pharmacology nearly matching that of the 5-ht$_7$ receptor (Kalkman *et al*., 1986).

On the other hand, methiothepin, mianserin, clozapine and risperidone similarly behaved as antagonists against the hypotensive effects of 5-CT. In this regard, though most of the drugs used in the present study display high affinity for 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors, and even for receptors other than 5-HT i.e. α_1-adrenoceptors and dopamine D_1 and D_2 receptors (Leysen, 1985; Cohen & Lipinski, 1986; Leysen *et al*., 1988; Hoyer *et al*., 1989; Meltzer *et al*., 1989; Roth *et al*., 1991; 1992), they share a high affinity binding for the cloned 5-ht$_7$ receptor (Table 1). In fact, methiothepin has been shown to antagonize the 5-HT-induced stimulation of adenosine 3′:5′-cyclic monophosphate (cyclic AMP) accumulation in transiently transfected COS-7 cells expressing the cloned mouse (Plassat *et al*., 1993) and human (Bard *et al*., 1993) 5-ht$_7$ receptor, whereas mianserin antagonized the 5-HT-induced stimulation of cyclic AMP accumulation in CHO cells expressing a rat 5-ht$_7$ receptor (Ruat *et al*., 1993). As one would expect from previous studies showing that risperidone has more than a 300 fold greater selectivity for the cloned 5-ht$_7$ receptor than for the cloned 5-ht$_6$ receptor (Roth *et al*., 1994; Table 1), the drug behaved as a very potent unsurmountable antagonist at the 5-HT receptor mediating long-lasting hypotension in the rat. The high potency of risperidone (78 nmol kg^{-1}, i.v.) was reflected as a 6 fold increase in the ED_{50} for 5-CT (Table 3) and is consistent with its nanomolar affinity at the 5-ht$_7$ receptor (Table 1); this antipsychotic, in contrast, displays very low affinity at the 5-HT$_{1A}$ receptor (Table 1) which further makes the participation of this receptor subtype unlikely.

Absence of indirect mechanisms in the hypotensive effect to 5-CT

Numerous studies in isolated arteries and veins have demonstrated that 5-HT elicits relaxation through both direct and indirect mechanisms (see Martin, 1994). The direct mechanism, showing an agonist profile of 5-CT > 5-HT >> α-methyl-5-HT $\geqslant$ sumatriptan and being highly sensitive to blockade by methiothepin, methysergide and the antipsychotic drug, spiperone, involves a 5-HT receptor the pharmacological properties of which closely match those of the 5-HT receptor mediating long-lasting hypotension in the rat (Martin *et al*., 1987; present study). The indirect mechanism involves the release of an endothelium-derived relaxing factor through either a 5-HT$_1$-like or an 'orphan' 5-HT receptor (Martin, 1994). Although the pharmacological properties of the 'hypotensive' 5-HT receptor in the rat suggests a major role of the direct vasorelaxant mechanism (Martin *et al*., 1987), it was of interest to determine if an indirect mechanism participates in the long-lasting hypotensive effects of 5-CT. Thus, the inhibition of the prostaglandin-forming cyclo-oxygenase and nitric oxide synthase with indomethacin (14 μmol kg^{-1}, i.v.) and L-NAME (120 μmol kg^{-1}, i.v.), respectively, produced no significant changes in the hypotensive response to 5-CT thereby supporting the possibility that indirect mechanisms are not involved. On this basis, it appears reasonable to suggest that the 'hypotensive' 5-HT receptor is mainly located in the vascular smooth muscle of the systemic resistance vessels.

In conclusion, it is suggested that the long-lasting hypotensive effect of 5-HT in the rat is mediated by a receptor similar to the cloned 5-ht$_7$ subtype. The pharmacological profile of this 'hypotensive' 5-HT receptor closely matches that previously described for a 5-ht$_7$-like receptor mediating direct smooth muscle relaxation in other vascular and nonvascular preparations (Carter *et al*., 1995; Martin & Wilson, 1995; Cushing *et al*., 1996; Terrón, 1996a, b). Thus, based upon these findings in pithed rats, it could be speculated that the 5-ht$_7$ receptor plays a role in the regulation of arterial blood pressure and may therefore represent another target for antihypertensive therapy. Indeed, the ability of 5-CT, provided as a chronic i.v. treatment, to significantly decrease blood pressure in Wistar-Kyoto and spontaneously hypertensive rats (Balasubramaniam *et al*., 1993) strongly supports this contention and highlights the possibility of developing selective 5-ht$_7$ receptor agonists as a novel therapeutic strategy in the treatment of hypertension.

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