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Pharmacological profile of the 5-HT-induced inhibition of cardioaccelerator sympathetic outflow in pithed rats: correlation with 5-HT_1 and putative 5-$ht_{5A/5B}$ receptors

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1 Continuous infusions of 5-hydroxytryptamine (5-HT) inhibit the tachycardiac responses to preganglionic (C_7-T_1) sympathetic stimulation in pithed rats pretreated with desipramine. The present study identified the pharmacological profile of this inhibitory action of 5-HT.

2 The inhibition induced by intravenous (i.v.) continuous infusions of 5-HT (5.6 $\mu g\,kg^{-1}\,min^{-1}$) on sympathetically induced tachycardiac responses remained unaltered after i.v. treatment with saline or the antagonists GR 127935 (5-$HT_{1B/1D}$), the combination of WAY 100635 (5-HT_{1A}) plus GR 127935, ritanserin (5-HT_2), tropisetron (5-$HT_{3/4}$), LY215840 (5-HT_7) or a cocktail of antagonists/inhibitors consisting of yohimbine (α_2), prazosin (α_1), ritanserin, GR 127935, WAY 100635 and indomethacin (cyclooxygenase), but was abolished by methiothepin (5-$HT_{1/2/6/7}$ and recombinant 5-$ht_{5A/5B}$). These drugs, used in doses high enough to block their respective receptors/mechanisms, did not modify the sympathetically induced tachycardiac responses *per se*.

3 I.v. continuous infusions of the agonists 5-carboxamidotryptamine (5-CT; 5-$HT_{1/7}$ and recombinant 5-$ht_{5A/5B}$), CP 93,129 (*r*5-HT_{1B}), sumatriptan (5-$HT_{1B/1D}$), PNU-142633 (5-HT_{1D}) and ergotamine (5-$HT_{1B/1D}$ and recombinant 5-$ht_{5A/5B}$) mimicked the above sympatho-inhibition to 5-HT. In contrast, the agonists indorenate (5-HT_{1A}) and LY344864 (5-ht_{1F}) were inactive. Interestingly, 5-CT-induced cardiac sympatho-inhibition was abolished by methiothepin, the cocktail of antagonists/inhibitors, GR 127935 or the combination of SB224289 (5-HT_{1B}) plus BRL15572 (5-HT_{1D}), but remained unchanged when SB224289 or BRL15572 were given separately.

4 Therefore, 5-HT-induced cardiac sympatho-inhibition, being unrelated to 5-HT_2, 5-HT_3, 5-HT_4, 5-ht_6, 5-HT_7 receptors, $\alpha_{1/2}$-adrenoceptor or prostaglandin synthesis, seems to be primarily mediated by (i) 5-HT_1 (probably 5-$HT_{1B/1D}$) receptors and (ii) a novel mechanism antagonized by methiothepin that, most likely, involves putative 5-$ht_{5A/5B}$ receptors.
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Abbreviations: BRL15572, (1-(3-chlorophenyl)-4-[3,3-diphenyl(2-(*S*,*R*)hydroxypropanyl) piperazine]) hydrochloride; CP 93,129, 3-(1,2,5,6-tetrahydropyrid-4-yl)pyrrolo[3,2-b]pyrid-5-one; 5-CT, 5-carboxamidotryptamine; GR 127935, *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; LY215840, *cis*-*n*-(2-hydroxycyclopentyl)-6-methyl-1-(1-methylethyl)ergoline-8-carboxamide; LY344864, *N*-[3-(dimethylamino)-2,3,4,9-tetrahydro-1*H*-carbazol-6-yl]-4-fluorobenzamide; PNU-142633, (s)-3,4-dihydro-1-[2-[4-[4-aminocarbonyl)phenyl]-1-perazinyl]ethyl]-*N*-methyl-1*H*-2-benzopyran-6-carboximide; SB224289, 2,3,6,7-tetrahydro-1′-methyl-5-[2′-methyl-4′(5-methyl-1,2,4-oxadiazol-3-yl)biphenyl-4-carbonyl]furo [2,3-f]indole-3-spiro-4′-piperidine)hydrochloride; WAY 100635, *N*-{2-[4-(2-methoxy-phenyl)-1-piperazinyil] ethyl}-*N*-(2-pyridinyl)cyclohexane carboxamide trihydrochloride

Introduction

The complexity of cardiovascular effects produced by serotonin (5-hydroxytryptamine; 5-HT), including bradycardia or tachycardia, hypotension or hypertension and vasodilatation or vasoconstriction, has been explained by the capability of this monoamine to interact with different receptors in the central nervous system, on the autonomic ganglia and postganglionic nerve endings, on vascular smooth muscle and endothelium and on the cardiac tissue (see Saxena & Villalón, 1990; 1991; Villalón *et al*., 1997; Saxena *et al*., 1998). With respect to 5-HT-induced tachycardia, this is notoriously species-dependent and is mediated, directly and/or indirectly, either by 5-HT_{2A} (rat), 5-HT_3 (rabbit), 5-HT_4 (human, pig) or 5-HT_7 (cat) receptors, or by tyramine-like (guinea-pig) or unidentified mechanisms (see Saxena &

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Villalón, 1990; 1991; Villalón *et al*., 1997; Centurión *et al*., 2002).

5-HT interferes with sympathetic neurotransmission by stimulating 5-HT_1 receptors located prejunctionally in a wide variety of blood vessels and other organs (see Saxena *et al*., 1998), including the systemic vasculature in pithed rats (Villalón *et al*., 1995; 1998). Interestingly, our group has also shown in pithed rats that continuous infusions of 5-HT dose-dependently inhibited the tachycardiac responses to selective stimulation of the preganglionic (C_7-T_1) cardiac sympathetic outflow (Villalón *et al*., 1999). Admittedly, this study did not measure sympathetic nerve activity directly, but the electrically induced noradrenaline release could be estimated indirectly by measurement of the evoked tachycardiac response. Under these conditions, the responses to 5-HT were considered to be sympatho-inhibitory, as the monoamine is capable of inhibiting the tachycardiac responses to sympathetic stimulation, but not those to exogenous noradrenaline (Villalón *et al*., 1999).

On this basis, the present study was designed to characterize the pharmacological profile of the above 5-HT-induced cardiac sympatho-inhibition. Therefore, based on the classification schemes proposed by the NC-IUPHAR subcommittee on 5-HT receptors (see Hoyer *et al*., 1994; Saxena *et al*., 1998), we investigated if the sympatho-inhibitory action of 5-HT could be: (i) blocked by the antagonists methiothepin (5-$HT_{1/2/6/7}$ and recombinant 5-$ht_{5A/5B}$), ritanserin (5–HT_2), tropisetron (5-$HT_{3/4}$), LY215840 (5-HT_7), GR 127935 (5-$HT_{1B/1D}$), the combination of WAY 100635 (5-HT_{1A}) plus GR 127935 or a cocktail of antagonists/inhibitors consisting of yohimbine (α_2), prazosin (α_1), ritanserin, GR 127935, WAY 100635 and indomethacin (cyclooxygenase) and (ii) mimicked by the agonists 5-carboxamidotryptamine (5-CT; 5-$HT_{1/7}$ and recombinant 5-$ht_{5A/5B}$), indorenate (5-HT_{1A}), CP 93,129 (*r*5-HT_{1B}), sumatriptan (5-$HT_{1B/1D}$), PNU-142633 (5-HT_{1D}), LY344864 (5-ht_{1F}) or ergotamine (5-$HT_{1B/1D}$ and recombinant 5-$ht_{5A/5B}$) (see Table 1 for affinity constants). Moreover, 5-CT-induced sympatho-inhibition was analysed after some of the above antagonists, as well as after SB224289 or BRL15572 (given alone or in combination). A preliminary account of this investigation was presented at the Satellite Meeting 'Serotonin: From the Molecule to the Clinic' (Villalón *et al*., 2000).

Methods

Animals

Male Wistar normotensive rats (240–280 g) were used in the present experiments. The animals were maintained at a 12/12-h light–dark cycle (light beginning at 07:00) and housed in a special room at constant temperature ($22\pm2^{\circ}C$) and humidity (50%), with food and water freely available in their home cages.

General methods

Experiments were carried out in a total of 151 rats. After anaesthesia with ether and cannulation of the trachea, the rats were pithed by inserting a stainless-steel rod through the orbit and *foramen magnum* into the vertebral *foramen* (Shipley & Tilden, 1947). The animals were artificially ventilated with room air using an Ideal Palmer pump (56 strokes min^{-1} and a stroke volume of 20 ml kg^{-1}), as previously established by Kleinman & Radford (1964). Subsequently, the pithing rod was replaced by an electrode and enamelled, except for 1 cm length 7 cm from the tip, so that the uncovered segment was situated at C_7-T_1 of the spinal cord to allow selective stimulation of the cardiac sympathetic outflow (Gillespie *et al*., 1970; Villalón *et al*., 1999). 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 XL), for the recording of blood pressure. Heart rate was

Table 1 Receptor-binding affinity (pK_i) of the drugs used in the present study at 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-ht_{1E}, 5-ht_{1F}, 5-ht_{5A} and 5-ht_{5B} receptors

	Receptor						
Drug	*5-HT$_{1A}$*	*5-HT$_{1B}$*	*5-HT$_{1D}$*	*5-ht$_{1E}$*	*5ht$_{1F}$*	*5-ht$_{5A}$*	*5-ht$_{5B}$*
BRL15572	7.7[i]	6.1[i]	7.9[i]	5.2[i]	6.0[i]	–	–
5-CT	10.3[f]	8.9[b]	9.2[g]	5.1[d]	5.8[d]	9.5[v]	7.4[v]
CP 93,129	5.5[l]	8.1[b]	5.7[l]	–	–	–	–
Ergotamine	8.4[k]	8.7[k]	7.9	6.2[d]	6.8[d]	8.4[v]	8.5[v]
GR 127935	6.9[e]	8.5[e]	8.9[e]	6.2[e]	7.3[e]	5.2[t]	–
5-HT	9.2[f]	8.6[b]	8.4[g]	8.0[d]	8.0[d]	8.1[v]	6.6[v]
Indorenate	7.8[s]	5.4[s]	6.7[s]	–	–	–	–
LY215840	–	–	–	–	–	–	–
LY344864	6.3[m]	6.3[m]	6.2[m]	5.8[m]	8.2[m]	–	–
Methiothepin	7.7[a]	7.2[b]	7.7[c]	6.7[d]	6.2[d]	7.0[v]	7.8[v]
PNU-142633	–	4.8[n]	8.3[n]	–	–	–	–
Prazosin	5.0[k]	5.1[k]	7.1[k]	–	–	–	–
Ritanserin	5.7[q]	4.0[k]	5.4[r]	–	–	–	–
SB224289	5.5[j]	8.0[j]	6.2[j]	<5.0[j]	<5.0[j]	–	–
Sumatriptan	6.4[h]	7.3[b]	8.5[h]	5.6[d]	7.6[d]	5.1[u]	6.1[u]
Tropisetron	–	–	–	–	–	<5.0[v]	<4.5[v]
WAY100635	9.6[o]	5.1[p]	5.6[p]	–	–	–	–
Yohimbine	6.9[k]	5.5[k]	–	5.9[d]	7.0[d]	6.0[v]	6.0[v]

[a]Fozard *et al*. (1987); [b]Beer *et al*. (1998); [c]Pauwels *et al*. (1996); [d]Adham *et al*. (1993); [e]Pauwels (1996); [f]Newman-Tancredi *et al*. (1997); [g]Weinshank *et al*. (1992); [h]Leysen *et al*. (1996); [i]Price *et al*. (1997); [j]Hagan *et al*. (1997); [k]Hoyer (1988); [l]Macor *et al*. (1990); [m]Phebus *et al*. (1997); [n]Pregenzer *et al*. (1999); [o]Johansson *et al*. (1997); [p]Mos *et al*. (1997); [q]Arnt & Hyttel (1989); [r]Peroutka & McCarthy (1989); [s]Hoyer, D. personal communication; [t]Skingle *et al*. (1996); [u]Boess & Martin (1994); [v]Hoyer *et al*. (1994). –, Unknown.

measured with a tachograph (7P4, 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 7 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 sympatho-inhibitory responses to 5-HT are particularly more pronounced at lower frequencies of stimulation, all animals were systematically pretreated with 50 μg kg^{-1} (i.v.) of desipramine (a noradrenaline-reuptake inhibitor) before each stimulus–response curve (S–R curve), as previously described (Villalón *et al*., 1999). The body temperature of each pithed rat was maintained at 37°C by a lamp and monitored with a rectal thermometer.

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 preganglionic cardiac sympathetic outflow was stimulated to elicit tachycardiac responses by applying trains of 10 s (monophasic rectangular pulses of 2 ms duration and 50 V), at increasing frequencies of stimulation (0.01, 0.03, 0.1, 0.3, 1 and 3 Hz). When the heart rate had returned to baseline levels, the next frequency was applied; this procedure was systematically performed until the S–R curve was completed (about 30 min). At this point, the animals (151 in total) were divided into five groups.

The first group ($n=33$) was subdivided into six subgroups that received an i.v. bolus injection of, respectively: (i) physiological saline (control; 1 ml kg^{-1}; $n=5$) or the 5-HT receptor antagonists (see Table 1): (ii) methiothepin (5-HT$_{1/2/6/7}$ and recombinant 5-ht$_{5A/5B}$; 300 μg kg^{-1}; $n=6$); (iii) ritanserin (5-HT$_2$; 100 μg kg^{-1}; $n=5$); (iv) tropisetron (5-HT$_{3/4}$; 3000 μg kg^{-1}; $n=5$); (v) LY215840 (5-HT$_7$; 1000 μg kg^{-1}; $n=6$); or (vi) a cocktail of antagonists/inhibitors ($n=6$) consisting of yohimbine (α_2-adrenoceptors; 1000 μg kg^{-1}), prazosin (α_1-adrenoceptors; 100 μg kg^{-1}), ritanserin (5-HT$_2$; 100 μg kg^{-1}), GR 127935 (5-HT$_{1B/1D}$; 300 μg kg^{-1}), WAY 100635 (5-HT$_{1A}$; 30 μg kg^{-1}) and indomethacin (cyclooxygenase; 5000 μg kg^{-1}). This cocktail was used in an attempt to cover as much as possible the spectrum of blockade of methiothepin (except the blockade of putative 5-ht$_{5A/5B}$ receptors), a nonselective drug which: (i) displays high affinity for (and blocks in several *in vivo* preparations) a wide variety of receptors including $\alpha_{1/2}$-adrenoceptors, 5-HT$_{1A/1B/1D}$ and 5-HT$_2$ receptors (Leysen, 1985; Hoyer, 1988; Saxena & Villalón, 1990; 1991; Hoyer *et al*., 1994; Villalón *et al*., 1995; 1997) and (ii) inhibits cyclooxygenase (see Leysen, 1985; Martin, 1994). At 10 min after the administration of above compounds, an S–R curve was elicited again, as described above, to analyse their effects on the sympathetically induced tachycardiac responses *per se*.

The second group ($n=27$) was subdivided into five subgroups that received an i.v. bolus injection of, respectively: (i) physiological saline (1 ml kg^{-1}; $n=5$); (ii) methiothepin (300 μg kg^{-1}; $n=6$); (iii) ritanserin (100 μg kg^{-1}; $n=6$); (iv) tropisetron (3000 μg kg^{-1}; $n=5$); or (v) LY215840 (1000 μg kg^{-1}; $n=5$). At 10 min after the dose of saline or the corresponding antagonist, all the subgroups received an i.v. continuous infusion of 5-HT (5.6 μg kg^{-1} min^{-1}) by a WPI model sp100i pump (World Precision Instruments Inc., Sarasota, FL, U.S.A.). At 20 min after starting the infusion, an S–R curve was elicited, as described above *during* the infusion of 5-HT. Once the S–R curve was completed, the infusion was stopped.

The third group ($n=42$) was subdivided into eight subgroups that received, respectively, an i.v. continuous infusion of: (i) physiological saline (control; 0.02 ml min^{-1}; $n=4$) twice; as well as the agonists (see Table 1); (ii) 5-CT (5-HT$_{1/7}$ and recombinant 5-ht$_{5A/5B}$; 0.1 and 0.3 μg kg^{-1} min^{-1}; $n=4$); (iii) indorenate (5-HT$_{1A}$; 30 and 100 μg kg^{-1} min^{-1}; $n=6$); (iv) CP 93,129 (r5-HT$_{1B}$; 30 and 100 μg kg^{-1} min^{-1}; $n=7$); (v) sumatriptan (5-HT$_{1B/1D}$; 30 and 100 μg kg^{-1} min^{-1}; $n=6$); (vi) PNU–142633 (5-HT$_{1D}$, 30 and 100 μg kg^{-1} min^{-1}; $n=5$); (vii) LY344864 (5-ht$_{1F}$; 30 and 100 μg kg^{-1} min^{-1}; $n=5$); or (viii) ergotamine (5-HT$_{1B/1D}$ and recombinant 5-ht$_{5A/5B}$; 1 and 1.8 μg kg^{-1} min^{-1}; $n=5$). At 20 min after starting each infusion, an S–R curve was elicited, as described above, *during* the infusion of the corresponding compound.

The fourth group ($n=16$) was subdivided into three subgroups that received, respectively, an i.v. bolus injection of the 5-HT$_1$ receptor antagonists (see Table 1): (i) GR 127935 (5-HT$_{1B/1D}$; 300 μg kg^{-1}; $n=5$); (ii) the combination of WAY 100635 (5-HT$_{1A}$; 30 μg kg^{-1}) plus GR 127935 (300 μg kg^{-1}) ($n=5$); or (iii) the cocktail of drugs previously described ($n=6$; see the experimental protocol of the first group). At 10 min after the administration of antagonists, the three subgroups received an i.v. continuous infusion of 5-HT (5.6 μg kg^{-1} min^{-1}), as described above. At 20 min after starting the infusion, an S–R curve was elicited again, *during* the infusion of 5-HT.

The fifth group ($n=33$) was subdivided into seven subgroups that received an i.v. bolus injection of, respectively: (i) physiological saline (1 ml kg^{-1}; $n=5$), or the antagonists: (ii) methiothepin (300 μg kg^{-1}; $n=5$); (iii) the cocktail of drugs previously described ($n=4$; see the experimental protocol of the first group); (iv) GR 127935 (5-HT$_{1B/1D}$; 300 μg kg^{-1}; $n=4$); (v) SB224289 (selective 5-HT$_{1B}$; 300 μg kg^{-1}; $n=5$); (vi) BRL15572 (selective 5-HT$_{1D}$; 300 μg kg^{-1}; $n=5$); or (vii) the combination of SB224289 plus BRL15572 (300 μg kg^{-1} each; $n=5$). At 10 min after the administration of saline or the corresponding antagonist, all the subgroups received an i.v. continuous infusion of 5-CT (0.1 μg kg^{-1} min^{-1}), as described above. At 20 min after starting the infusion, an S–R curve was elicited again, *during* the infusion of 5-CT. Once the S–R curve was completed, the infusion was stopped.

The Ethical Committee of the CINVESTAV-IPN (CICUAL) dealing with the use of animals in scientific experiments approved the protocols of the present investigation.

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, gallamine triethiodide, ritanserin, yohimbine hydrochloride and *N*-{2-[4-(2-methoxy-phenyl)-1-piperazinyl]ethyl}-*N*-(2-pyridinyl)cyclohexane carboxamide trihydrochloride (WAY 100635) (Sigma Chemical Co., St Louis, MO, U.S.A.); indomethacin, desipramine hydrochloride and prazosin hydrochloride (Research Biochemicals Int., Natick, MA, U.S.A.); methiothepin maleate (gift from Hoffman-La Roche Ltd,

Basel, Switzerland); tropisetron (ICS 205-930: 3α-tropanyl-1*H*-indole-3-carboxylic acid ester) (gift from Sandoz A.C., Basel, Switzerland); 5-carboxamidotryptamine maleate, sumatriptan succinate and *N*-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl) [1,1,-biphenyl]-4-carboxamide hydrochloride monohydrate (GR 127935) (gifts from GlaxoSmithKline, Stevenage, Hertfordshire, U.K.); indorenate (5-methoxytryptamine-β-methylcarboxylate hydrochloride; gift from Professor Enrique Hong, CINVESTAV-IPN, Mexico City, Mexico); [3-(1,2,5,6-tetrahydropyrid-4-yl)pyrrolo[3,2-b]pyrid-5-one] (CP 93,129) (gift from Pfizer Inc., Groton, U.S.A.); ergotamine tartrate (gift from Sandoz de Mexico, Mexico City, Mexico); *N*-[3-(dimethylamino)-2,3,4,9-tetrahydro-1*H*-carbazol-6-yl]-4-fluorobenzamide (LY344864) and *cis*-*n*-(2-hydroxycyclopentyl)-6-methyl-1-(1-methylethyl)ergoline-8-carboxamide (LY215840) (both gifts from Eli Lilly & Co., Indianapolis, IN, U.S.A.); (*S*)-(−)-3,4-dihydro-1-[2-[4-aminocarbonyl)phenyl]-1-piperazinyl]ethyl]-*N*-methyl-1*H*-2-benzopyran-6-carboxamide (PNU-142633) (gift from Dr R.B. McCall, Pharmacia & Upjohn, Kalamazoo, MI, U.S.A.) and 2,3,6,7-tetrahydro-1′-methyl-5-[2′-methyl-1,2,4-oxadiazol-3-yl) biphenyl-4-carbonyl] furo [2,3f]indole-3-spiro-4′-piperidine hydrochloride (SB224289) and 1-(3-chlorophenyl)4-[3,3-diphenyl (2-(*S*,*R*)hydroxy propanyl) piperazine] hydrochloride (BRL15572) (both gifts from Dr A.A. Parsons, GlaxoSmithKline, Harlow, Essex, U.K.).

All compounds were dissolved in saline, except: (i) GR 127935, which was dissolved according to the instructions of the supplier by the dispersion in distilled water to about 70°C for 10 s and then allowing to cool down to room temperature and (ii) ergotamine, which was initially dissolved in 10% of propylene glycol. When needed, some drops of 20% ($v\,v^{-1}$) DMSO and 20% ($v\,v^{-1}$) propylene glycol were used to dissolve, respectively, SB224289 and BRL15572, and then, the resulting solution was finally gauged with physiological saline. These vehicles had no effect on the baseline values of diastolic blood pressure or heart rate (not shown). Fresh solutions were prepared for each experiment. The doses mentioned in the text refer to the salts of substances except in the case of 5-HT, 5-CT, indorenate, CP 93,129, PNU-142633, sumatriptan, LY334864 and ergotamine, where they refer to the free base.

Data presentation and statistical evaluation

All data in the text and figures are presented as mean ± s.e.m. The peak changes in heart rate produced by electrical stimulation in the saline- and the agonist-infused animals were determined. The difference between the changes in heart rate within one subgroup of animals was evaluated by Student–Newman–Keuls' test, once a two-way repeated-measures analysis of variance (randomized block design) had revealed that the samples represented different populations (Steel & Torrie, 1980). Statistical significance was accepted at $P<0.05$ (two-tailed). The F-values, degrees of freedom and *P*-values are reported for the two factors (i.e. F_1 and P_1 refer to factor 1, the pharmacological treatment, and F_2 and P_2 refer to factor 2, the frequency of stimulation), and the subscript values in the parentheses close to F represent the degrees of freedom of each factor.

Results

Systemic haemodynamic variables

The baseline values of diastolic blood pressure and heart rate in the 151 rats were, respectively, 53 ± 2 mmHg and 281 ± 3 beats min^{-1}. After the first i.v. bolus injection of desipramine (50 μg kg^{-1}), the baseline values of the aforementioned variables significantly increased to 57 ± 3 mmHg and 287 ± 3 beats min^{-1}, respectively. The subsequent treatments with desipramine did not change the baseline values of diastolic blood pressure or heart rate.

In the different subgroups of rats pretreated with desipramine, the baseline values of diastolic blood pressure and heart rate were not significantly modified by: (i) the i.v. bolus injections of saline, methiothepin, ritanserin, tropisetron, LY215840, GR 127935, the combination of WAY 100635 plus GR 127935, SB224289, BRL15572, the combination of SB224289 plus BRL15572 or the cocktail of drugs previously described (data not shown), or (ii) the continuous infusions of saline, 5-HT, indorenate, CP 93,129, PNU-142633, sumatriptan or LY344864 (data not shown). Only 5-CT and ergotamine produced, respectively, moderate decreases and increases in blood pressure without changes in heart rate (data not shown).

Electrical stimulation of the preganglionic (C_7-T_1) cardiac sympathetic outflow

The onset of the responses induced by stimulation (0.01–3 Hz) of the preganglionic sympathetic nerves (C_7-T_1) was immediate, and resulted in frequency-dependent increases in heart rate (see below). It must be emphasized that, *in all cases*, these increases in heart rate were statistically significant when compared to baseline values ($P_2<0.001$). These tachycardiac responses are due to selective cardiostimulation, since only negligible and inconsistent increases in diastolic blood pressure were observed (data not shown), as previously shown (Gillespie *et al*., 1970; Villalón *et al*., 1999).

Effect of saline, 5-HT receptor antagonists or the cocktail of drugs on the tachycardiac responses to cardiac sympathetic nerve stimulation per se

Figure 1 shows the sympathetically induced tachycardiac responses before (control *S*–*R* curves) and after i.v. treatment with saline (1 ml kg^{-1}), methiothepin (300 μg kg^{-1}), ritanserin (100 μg kg^{-1}), tropisetron (3000 μg kg^{-1}), LY215840 (1000 μg kg^{-1}) or the cocktail of drugs consisting of yohimbine (1000 μg kg^{-1}), prazosin (100 μg kg^{-1}), ritanserin (100 μg kg^{-1}), GR 127935 (300 μg kg^{-1}), WAY 100635 (30 μg kg^{-1}) and indomethacin (5000 μg kg^{-1}). As can be observed, the sympathetically induced tachycardiac responses remained without significant changes after the i.v. administration of saline (Figure 1a; $F_{1(1,4)}=1.75$; $P_1=0.256$ and $F_{2(5,20)}=53.72$), methiothepin (Figure 1b; $F_{1(1,5)}=0.505$; $P_1=0.509$ and $F_{2(5,25)}=2.03$), ritanserin (Figure 1c; $F_{1(1,4)}=0.377$; $P_1=0.575$ and $F_{2(5,20)}=25.81$), tropisetron (Figure 1d; $F_{1(1,4)}=0.413$; $P_1=0.555$ and $F_{2(5,20)}=24.94$), LY215840 (Figure 1e; $F_{1(1,5)}=4.262$; $P_1=0.094$ and $F_{2(5,25)}=101.16$) or the cocktail of drugs (Figure 1f; $F_{1(1,5)}=1.111$; $P_1=0.340$ and $F_{2(5,25)}=38.31$). These findings indicate that the above

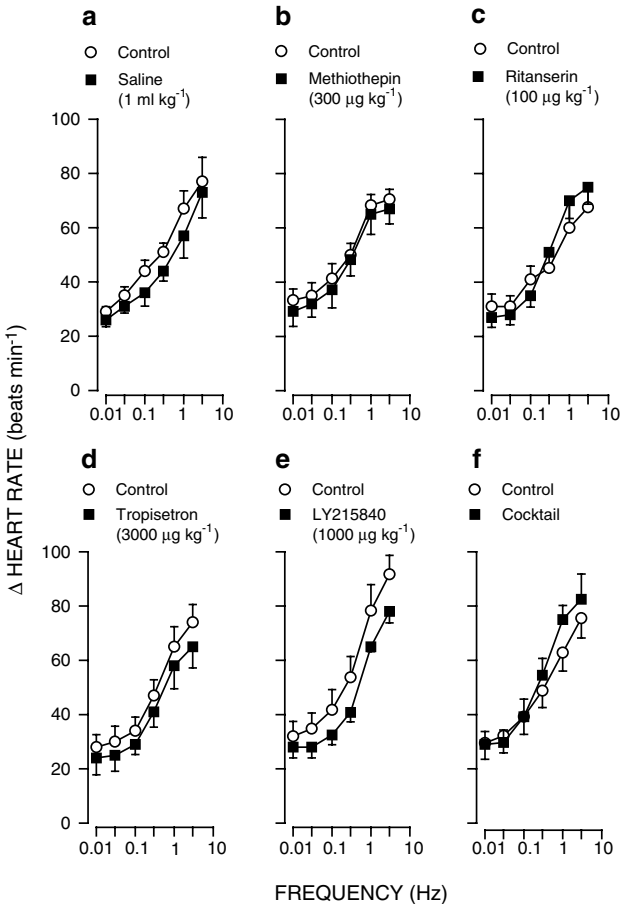

Figure 1 Effects of i.v. bolus injections of: (a) physiological saline ($n=5$), (b) methiothepin ($n=6$), (c) ritanserin ($n=5$), (d) tropisetron ($n=5$), (e) LY215840 ($n=6$) or (f) the cocktail of antagonists/inhibitors (consisting of yohimbine, 1000 $\mu g\,kg^{-1}$; prazosin, 100 $\mu g\,kg^{-1}$; ritanserin, 100 $\mu g\,kg^{-1}$; GR 127935, 300 $\mu g\,kg^{-1}$; WAY 100635, 30 $\mu g\,kg^{-1}$ and indomethacin, 5000 $\mu g\,kg^{-1}$; $n=6$) *per se* on the increases in heart rate produced by stimulation of the cardiac sympathetic outflow ($S-R$ curves). Note that there were no significant differences ($P>0.05$) in the $S-R$ curves obtained before (control) and after administration of the different compounds.

compounds were devoid of any effects on the sympathetically induced tachycardiac responses *per se*.

Effect of saline or 5-HT receptor antagonists on the 5-HT-induced inhibition of tachycardiac responses to cardiac sympathetic nerve stimulation

Figure 2 illustrates the sympathetically induced tachycardiac responses before (control $S-R$ curves) and after i.v. treatment with saline, methiothepin, ritanserin, tropisetron or LY215840 (at the doses previously indicated), respectively, followed by the infusion of 5-HT (5.6 $\mu g\,kg^{-1}\,min^{-1}$, i.v.). Consistent with previous findings in pithed rats (Villalón *et al*., 1999), the infusion of 5-HT elicited a reproducible inhibition of the sympathetically induced tachycardiac responses in the saline-treated animals (Figure 2a; $F_{1(1,4)}=40.21$; $P_1=0.0032$ and $F_{2(5,20)}=74.69$). This 5-HT-induced cardiac sympatho-inhibition was completely antagonized in the animals treated with methiothepin (Figure 2b; $F_{1(1,5)}=2.02$; $P_1=0.214$ and $F_{2(5,25)}=12.25$), but remained unaffected in those treated with

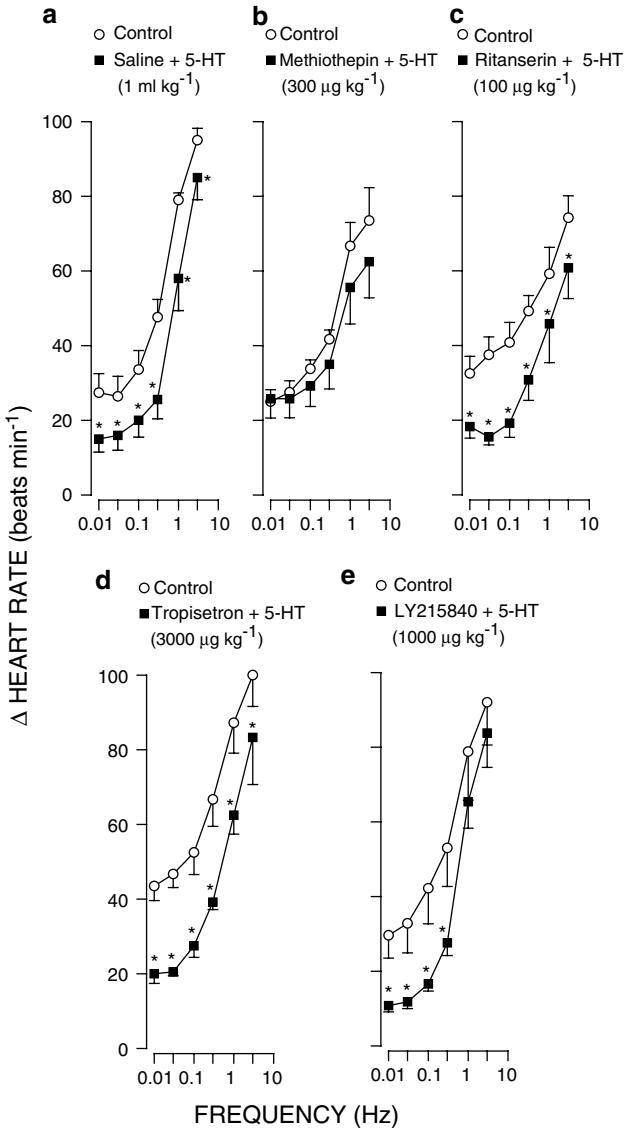

Figure 2 Effects of i.v. bolus injections of: (a) physiological saline ($n=5$), (b) methiothepin ($n=6$), (c) ritanserin ($n=6$), (d) tropisetron ($n=5$) or (e) LY215840 ($n=5$) on the inhibition of sympathetically induced tachycardiac responses induced by 5-HT (5.6 $\mu g\,kg^{-1}\,min^{-1}$ i.v.). The above compounds were injected after concluding the control $S-R$ curve, 10 min before starting the infusion of 5-HT. *$P<0.05$ vs the control response.

ritanserin (Figure 2c; $F_{1(1,5)}=75.70$; $P_1=0.0003$ and $F_{2(5,25)}=34.22$), tropisetron (Figure 2d; $F_{1(1,4)}=18.70$; $P_1=0.0124$ and $F_{2(5,20)}=25.25$) or LY215840 (Figure 2e; $F_{1(1,4)}=7.04$; $P_1=0.047$ and $F_{2(5,20)}=71.42$) in doses high enough to antagonize their respective receptors (Villalón *et al*., 1996; Centurión *et al*., 2002).

Effect of some 5-HT receptor agonists on the tachycardiac responses to cardiac sympathetic nerve stimulation

In contrast to the i.v. continuous infusions of saline (0.02 $ml\,min^{-1}$ twice; Figure 3a; $F_{1(2,6)}=2.43$; $P_1=0.169$ and $F_{2(5,15)}=182.98$), the continuous infusions of the agonists, 5-CT (0.1 and 0.3 $\mu g\,kg^{-1}\,min^{-1}$; Figure 3b; $F_{1(2,6)}=20.37$; $P_1=0.0021$ and $F_{2(5,15)}=53.48$), CP 93,129 (30 and 100 $\mu g\,kg^{-1}\,min^{-1}$;

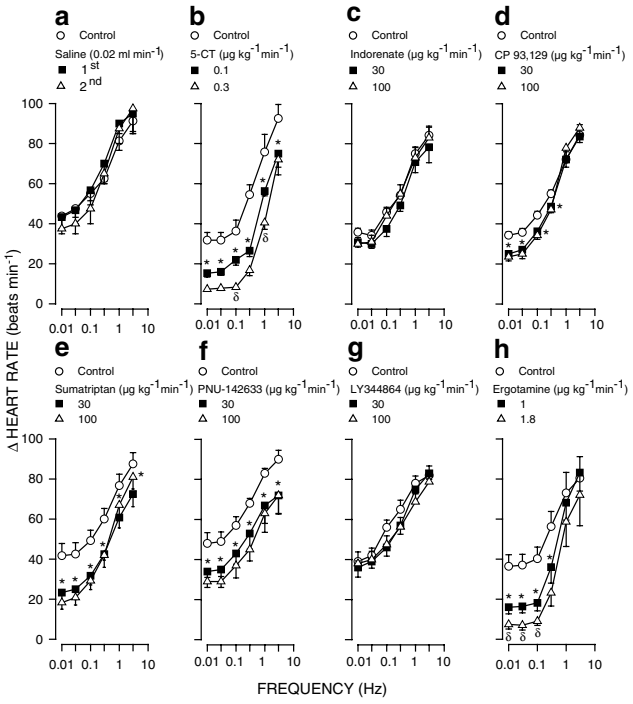

Figure 3 Increases in heart rate produced by stimulation of the cardiac sympathetic outflow ($S-R$ curves) before (control) and during i.v. continuous infusions of: (a) physiological saline ($n=4$), (b) 5-CT ($n=4$), (c) indorenate ($n=6$), (d) CP 93,129 ($n=7$), (e) sumatriptan ($n=6$), (f) PNU-142633 ($n=5$), (g) LY344864 ($n=5$) or (h) ergotamine ($n=5$). *$P<0.05$ vs the control response; all the other graphs after the starred (*) graph are also significantly different from the control response. δ, $P<0.05$ vs the response produced by the previous infusion of either 5-CT (0.1 μg kg^{-1} min^{-1}) or ergotamine (1 μg kg^{-1} min^{-1}).

Figure 3d; $F_{1(2,12)}=5.74$; $P_1=0.0174$ and $F_{2(5,30)}=186.23$), sumatriptan (30 and 100 μg kg^{-1} min^{-1}; Figure 3e; $F_{1(2,10)}=12.63$; $P_1=0.0018$ and $F_{2(5,25)}=15.27$), PNU-142633 (30 and 100 μg kg^{-1} min^{-1}; Figure 3f; $F_{1(2,8)}=10.88$; $P_1=0.0052$ and $F_{2(5,20)}=27.12$) and ergotamine (1 and 1.8 μg kg^{-1} min^{-1}; Figure 3h; $F_{1(2,8)}=17.37$; $P_1=0.0012$ and $F_{2(5,20)}=47.13$) mimicked 5-HT producing a significant inhibition of the sympathetically induced tachycardiac responses. The apparent rank order of agonist potency was 5-CT$\geqslant$ergotamine$>$5-HT$>$sumatriptan$=$PNU-142633$\geqslant$CP93,129, with both 5-CT and ergotamine being about 2 log units more potent than the rest of agonists. This sympatho-inhibition was particularly more pronounced at lower frequencies of stimulation (0.01–0.3 Hz). In contrast, the infusions of indorenate (Figure 3c; $F_{1(2,10)}=1.61$; $P_1=0.2471$ and $F_{2(5,25)}=7.40$) and LY344864 (Figure 3g; $F_{1(2,8)}=0.452$; $P_1=0.6565$ and $F_{2(5,20)}=2.12$) (30 and 100 μg kg^{-1} min^{-1} each) were inactive.

Effect of some antagonists on the 5-HT-induced inhibition of tachycardiac responses to cardiac sympathetic nerve stimulation

Figure 4 shows the sympathetically induced tachycardiac responses before (control $S-R$ curves) and after i.v. treatment with GR 127935 (300 μg kg^{-1}), the combination of WAY 100635 (30 μg kg^{-1}) plus GR 127935 (300 μg kg^{-1}) or the cocktail of drugs (see above for doses and Figure 4c), respectively, followed by the infusion of 5-HT (5.6 μg kg^{-1} min^{-1}, i.v.). Interestingly, under these experimental conditions, the 5-HT-induced cardiac sympatho-inhibition was resistant to antagonism by GR 127935 (Figure 4a; $F_{1(1,4)}=14.46$; $P_1=0.0191$ and $F_{2(5,20)}=65.78$), WAY 100635 plus GR 127935 (Figure 4b; $F_{1(1,4)}=16.24$; $P_1=0.0157$ and $F_{2(5,20)}=148.16$) or the cocktail of drugs (Figure 4c; $F_{1(1,5)}=7.66$; $P_1=0.039$ and $F_{2(5,25)}=38.20$). It should be stressed that the doses of the above compounds were high enough to block their respective receptors/mechanisms (Rosenblum & Nelson, 1988; Villalón *et al*., 1995; 1998; 1999).

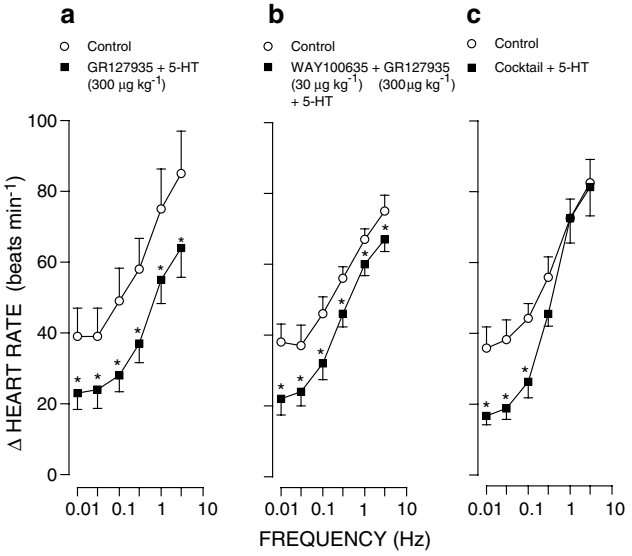

Figure 4 Effects of i.v. bolus injections of: (a) GR 127935 ($n=5$), (b) the combination of WAY 100635 plus GR 127935 ($n=5$) or (c) the cocktail of antagonists/inhibitors (consisting of yohimbine, 1000 μg kg^{-1}; prazosin, 100 μg kg^{-1}; ritanserin, 100 μg kg^{-1}; GR 127935, 300 μg kg^{-1}; WAY 100635, 30 μg kg^{-1}; and indomethacin, 5000 μg kg^{-1}; $n=6$) on the inhibition of sympathetically induced tachycardiac responses induced by 5-HT (5.6 μg kg^{-1} min^{-1} i.v.). The above compounds were injected after concluding the control $S-R$ curve, 10 min before starting the infusion of 5-HT. *$P<0.05$ vs the control response.

Effect of saline or some antagonists on the 5-CT-induced inhibition of tachycardiac responses to cardiac sympathetic nerve stimulation

Figure 5 shows the sympathetically induced tachycardiac responses before (control $S-R$ curves) and after i.v. treatment with physiological saline (1 ml kg^{-1}), methiothepin (300 μg kg^{-1}), the cocktail of drugs (see above for doses and Figure 5c), GR 127935 (300 μg kg^{-1}), SB224289 (300 μg kg^{-1}), BRL15572 (300 μg kg^{-1}) or the combination of SB224289 plus BRL15572 (300 μg kg^{-1} each), respectively, followed by the infusion of 5-CT (0.1 μg kg^{-1} min^{-1}, i.v.). Thus, the sympatho-inhibitory responses to 5-CT, which remained unaltered after saline (Figure 5a; $F_{1(1,4)}=16.44$; $P_1=0.0154$ and $F_{2(5,20)}=40.15$), were: (i) abolished after treatment with methiothepin (Figure 5b; $F_{1(1,4)}=4.69$; $P_1=0.0962$ and $F_{2(5,20)}=196.74$), the cocktail of drugs (Figure 5c; $F_{1(1,3)}=2.02$; $P_1=0.250$ and $F_{2(5,15)}=36.82$), GR 127935 (Figure 5d; $F_{1(1,3)}=1.14$; $P_1=0.3634$ and

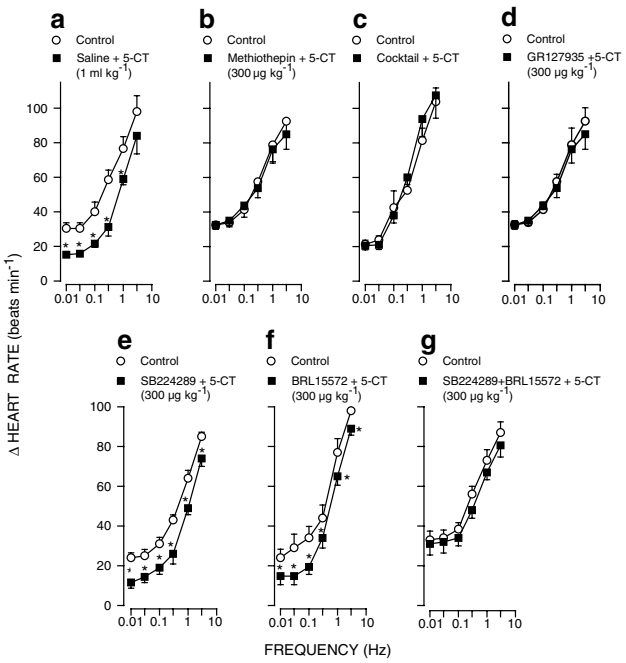

Figure 5 Effects of i.v. bolus injections of: (a) physiological saline ($n=5$), (b) methiothepin ($n=5$), (c) the cocktail of antagonists/inhibitors (consisting of yohimbine, 1000 μg kg^{-1}; prazosin, 100 μg kg^{-1}; ritanserin, 100 μg kg^{-1}; GR 127935, 300 μg kg^{-1}; WAY 100635, 30 μg kg^{-1} and indomethacin, 5000 μg kg^{-1}; $n=4$), (d) GR 127935 ($n=4$), (e) SB224289 ($n=5$), (f) BRL15572 ($n=5$) or (g) the combination of SB224289 plus BRL15572 ($n=5$) on the inhibition of sympathetically induced tachycardiac responses induced by 5-CT (0.1 μg kg^{-1} min^{-1} i.v.). The above compounds were injected after concluding the control $S-R$ curve, 10 min before starting the infusion of 5-CT. *$P<0.05$ vs the control response.

$F_{2(5,15)}=56.43$) or the combination of SB224289 plus BRL15572 (Figure 5g; $F_{1(1,4)}=1.40$; $P_1=0.3024$ and $F_{2(5,20)}=132.01$) at the same doses previously used with 5-HT (see above), and (ii) resistant to blockade after treatment with SB224289 (Figure 5e; $F_{1(1,4)}=45.50$; $P_1=0.025$ and $F_{2(5,20)}=103.06$) or BRL15572 (Figure 5f; $F_{1(1,4)}=8.99$; $P_1=0.040$ and $F_{2(5,25)}=186.084$) at doses high enough to block, respectively, 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors producing sympatho-inhibition (Villalón *et al*., 2001).

Discussion

General

Apart from the implications discussed below, our study shows that cardiac sympatho-inhibition to 5-HT in pithed rats is most probably mediated by two main sympatho-inhibitory mechanisms, namely: (i) 5-HT$_1$ receptors and (ii) a novel mechanism involving putative 5-ht$_{5A/5B}$ receptors. For this reason, from now on, 5-CT, ergotamine and methiothepin will be described as compounds with activity at 5-HT$_{1B/1D}$ and recombinant 5-ht$_{5A/5B}$ receptors (see Table 1).

Systemic haemodynamic changes

The fact that diastolic blood pressure and heart rate were significantly increased after desipramine can be attributed to an inhibition of noradrenaline uptake (Bechtel *et al*., 1986). Moreover, the potentiation of the sympathetically induced tachycardiac responses after desipramine (for comparison, see Villalón *et al*., 1995; 1999) has relevance for the purpose of the present study. Thus, the cardiac sympatho-inhibitory effects of 5-HT (see Figure 2a) were, coincidentally, more pronounced at lower frequencies of stimulation (see Langer, 1980). However, it could be alternatively argued that the proposed sympatho-inhibitory action of 5-HT (Figure 2) and related agonists (Figure 3) may have been due to tachyphylaxis of the sympathetically induced tachycardiac responses. However, this is unlikely since such responses remained unchanged after i.v. saline (Figures 1a and 3a); an additional conclusion drawn from this is that no time-dependent changes occurred in our experiments.

The fact that the sympathetically induced tachycardiac responses remained essentially unchanged after i.v. saline, methiothepin, ritanserin, tropisetron, LY215840 or the cocktail of drugs (see Figure 1) indicates that these compounds were devoid of any effects on the above tachycardiac responses *per se*. These findings, coupled to the lack of effect of the above compounds on baseline diastolic blood pressure and heart rate (not shown), suggest that any effect of a given antagonist on 5-HT-induced cardiac sympatho-inhibition is due to a direct interaction of the antagonist with its respective receptors on the cardiac sympathetic nerves.

Pharmacological profile of the cardiac sympatho-inhibitory action of 5-HT in the rat

Taken collectively, our findings support the notion that 5-HT$_1$ (but not 5-HT$_2$, 5-HT$_3$, 5-HT$_4$, 5-ht$_6$ and 5-HT$_7$) receptors may mediate, at least in part, the cardiac sympatho-inhibitory action to 5-HT, since this response was: (i) resistant to blockade by ritanserin, tropisetron and LY215840 (see Figure 2) at doses that are sufficient to antagonize, respectively, 5-HT$_2$ (Centurión *et al*., 2002), 5-HT$_{3/4}$ (Villalón *et al*., 1990; 1991) and 5-HT$_7$ (Centurión *et al*., 2000) receptors mediating cardiovascular responses and (ii) antagonized by methiothepin (Figure 2b) at doses that are sufficient to antagonize '5-HT$_1$-like' receptors (Saxena & Villalón, 1990; 1991; Villalón *et al*., 1995). Nevertheless, based on the current classification scheme of 5-HT receptors (see Hoyer *et al*., 1994; Saxena *et al*., 1998), the above notion supporting the involvement of methiothepin-sensitive 5-HT$_1$ receptors is admittedly weak, since: (i) the 5-HT$_1$ receptor family is highly heterogeneous and includes 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1D}$, 5-ht$_{1E}$ and 5-ht$_{1F}$ receptor subtypes and (ii) methiothepin blocks, in addition to all of these 5-HT$_1$ subtypes, recombinant 5-ht$_{5A/5B}$ receptors. Evidently, the use of selective agonists and antagonists at the different 5-HT$_1$ receptor subtypes (see below) becomes crucial to confirm the above notion.

Apparent rank order of potency of some agonists causing cardiac sympatho-inhibition and its blockade by some antagonists

Our results show that 5-HT-induced cardiac sympatho-inhibition cannot be: (i) mimicked by indorenate or LY344864 (see Figure 3) which are agonists at, respectively, 5-HT$_{1A}$ (Dompert *et al*., 1985) and 5-ht$_{1F}$ (Phebus *et al*., 1997) receptors and (ii) blocked by WAY 100635 (see Figure 4b and c), a potent

5-HT_{1A} receptor antagonist (Fletcher *et al*., 1996), at doses sufficient to antagonize sympatho-inhibitory 5-HT_{1A} receptors (Villalón *et al*., 1998). Hence, these results strongly suggest that 5-HT_{1A} and 5-ht_{1F} receptors are not involved.

In contrast, the 5-HT receptor agonists, 5-CT, ergotamine (both 5-$HT_{1B/1D}$ and recombinant 5-$ht_{5A/5B}$; Hoyer *et al*., 1994), CP 93,129 (r5-HT_{1B}; Macor *et al*., 1990), sumatriptan (5-$HT_{1B/1D}$; Hoyer *et al*., 1994) and PNU-142633 (5-HT_{1D}; Pregenzer *et al*., 1999), which failed to modify the tachycardiac responses to exogenous noradrenaline (data not shown), mimicked the sympatho-inhibition to 5-HT with an apparent rank order of agonist potency of 5-CT $\geqslant$ ergotamine $>$ 5-HT $>$ sumatriptan = PNU-142633 $\geqslant$ CP 93,129. Pending the use of selective antagonists, this suggests a possible correlation with the 5-HT_{1B} and 5-HT_{1D} receptor subtypes. Several possible explanations for these differences in agonist potencies may include: (i) the use of 'second messengers' and functional (cardiac sympatho-inhibition) responses; (ii) tissue-dependent factors such as the density of receptors and coupling efficiency and/or (iii) drug-dependent factors such as the affinity of 5-HT and related agonists for each of these receptors (i.e. 5-HT_{1B}, 5-HT_{1D} and 5-$ht_{5A/5B}$; see Table 1).

The possible involvement of 5-$HT_{1B/1D}$ receptors gains weight by the fact that 5-CT-induced sympatho-inhibition was abolished by: (i) the 5-$HT_{1B/1D}$ receptor antagonist GR 127935 (Skingle *et al*., 1996) (see Figure 5c and d) at doses that antagonize sympatho-inhibitory 5-$HT_{1B/1D}$ receptors (Villalón *et al*., 1998) and (ii) the combination of SB224289 plus BRL15572 (see Figure 5g), which are selective antagonists at, respectively, 5-HT_{1B} and 5-HT_{1D} receptors (Hagan *et al*., 1997) at doses that completely block 5-HT_{1B} and 5-HT_{1D} receptors *in vivo* (De Vries *et al*., 1998). Interestingly, the fact that SB224289 alone apparently failed to block the responses to 5-CT (see Figure 5e) may be explained by the additional capability of 5-CT to stimulate 5-HT_{1D} (and probably also 5-$ht_{5A/5B}$) receptors; this may have overshadowed the selective antagonism of 5-HT_{1B} receptors with SB224289. A similar line of reasoning may apply to the selective antagonism of 5-HT_{1D} receptors with BRL15572 alone (Figure 5f). This led us to hypothesize that if the sympatho-inhibitory responses to 5-CT involve the stimulation of two (or even three) receptors, the antagonism of *at least* two of them (with GR 127935 or the combination of SB224289 plus BRL15572) would be mandatory to produce a complete blockade. This view is reinforced by the fact that: (i) methiothepin (which antagonizes 5-$HT_{1B/1D}$ and recombinant 5-$ht_{5A/5B}$ receptors) also abolished the responses to 5-CT (see Figure 5b) and (ii) the cardiac sympatho-inhibition to the selective agonists CP 93,129 (5-HT_{1B}) and PNU-142633 (5-HT_{1D}) are completely and specifically blocked by, respectively, SB224289 (5-HT_{1B}) and BRL15572 (5-HT_{1D}) (Villalón *et al*., 2000). Admittedly, 5-HT displays high affinity for 5-ht_{1E} (and 5-ht_{1F}) receptors; however, their involvement seems unlikely as 5-CT displays a very low affinity for 5-ht_{1E} (and 5-ht_{1F}) receptors (Adham *et al*., 1993).

Apparent failure of GR 127935, the combination of WAY 100635 plus GR 127935 or the cocktail of drugs to antagonize the 5-HT-induced cardiac sympatho-inhibition

In order to confirm the previously suggested involvement of GR 127935-sensitive 5-$HT_{1B/1D}$ receptors with 5-HT, it was decided to analyse, in principle, the effect of GR 127935 on 5-HT-induced cardiac sympatho-inhibition. Unexpectedly, and contrasting with methiothepin (Figure 2b), GR 127935 given alone (Figure 4a) or in combination with WAY 100635 (Figure 4b) failed to antagonize the response to 5-HT at doses high enough to completely antagonize, respectively, the 5-$HT_{1B/1D}$ and 5-HT_{1A} receptors (Villalón *et al*., 1998). In view of this lack of blockade by GR 127935, we considered it unnecessary to investigate the potential blockade of 5-HT-induced sympatho-inhibition by SB224289 and/or BRL15572. Although the simplest interpretation of these findings would apparently suggest that 5-$HT_{1B/1D}$ receptors are not involved, it should be kept in mind that 5-HT, as an endogenous ligand, can stimulate a wide variety of known (5-HT_1-5-HT_7) and unknown (orphan, novel, unclassified) receptors. Therefore, it is reasonable to assume that, besides 5-$HT_{1B/1D}$ receptors, 5-HT-induced sympatho-inhibition involves the additional participation of a novel mechanism which overshadows the blockade of 5-$HT_{1B/1D}$ receptors with GR 127935. This novel mechanism, as discussed below, seemingly displays the pharmacological profile of putative 5-$ht_{5A/5B}$ receptors.

Possible coinvolvement of 5-$HT_{1B/1D}$ and putative 5-$ht_{5A/5B}$ receptors in the 5-HT-induced cardiac sympatho-inhibition

Our suggestion that 5-HT-induced sympatho-inhibition involves the additional participation of a novel mechanism (probably resembling putative 5-$ht_{5A/5B}$ receptors) is reinforced when considering that: (i) methiothepin, which displays a high affinity for recombinant 5-$ht_{5A/5B}$ and 5-$HT_{1B/1D}$ receptors (see Table 1), was *the only antagonist* that abolished 5-HT-induced sympatho-inhibition (compare Figures 2 and 4); (ii) GR127935, which displays a high affinity for 5-$HT_{1B/1D}$ receptors, but low affinity for recombinant 5-ht_{5A} receptors (see Table 1), abolished the sympatho-inhibition to 5-CT, *but not that to 5-HT*, and (iii) 5-CT, methiothepin and ergotamine display a relatively high affinity for recombinant 5-ht_{5A} and 5-ht_{5B} receptors in addition to 5-$HT_{1B/1D}$ receptors (see Table 1). Consistent with these findings, Figure 3 reveals that the sympatho-inhibitory potencies of 5-CT and ergotamine (both with high affinities at 5-$HT_{1B/1D}$ and recombinant 5-$ht_{5A/5B}$ receptors) were about 2 log units higher than that of CP 93,129, sumatriptan or PNU-142633 (selective 5-HT_1 receptor agonists) which display low affinities for 5-$ht_{5A/5B}$ receptors (see Table 1).

Additional evidence in favour of the possible coinvolvement of putative 5-$ht_{5A/5B}$ receptors

Since, as previously pointed out, methiothepin is a nonselective drug, an attempt was made to cover as much as possible its spectrum of blockade (except the blockade of putative 5-$ht_{5A/5B}$ receptors), by studying the effects of the cocktail of drugs (see Experimental protocol) on 5-HT-induced cardiac sympatho-inhibition. Nevertheless, this response remained un-altered after the cocktail (Figure 4c), a finding which demonstrates that simultaneous antagonism/inhibition of the above receptors/mechanisms *does not* play a role in the antagonism produced by methiothepin (Figure 2b). The fact that this cocktail abolished the sympatho-inhibition to 5-CT (Figure 5c) is attributable to the dose of GR 127935 present in it (see above). It is to be highlighted that the doses of these drugs in the cocktail were high enough to antagonize/inhibit, respectively, $\alpha_{1/2}$-adreno-

ceptors (Willems *et al*., 2001a, b), 5-HT_{1A} receptors (Villalón *et al*., 1998), 5-$HT_{1B/1D}$ receptors (Villalón *et al*., 1996; 1998), 5-HT_2 receptors (Centurión *et al*., 2002) and cyclooxygenase (Rosenblum & Nelson, 1988). Obviously, these findings exclude the involvement of $\alpha_{1/2}$-adrenoceptors (Rand *et al*., 1987; Boehm & Kubista, 2002) and the release of prostanoids (Kokkas & Boeynaems, 1988) in our experimental model.

Exclusion of the involvement of 5-ht$_6$ and 5-HT$_7$ receptors

Despite the above lines of pharmacological evidence, it could still be argued that 5-ht_6 and 5-HT_7 receptors may be involved, as methiothepin (which abolished 5-HT- and 5-CT-induced sympatho-inhibition; see Figures 2b and 5b) displays high affinity for both receptors (Plassat *et al*., 1993). However, this seems unlikely, based on: (i) the inactivity of sumatriptan, CP 93,129 and PNU-142633 (as agonists or antagonists) at 5-ht_6 and 5-HT_7 receptors and (ii) the apparent rank order of agonist potency of 5-HT and 5-CT at 5-ht_6 (5-HT > 5-CT) and 5-HT_7 (5-CT $\gg$ 5-HT) receptors (see Hoyer *et al*., 1994; Villalón *et al*., 1997).

Transductional evidence in favour of the involvement of 5-HT$_1$ and 5-ht$_{5A}$ receptors

Admittedly, our study does not provide direct evidence that the sympatho-inhibition to 5-HT and the rest of agonists involves inhibition of adenylyl cyclase. Nevertheless, it is worthy of note that the 5-HT_1 receptor subtypes (Hoyer *et al*., 1994) as well as the 5-ht_{5A} receptor (Carson *et al*., 1996; Grailhe *et al*., 2001) are negatively coupled to adenylyl cyclase and/or positively coupled to the inwardly rectifying K^+ channel; these are signal transduction systems usually associated with sympatho-inhibition (Langer, 1980; Rand *et al*., 1987; Boehm & Kubista, 2002). In marked contrast, 5-HT_4, 5-ht_6 and 5-HT_7 receptors are, by definition, positively coupled to adenylyl cyclase (Plassat *et al*., 1993; Hoyer *et al*., 1994), and this is associated with sympatho-excitation (Langer, 1980; Rand *et al*., 1987; Boehm & Kubista, 2002). Similarly, activation of 5-HT_2 (positively coupled to phospholipase C) and 5-HT_3 (coupled to ligand-operated Na^+/K^+channels which trigger a rapid neuronal depolarization) receptors is associated with increases in the cytosolic concentration of Ca^{2+}, and a consequent stimulation (rather than inhibition) of the release of neurotransmitters (Rand *et al*., 1987; Boehm & Kubista, 2002).

Possible locus of the cardiac sympatho-inhibitory 5-HT$_{1B/1D}$ and putative 5-ht$_{5A/5B}$ receptors

Although central mechanisms are not operative in our experimental model since pithed rats were used, we cannot exclude an action of 5-HT and related agonists at both the sympathetic ganglia (see Fozard, 1984) and postganglionic sympathetic neurons (see Saxena & Villalón, 1990; 1991) which have modulatory 5-HT receptors. Indeed, 5-HT_1 receptors may inhibit the sympathetic ganglionic transmission in rats (5-HT_{1A} subtype; Ireland & Jordan, 1987) or cats (5-$HT_{1B/1D}$ subtypes; Jones *et al*., 1995). With respect to postganglionic sympathetic neurons, prejunctional 5-HT_{1D} receptors mediate the inhibition of noradrenaline release in human atrium (Molderings *et al*., 1996); in contrast, 5-HT_{1B} receptors mediate this response in rat vena cava (Molderings *et al*., 1987). Admittedly, further studies in rats will be required to ascertain the functional role of 5-$HT_{1B/1D}$ and putative 5-$ht_{5A/5B}$ receptors on ganglia and postganglionic sympathetic neurons.

In conclusion, the above results suggest that 5-HT-induced cardiac sympatho-inhibition in pithed rats is primarily mediated by two mechanisms, namely: (i) GR 127935-sensitive 5-$HT_{1B/1D}$ receptors and (ii) a novel (methiothepin-sensitive, but GR 127935-resistant) mechanism most probably involving putative 5-$ht_{5A/5B}$ receptors. Pending the advent of selective ligands at 5-$ht_{5A/5B}$ receptors, our results would seem to be the first evidence showing a functional role of putative 5-$ht_{5A/5B}$ receptors in the peripheral nervous system, contrasting with its well-established distribution in the central nervous system (Grailhe *et al*., 2001).

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