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The relaxant 5-HT receptor in the dog coronary artery smooth muscle: pharmacological resemblance to the cloned 5-ht$_7$ receptor subtype

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1 The relaxant effect of 5-hydroxytryptamine (5-HT) in the dog isolated coronary artery deprived of endothelium is mediated by a receptor unrelated to the 5-HT$_1$, 5-HT$_2$, 5-HT$_3$ or 5-HT$_4$ types. Based upon the pharmacological characteristics of this relaxant 5-HT receptor and those reported for the new members of the 5-HT receptor family, the present study explored the possibility that the relaxant 5-HT receptor referred to above, corresponds to the cloned 5-ht$_7$ subtype. Thus, the relaxing and/or blocking effects of several 5-HT receptor drugs as well as some typical and atypical antipsychotic drugs with high affinity for the cloned 5-ht$_7$ receptor in precontracted ring segments were analyzed.

2 5-HT, 5-carboxamidotryptamine (5-CT) and 5-methoxytryptamine, but not 8-OH-DPAT or sumatriptan, produced concentration-dependent relaxations in endothelium-denuded canine coronary artery rings precontracted with prostaglandin $F_{2\alpha}$ (2 μM). Clozapine (1 μM) produced in some cases a small relaxing effect and antagonized 5-HT- and 5-CT-induced relaxation suggesting a partial agonist effect. In the presence of the 5-HT$_{1D}$ receptor antagonist, GR127935 (100 nM), the rank order of agonist potency was 5-CT > 5-HT > clozapine $\geqslant$ 5-methoxytryptamine. 8-OH-DPAT and sumatriptan remained inactive as agonists.

3 In GR127935-treated preparations, methiothepin (3 nM) and mianserin (1 μM), as well as the antipsychotics, clozapine (1 μM), pimozide (300 nM), risperidone (3 nM) and spiperone (1 μM), failed to induce a significant relaxation in prostaglandin $F_{2\alpha}$-precontracted vessels, but produced significant rightward displacements of the concentration-response curves to 5-HT and 5-CT without significantly reducing the E_{max}. In a final set of experiments with 5-CT, metergoline (100 nM) and mesulergine (300 nM) behaved as competitive antagonists. In contrast, lisuride (3 nM) noncompetitively antagonized 5-CT-induced relaxation. The estimated affinity (apparent pK_B values) of the above antagonist drugs for the relaxant 5-HT receptor significantly correlated with their reported affinity at the cloned 5-ht$_7$ receptor.

4 Taken together, the above pharmacological data may suggest that the relaxant 5-HT receptor in the smooth muscle of the canine coronary artery is similar to the cloned 5-ht$_7$ receptor subtype.

Keywords: Antipsychotic drugs; canine coronary artery; 5-hydroxytryptamine; 5-ht$_7$ receptor; relaxation; vascular smooth muscle

Introduction

It has been shown that 5-hydroxytryptamine (5-HT), in addition to producing contraction, also induces, at high concentrations, endothelium-independent relaxation in the canine coronary artery (Houston & Vanhoutte, 1988; Cushing & Cohen, 1992a). Based upon the low potency of 5-HT and the failure of ketanserin to block 5-HT-induced relaxation, it was stressed that the relaxant 5-HT receptor did not conform to the 5-HT$_1$-like or 5-HT$_2$ categories (Houston & Vanhoutte, 1988). Further pharmacological data using a wide range of drugs with relative selectivity for the 5-HT$_1$-like, 5-HT$_2$, 5-HT$_3$ and 5-HT$_4$ receptors confirmed the above observations and suggested that the relaxant 5-HT receptor is dissimilar to these main types (Cushing & Cohen, 1992a).

Although the above relaxant 5-HT receptor displays some 5-HT$_1$-like characteristics such as the high agonist potency of 5-carboxamidotryptamine (5-CT; Toda & Okamura, 1990; Cushing & Cohen, 1992a) and the high antagonist potency of methiothepin (Houston & Vanhoutte, 1988), the low potency of 5-HT in conjunction with the failure of sumatriptan to produce relaxation can be regarded as one distinctive operational feature of this relaxant 5-HT receptor (Cushing & Cohen, 1992a).

Taking into consideration the recent classification scheme of 5-HT receptors which includes several additional members of the 5-HT receptor family such as the 5-ht$_{5A/B}$, 5-ht$_6$ and 5-ht$_7$ subtypes (see Hoyer *et al.*, 1994 for review), one could suggest that the 5-HT receptor mediating relaxation in the dog coronary artery smooth muscle may correspond to one of these recent subtypes. Indeed, the cloned 5-ht$_7$ receptor exhibits a pharmacological profile (high affinity for 5-CT and methiothepin, 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) closely resembling that of the above relaxant 5-HT receptor (Houston & Vanhoutte, 1988; Cushing & Cohen, 1992a).

On the basis of the above, the present study investigated the possibility that the relaxant 5-HT receptor in the smooth muscle of the canine coronary artery corresponds to the cloned 5-ht$_7$ receptor subtype. For this purpose, the effects of several 5-HT receptor agonists and antagonists displaying high affinity for this novel receptor subtype, including some typical and atypical antipsychotic drugs (Roth *et al.*, 1994), in endothelium-denuded canine coronary artery rings were analyzed. Since 5-HT and other 5-HT receptor agonists produce contraction in this tissue via stimulation of vascular 5-HT$_{1D}$ receptors (Cushing & Cohen, 1992b; Cushing *et al.*, 1994; Terrón, 1996a), the experiments with antagonists were conducted in the presence of GR127935, a potent and selective 5-HT$_{1D}$ receptor antagonist (Skingle *et al.*, 1993). Preliminary results of this investigation have been communicated to the Western Pharmacology Society (Terrón, 1996b).

Methods

Tissue preparation

A total of 32 mongrel dogs (15–25 kg) of either sex were anaesthetized with sodium pentobarbitone (30 mg kg^{-1}) and killed by rapid exsanguination from the carotid arteries. Hearts were removed and the circumflex coronary arteries were placed in Krebs bicarbonate solution of the following composition (millimolar concentrations): NaCl 118, KCl 4.8, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 24, KH_2PO_4 1.2, dextrose 11 and Ca_2EDTA 0.026. The vessels were cleaned of connective tissue and adherent fat and cut into ring segments, 4 mm in length. Up to eight adjacent rings from the same vessel were used as experimental and control rings. In order to remove the endothelium, a specially designed nikrom stick with a rough surface and sharp tip was inserted into the lumen of each ring so that the intimal surface could be rubbed gently.

Organ chamber studies

The rings were suspended horizontally in a jacketed organ bath by two stainless-nikrom wire hooks; the lower hook was connected by a clamp to a tissue holder whilst the upper hook was connected directly to an isometric force displacement transducer (Grass FT03C) so that isometric changes in force could be recorded on a Grass model 7D Polygraph. The rings were mounted in organ chambers containing 10 ml of Krebs bicarbonate solution maintained at 37°C and aerated continuously with 95% O_2 and 5% CO_2 to give pH 7.4. Tissues were gradually stretched over a 90 min period to a tension of 10 g. This has been found to be optimal for contraction of the coronary artery by testing repeated contractile responses to potassium chloride (KCl, 20 mM; Cohen *et al.*, 1983; Houston & Vanhoutte, 1988; Terrón, 1996a). Rings were contracted 2 or 3 times by depolarization with KCl (20 mM) until contractions were reproducible; a final depolarization with KCl (40 mM), which preliminary experiments had shown to evoke a submaximal contractile response, was elicited. After repetitive washout every 15 min, the ring segments were contracted with prostaglandin $F_{2\alpha}$ (2 μM) and tested for the absence of functional endothelium by exposure to acetylcholine (1 μM). Those rings that relaxed to acetylcholine were not used for the study. As some of the results with the dog coronary artery have suggested that the responses to 5-HT are susceptible to the neuronal uptake process (Cohen, 1986), the experiments with this monamine were conducted in the presence of desipramine (1 μM) and deoxycorticosterone (10 μM) to inhibit neuronal and extraneuronal uptake, respectively.

Experimental protocols

Once the tissues were equilibrated and tested for viability with KCl, three protocols were followed. The first protocol was designed to evaluate the relaxant activity of 5-HT (10 nM–100 μM), 5-CT (1 nM–10 μM), 5-methoxytryptamine (100 nM–1 mM), 8-OH-DPAT (1 nM–100 μM) and sumatriptan (1 nM–100 μM) in rings preincubated with either vehicle or a high concentration (100 nM) of the 5-HT$_{1D}$ receptor antagonist, GR127935, to ensure complete blockade of the contractile 5-HT$_{1D}$ receptors (Terrón, 1996a). Thus, after 30 min of equilibration with GR127935, the preparations were contracted with prostaglandin $F_{2\alpha}$ (2 μM). In the plateau phase of the contraction, which developed in about 30 min, the agonists were added cumulatively so that complete concentration-response (C–R) curves could be obtained. Therefore, the incubation time with vehicle or GR127935 approximated 60 min. It should be emphasized that during the interaction experiments in which the potential ability of several 5-HT receptor antagonists and some antipsychotic drugs to block agonist-induced relaxations was evaluated (see below), it was noticed that, in two experiments, clozapine (1 μM) produced a small relaxant effect, accounting for about 5% of the prostaglandin $F_{2\alpha}$ contraction. Therefore, in other experiments, the effects of higher concentrations of clozapine were elicited in order to obtain complete C–R curves. The effect of GR127935 (100 nM) on clozapine-induced responses was also evaluated.

The second protocol explored the effects of methiothepin (3 nM), mianserin (1 μM) or ketanserin (1 μM) as well as those of the antipsychotics, pimozide (30 nM), spiperone (1 μM), clozapine (1 μM) or risperidone (3 nM) on the relaxation induced by 5-HT and 5-CT. These experiments were conducted in the presence of GR127935 (100 nM). Then, cumulative C–R curves for 5-HT and 5-CT were generated in tissues incubated with either vehicle or antagonist for 1 h. These experiments with antagonists were performed in parallel with the controls. It should be noted that in some experiments each antagonist was added during the plateau phase of the contraction to prostaglandin $F_{2\alpha}$ so that the potential ability of these drugs to produce relaxation could be verified. Each concentration of 5-HT and 5-CT (spaced by a factor of $10^{1/2}$) was added only after the maximum response to the previous concentration had been attained. Responses to 5-HT and 5-CT in vehicle- and antagonist-treated tissues were elicited in separate rings so that only one C–R curve was obtained in each tissue. Lastly, the effects of metergoline (100 nM), mesulergine (300 nM) and lisuride (3 nM) on the relaxation induced by 5-CT were analyzed. The effects of these drugs on the responses produced by 5-HT were not evaluated because of the limited quantity of dogs available.

Data presentation and statistical evaluation

All data in the text and figures are expressed as the mean ± s.e.mean, where n represents the number of dogs from which the vessels were taken. In order to restrict the number of dogs used in the present study, no more than one tissue was used from each animal for any given treatment. Changes in tension are expressed as percentage of the contraction to prostaglandin $F_{2\alpha}$ (2 μM). Comparisons between vehicle- and antagonist-treated rings obtained from the same animal were performed in separate tissues and no tissue was used to generate more than one agonist C–R curve. The pD_2 values (negative logarithm of EC_{50}, the agonist concentration producing 50% of the maximum relaxant response, calculated by nonlinear regression analysis) and the maximum response (E_{max}) were determined from individual C–R curves.

Apparent antagonist dissociation constants (K_B) were determined for each antagonist according to the following equation:

$$K_B = [B]/(\text{dose ratio} - 1)$$

where [B] is the concentration of the antagonist and the dose ratio is the EC_{50} of the agonist in the presence of the antagonist divided by the EC_{50} of the agonist in vehicle-treated tissues. These results were then expressed as the negative logarithm of K_B ($-\log K_B = pK_B$).

Significant differences between values of pD_2 and E_{max} obtained in vehicle- and antagonist-treated preparations were determined with a Student's t test (Steel & Torrie, 1980). In an attempt to corroborate the nature of the antagonism in the interaction experiments, tests for parallel lines were applied to the linear portion of the C–R curves obtained with vehicle- and antagonist-treated tissues (Tallarida *et al.*, 1979). In all cases a P value of 0.05 or less (two-tailed) was considered statistically significant.

Drugs

Apart from the anaesthetic (sodium pentobarbitone), the drugs used in the present study (obtained from the sources indicated) were the following: 5-hydroxytryptamine creatinine sulphate, deoxycorticosterone and prostaglandin $F_{2\alpha}$ (Sigma Chemical Company, St. Louis, MO, U.S.A.); acetylcholine chloride, desipramine hydrochloride, (±)-8-hydroxy-dipropylaminotetralin hydrobromide (8-OH-DPAT) and lisuride hydrogen

maleate (Research Biochemicals Int., Natick, MA, U.S.A.); 5-carboxamidotryptamine maleate, N-[4-methoxy-3-(4-methyl-1-piperazinyl) phenyl]-2'-methyl-4'-5(-methyl-1,2,4-oxadiazol-3-yl)[1,1,-biphenyl]-4-carboxamidehydrochloride monohydrate (GR127935) and sumatriptan succinate (gift: Glaxo Group Research, Ware, U.K.); pimozide, risperidone and spiperone (gift: Janssen Pharmaceutica, Beerse, Belgium); methiothepin maleate (gift: Hoffman-La Roche Ltd., Basel, Switzerland); metergoline (gift: Farmitalia, Milan, Italy); mianserin hydrochloride (gift: Organon de México, Mexico city); and clozapine and mesulergine (gift: Sandoz A.G., Basel, Switzerland).

All compounds were dissolved in distilled water. When needed, 4% ascorbic acid (clozapine and metergoline) or 5% (v/v) propylene glycol (deoxycorticosterone) or dimethylsulphoxide (DMSO) (lisuride methiothepin, pimozide, risperidone and spiperone) was added. Fresh solutions were prepared for each experiment and vehicles had no effect on baseline tension or agonist-induced responses.

Results

Initial effects of agonist drugs in $PGF_{2\alpha}$-precontracted vessels

As previously noticed (Cushing & Cohen, 1992a), 5-HT, 5-CT and 5-methoxytryptamine (Figure 1), but not 8-OH-DPAT or sumatriptan ($n=3$ each; not shown), produced concentration-dependent relaxations in endothelium-denuded dog coronary artery rings. The responses to the relaxant agonists were biphasic i.e. they produced a small vasoconstriction followed by a more intense relaxation (Figure 1). Interestingly, the atypical antipsychotic drug, clozapine, mimicked the relaxing effects of 5-HT with lower potency and a slightly, though not significantly, lower efficacy. Thus, the rank order of agonist potency was 5-CT > 5-HT > clozapine ≥ 5-methoxytryptamine (Figure 1; see below).

Effects of GR127935 on agonist-induced responses

Incubation of endothelium-denuded prostaglandin $F_{2\alpha}$-precontracted vessels with the 5-HT_{1D} receptor antagonist, GR127935 (100 nM), not only abolished the initial vasoconstrictor phase of the response of 5-HT, 5-CT and 5-methoxytryptamine, but also increased significantly the potency of 5-HT ($pD_2 = 5.94 \pm 0.15$ and 6.7 ± 0.18 without and with GR127935, respectively; $n=4$) and 5-CT ($pD_2 = 7.04 \pm 0.15$ and 7.64 ± 0.13 without and with GR127935, respectively; $n=5$). The E_{max} of 5-HT and 5-CT was also significantly increased by GR127935 (see Figure 1). Although a similar pattern was observed with 5-methoxytryptamine, which apparently displayed a higher relaxing efficacy than 5-HT and 5-CT, no significant increase in the potency ($pD_2 = 4.42 \pm 0.26$ and 4.58 ± 0.02 without and with GR127935, respectively; $n=3$) and the E_{max} were observed as a result of preincubation with GR127935 (Figure 1). In contrast to these findings, neither the potency ($pD_2 = 5.13 \pm 0.07$ and 4.88 ± 0.29 without and with GR127935, respectively; $n=3$) nor the E_{max} of clozapine was modified by GR127935 (Figure 1).

Thus, these data clearly indicate that once the contractile 5-HT_{1D} receptors are selectively blocked with GR127935 (Terrón, 1996a), a full relaxing effect can be unmasked so that the actual and most accurate potency of the relaxant 5-HT receptor agonists can be determined. On these bases, the experiments with antagonist drugs were conducted in the presence of a high concentration (100 nM) of GR127935. The blockade of 5-HT_{1D} receptors did not alter the rank order of agonist potency. In addition, no relaxing activity of 8-OH-DPAT or sumatriptan could be unmasked by GR127935 (not shown).

Effects of methiothepin, mianserin and ketanserin on 5-HT- and 5-CT-induced relaxation

The effects of methiothepin, mianserin and ketanserin on the relaxant responses induced by 5-HT and 5-CT in rings precontracted with prostaglandin $F_{2\alpha}$ (2 μM) and preincubated with GR127935 (100 nM) are depicted in Figure 2a, b and c, respectively. It should be remarked that 5-HT and 5-CT were selected on the basis of their higher relaxing potency. Unlike ketanserin (1 μM), incubation with methiothepin (3 nM) and mianserin (1 μM), produced a rightward shift of the C–R curves for 5-HT and 5-CT. None of these antagonists produced relaxation when added to precontracted vessels. The finding that these antagonists did not significantly reduce the

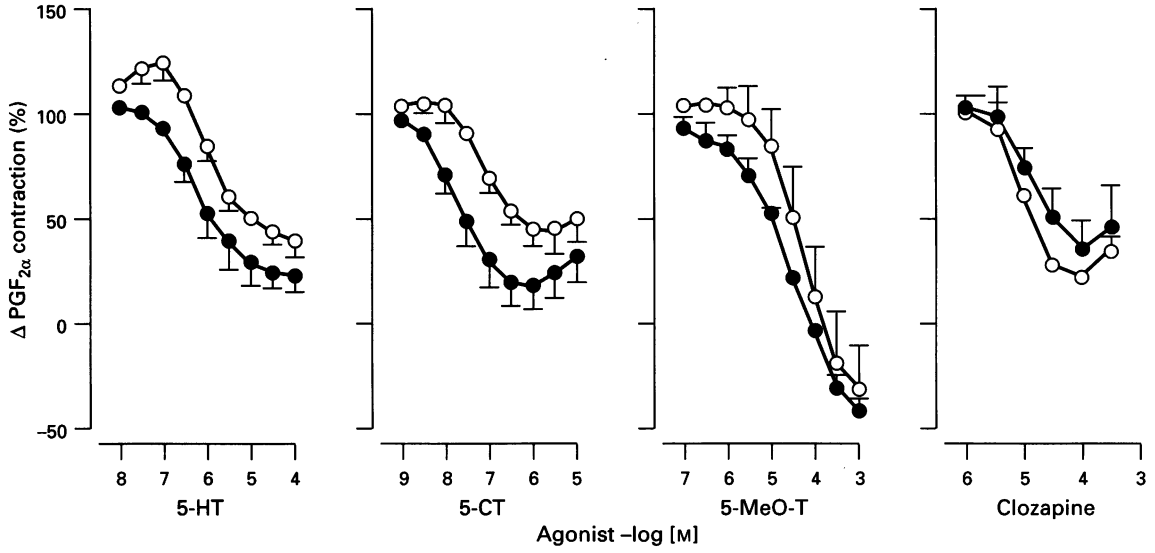

Figure 1 The effects of GR127935 (○, 0; and ●, 100 nM) on cumulative concentration-response curves to 5-HT, 5-CT, 5-methoxytryptamine (5-MeO-T) and clozapine in canine coronary artery rings without endothelium taken from the same animal. The rings had been precontracted with prostaglandin $F_{2\alpha}$ (2 μM) so that the changes in tension could be expressed as a percentage of that contraction. Note that GR127935 completely abolished the vasoconstrictor phase of the responses. Both the potency (pEC_{50}) and the maximum relaxing effect (E_{max}) of 5-HT and 5-CT were significantly increased by GR127935. Points are the mean, with s.e.mean of 3–5 observations. Contraction to prostaglandin $F_{2\alpha}$ was 5.2 ± 0.5 and 5.5 ± 0.6 g in vehicle- and GR127935-treated rings, respectively.

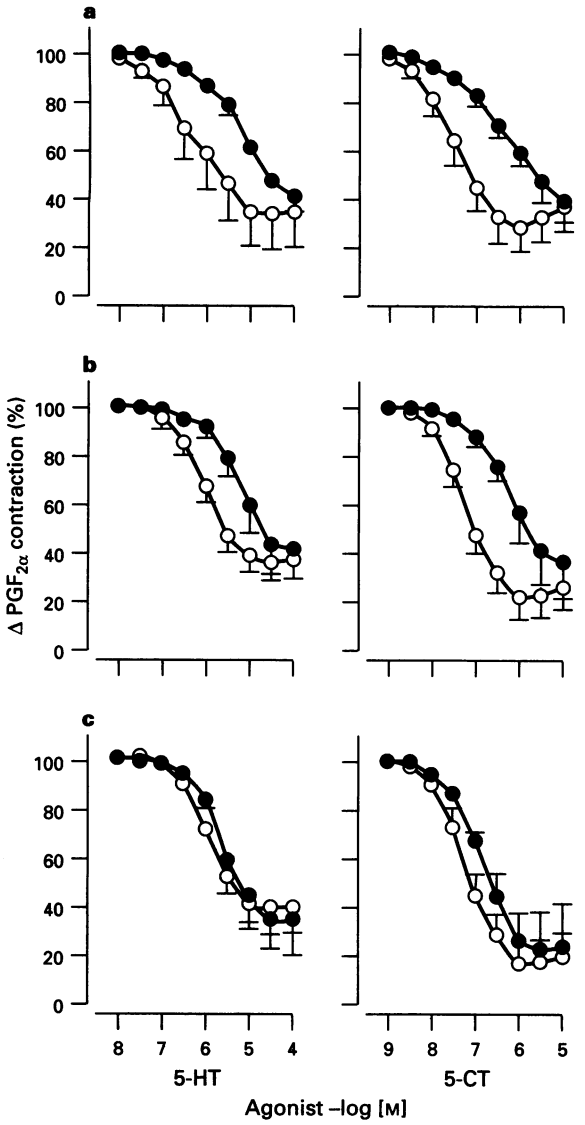

Figure 2 The effects of (a) methiothepin (○, 0; and ●, 3 nM), (b) mianserin (○, 0; and ●, 1 μM) or (c) ketanserin (○, 0; and ●, 1 μM) on cumulative concentration-relaxant response curves to 5-HT and 5-CT in canine coronary artery rings without endothelium taken from the same animal and preincubated with GR127935 (100 nM). The rings had been precontracted with prostaglandin $F_{2\alpha}$ (2 μM) so that the changes in tension could be expressed as a percentage of that contraction. Only in methiothepin- and mianserin-treated rings, were the pEC_{50} values between vehicle- and antagonist-treated tissues, but not the corresponding E_{max}, significantly different ($P<0.05$). Points are the mean with s.e.mean of 3–5 observations. Contraction to prostaglandin $F_{2\alpha}$ was 6.4±0.5 and 6±0.4 g in vehicle- and antagonist-treated rings, respectively.

corresponding E_{max} suggests a competitive interaction though, in one experiment, the E_{max} of both 5-HT and 5-CT was apparently reduced by methiothepin. In addition, it is worth noting that the potency of the above antagonists (estimated pK_B values) against 5-HT and 5-CT did not differ significantly (see Table 1).

Effects of some typical and atypical antipsychotic drugs on the relaxation induced by 5-HT and 5-CT

As noticed with methiothepin and mianserin, the antipsychotic drugs, pimozide (30 nM) and spiperone (1 μM) (typical; Figure 3a and b, respectively), and clozapine (1 μM) and risperidone (3 nM) (atypical; Figure 4a and b, respectively), all produced rightward displacements of the C–R curves for 5-HT and 5-CT without significantly reducing the corresponding E_{max}. With the exception of clozapine (1 μM), which in two experiments produced a slight relaxation (about 5% of the contraction to prostaglandin $F_{2\alpha}$), none of the above antipsychotics relaxed the precontracted preparations (not shown). Notwithstanding, it should be remarked that, in two experiments, risperidone, like methiothepin, apparently reduced the E_{max} of 5-HT and 5-CT. Significantly, all the antipsychotic drugs were equipotent in antagonizing the relaxant responses of 5-HT and 5-CT (see the corresponding pK_B values in Table 1).

Effects of metergoline, mesulergine and lisuride on the relaxation induced by 5-CT

The effects of metergoline (100 nM), mesulergine (300 nM) and lisuride (3 nM) on the 5-CT-induced relaxation are shown in Figure 5a, b and c, respectively. All of these drugs produced a significant blockade of the relaxant responses induced by 5-CT. In contrast to metergoline and mesulergine, both of which produced a parallel rightward displacement of the C–R curve for 5-CT, lisuride produced a nonparallel displacement to the right with a significant reduction ($P<0.05$) in E_{max} (see Figure 5c). As observed with the other antagonists, none of these drugs produced relaxant effects when added to precontracted vessels (not shown).

Discussion

The major finding of the present study was that the relaxant 5-HT receptor in the canine coronary artery smooth muscle is highly sensitive to blockade by several 5-HT receptor antagonists and some typical and atypical antipsychotic drugs, all of which display high affinity for the cloned 5-ht$_7$ receptor subtype. It should be stressed that a possible association of the above relaxant 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 an appropriate experimental design, a more direct analysis of the relaxant 5-HT receptor was attempted by blocking the 5-HT_{1D} receptor mediating contraction in this preparation (Cushing & Cohen, 1992b; Cushing *et al.*, 1994; Terrón, 1996a). Thus, in accordance with previous results showing the high potency of GR127935 in blocking the contraction induced by 5-HT and sumatriptan in the canine coronary artery (Terrón, 1996a), a high concentration (100 nM) of this antagonist completely abolished the vasoconstrictor component of the response induced by 5-HT, 5-CT and 5-methoxytryptamine. Accordingly, a significant increase in potency (see estimated pD_2 values in Results) and relaxing efficacy (E_{max}; see Figure 1) of 5-HT and 5-CT was observed in rings preincubated with GR127935. This finding undoubtedly confirms the counteracting influence of the contractile 5-HT receptor on the relaxant effects of the 5-HT receptor agonists and therefore justifies the use of GR127935 in the experiments with antagonist drugs.

Relaxant effects of 5-HT receptor agonists in the canine coronary artery smooth muscle

The rank order of agonist potency obtained after blockade of 5-HT_{1D} receptors in the canine coronary artery (present study) was the same as that reported by Cushing & Cohen (1992a) i.e. 5-CT > 5-HT > 5-methoxytryptamine. In the context of a possible involvement of the novel 5-HT receptor subtypes, this profile is similar to that reported at both the mouse (Lovenberg *et al.*, 1993; Plassat *et al.*, 1993), rat (Ruat *et al.*, 1993; Shen *et al.*, 1993) and human (Bard *et al.*, 1993) cloned 5-ht$_7$ subtype i.e. 5-CT > 5-methoxytryptamine ≥ 5-HT. These orders of agonist potency are in marked contrast with that reported at the cloned rat 5-ht$_6$ receptor i.e. 5-methoxytryptamine > 5-HT > 5-CT (Monsma *et al.*, 1993). In addition, although clo-

Table 1 Functional potencies of antagonists (pK_B) against 5-HT- and/or 5-CT-induced relaxation in the endothelium-denuded canine coronary artery

Antagonist	*Conc.*	pK_B *5-HT*	*5-CT*
Methiothepin	3 nM	9.4±0.4 (3)	9.7±0.1 (4)
Mianserin	1 μM	6.74±0.2 (5)	7.08±0.3 (5)
Metergoline	100 nM	ND	7.86±0.03 (3)
Mesulergine	300 nM	ND	7.17±0.1 (4)
Pimozide	30 nM	8.2±0.2 (4)	8.27±0.2 (4)
Spiperone	1 μM	6.87±0.3 (4)	7.14±0.3 (4)
Clozapine	1 μM	6.8±0.3 (3)	7.12±0.2 (3)
Risperidone	3 nM	9.15±0.2 (4)	9.4±0.3 (4)

Antagonist potencies (mean±s.e.mean for n experiments in parentheses) were determined as described in Methods (ND, not determined).

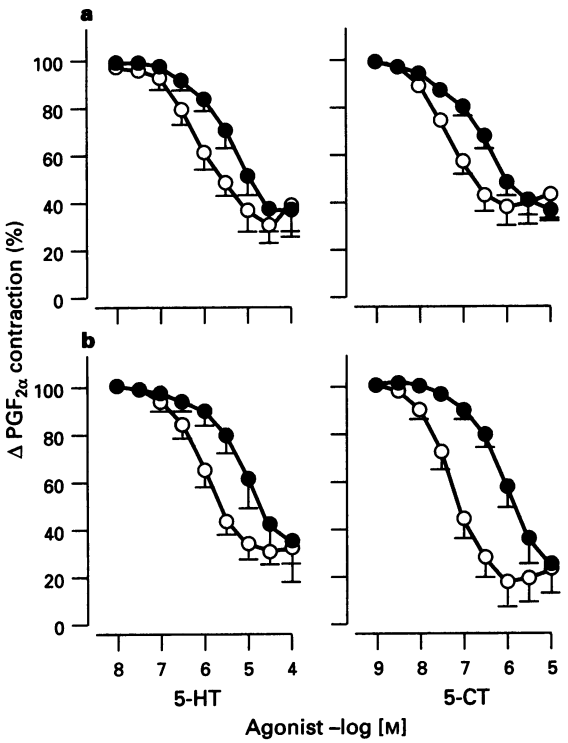

Figure 3 The effects of the typical antipsychotic drugs, (a) pimozide (○, 0; and ●, 30 nM) or (b) spiperone (○, 0; and ●, 1 μM), on cumulative concentration-relaxant response curves to 5-HT and 5-CT in canine coronary artery rings without endothelium taken from the same animal and preincubated with GR127935 (100 nM). The rings had been precontracted with prostaglandin $F_{2\alpha}$ (2 μM) so that the changes in tension could be expressed as a percentage of that contraction. In both cases, the pEC_{50} values between vehicle- and antagonist-treated tissues, but not the corresponding E_{max}, were significantly different ($P<0.05$). Points are the mean with s.e.mean of 3–4 observations. Contraction to prostaglandin $F_{2\alpha}$ was 7±0.6 and 6.6±0.5 g in vehicle- and antagonist-treated rings, respectively.

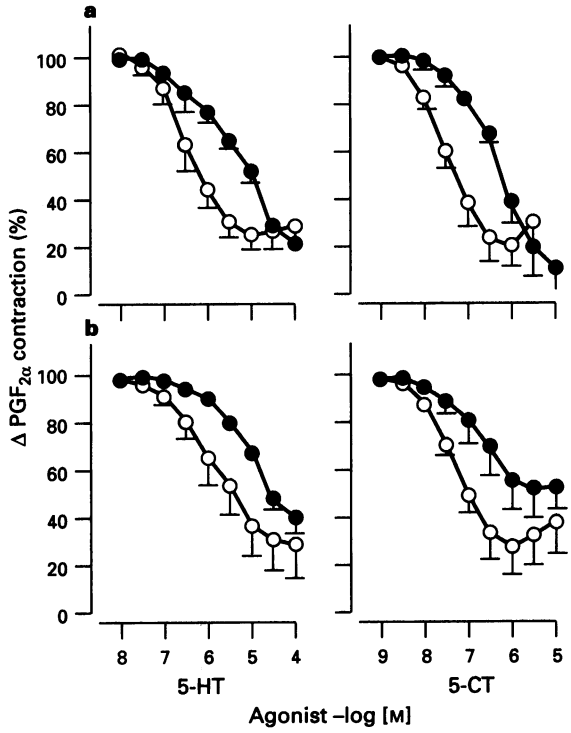

Figure 4 The effects of the atypical antipsychotic drugs, (a) clozapine (○, 0; and ●, 1 μM) or (b) risperidone (○, 0; and ●, 3 nM), on cumulative concentration-relaxant response curves to 5-HT and 5-CT in canine coronary artery rings without endothelium taken from the same animal and preincubated with GR127935 (100 nM). The rings had been precontracted with prostaglandin $F_{2\alpha}$ (2 μM) so that the changes in tension could be expressed as a percentage of that contraction. In both cases, the pEC_{50} values, but not the corresponding E_{max}, between vehicle- and antagonist-treated tissues were significantly different ($P<0.05$). Points are the mean with s.e.mean of 3–4 observations. Contraction to prostaglandin $F_{2\alpha}$ was 5.6±0.5 and 6.5±0.5 g in vehicle- and antagonist-treated rings, respectively.

zapine may be a partial agonist at the relaxant 5-HT receptor in the dog coronary artery smooth muscle, as suggested by its ability to antagonize 5-HT- and 5-CT-induced relaxation (Figure 4a; see below), it displayed a relaxing potency 2.5 log units lower than that of 5-CT in this tissue (see Results). This finding is in contrast to the 10 fold higher affinity of clozapine, with respect to 5-CT, for the cloned 5-ht$_6$ receptor (Monsma *et al.*, 1993).

Although the above agonists have been found to display nanomolar affinities for the cloned 5-ht$_7$ receptor (see for instance Bard *et al.*, 1993; Ruat *et al.*, 1993; Shen *et al.*, 1993), their affinity values in the dog coronary artery (see Results) are in close agreement with those reported in the rabbit femoral vein, another preparation containing relaxant 5-ht$_7$-like receptors (Martin & Wilson, 1995). Thus, there may be important differences in drug affinities between cloned and functional 5-ht$_7$ receptors.

As previously reported (Cushing & Cohen, 1992a), both 8-OH-DPAT and sumatriptan failed to induce relaxation in the canine coronary artery even in the presence of GR127935 (present results). Nevertheless, the failure of 8-OH-DPAT to mimic (Cushing & Cohen, 1992a; present results) or to an-

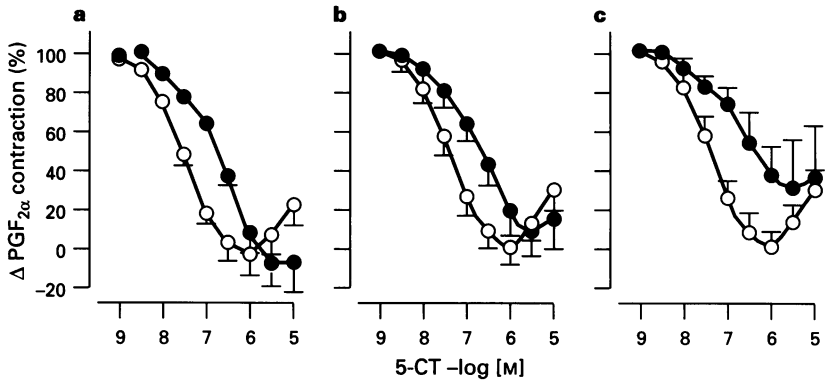

Figure 5 The effects of (a) metergoline (○, 0; and ●, 100 nM), (b) mesulergine (○, 0; and ●, 300 nM), or (c) lisuride (○, 0; and ●, 3 nM) on cumulative concentration-relaxant response curves to 5-CT in canine coronary artery rings without endothelium taken from the same animal and preincubated with GR127935 (100 nM). The rings had been precontracted with prostaglandin $F_{2\alpha}$ (2 μM) so that the changes in tension could be expressed as a percentage of that contraction. In all cases, the pEC_{50} values between vehicle- and antagonist-treated tissues were significantly different ($P<0.05$). Only in the case of lisuride, a significant reduction ($P<0.05$) in E_{max} was noticed. Points are the mean with s.e.mean of 3–4 observations. Contraction to prostaglandin $F_{2\alpha}$ was 5.8 ± 0.7 and 6.5 ± 0.6 g in vehicle- and antagonist-treated rings, respectively.

tagonize (Houston & Vanhoutte, 1988) the 5-HT-induced relaxation in this tissue may not represent an argument against the possible participation of a 5-ht$_7$-like receptor as this drug exhibits relatively high affinity for the mouse and rat (pK_i values between 7.5 and 7.3; Plassat *et al.*, 1993; Ruat *et al.*, 1993; Shen *et al.*, 1993), but not for the human ($pK_i = 6.3$; Bard *et al.*, 1993), 5-ht$_7$ receptor. In the case of sumatriptan, it shows low affinity at both the mouse, rat and human 5-ht$_7$ receptor (pK_i values between 6.3 and 6; Bard *et al.*, 1993; Plassat *et al.*, 1993; Ruat *et al.*, 1993; Shen *et al.*, 1993).

Effects of methiothepin, mianserin and ketanserin on 5-HT- and 5-CT-induced relaxation

The original experiments by Houston & Vanhoutte (1988) established that nanomolar concentrations of methiothepin antagonized the 5-HT-induced relaxation with a minimal shift of the contractile response. In accordance with these findings, a low concentration (3 nM) of methiothepin strongly antagonized the relaxation produced by 5-HT and 5-CT. Similar results were obtained with mianserin (1 μM), but not with ketanserin (1 μM). Although the E_{max} of both agonists was not significantly reduced by methiothepin or mianserin, which is suggestive of competitive interaction, a higher concentration (10 nM) of methiothepin produced unsurmountable antagonism (Terrón, unpublished). Interestingly, the estimated pK_B values of these antagonists against 5-HT and 5-CT did not differ significantly suggesting the involvement of a common receptor site. Since 5-HT_1, 5-HT_2, 5-HT_3 or 5-HT_4 receptors are not involved (Cushing & Cohen, 1992a; present results), the blocking effects of methiothepin and mianserin should be explained by interaction with a different 5-HT receptor subtype.

In this regard, the affinity of methiothepin and mianserin for the relaxant 5-HT receptors is in agreement with their affinity for the cloned 5-ht$_7$ receptor. Thus, the pK_B of methiothepin against 5-HT at the relaxant 5-HT receptor approximated 9.4 (Table 1) while its reported pK_i at the cloned 5-ht$_7$ receptor was 9.42 (Shen *et al.*, 1993). In fact, methiothepin was found to antagonize the 5-HT-induced stimulation of 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. In the case of mianserin, another drug showing antagonist effects at the relaxant 5-HT receptor (pK_B values between 6.7 and 7.1; Table 1), it also displayed affinity at the rat cloned 5-ht$_7$ receptors (pK_i values between 7.2 and 7.4; Ruat *et al.*, 1993; Shen *et al.*, 1993) and antagonized the 5-HT-induced stimulation of cyclic AMP accumulation in CHO cells expressing a rat 5-ht$_7$ receptor ($pK_B = 7.9$; Ruat *et al.*, 1993). The failure of ketanserin to block 5-HT- and 5-CT-induced relaxation also supports the possible involvement of a 5-ht$_7$-like receptor as this drug has very low affinity for the cloned 5-ht$_7$ receptor (pK_i values <6; Bard *et al.*, 1993; Ruat *et al.*, 1993; Shen *et al.*, 1993).

Effects of some typical and atypical antipsychotic drugs on the relaxation induced by 5-HT and 5-CT

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 (Monsma *et al.*, 1993; Shen *et al.*, 1993; Roth *et al.*, 1994). Accordingly, the effects of some of these drugs on the relaxant effects of 5-HT and 5-CT were tested. For this purpose, a concentration of the antipsychotics close to the reported pK_i values at the cloned 5-ht$_7$ receptor was selected for experimentation (Terrón, 1996b; Table 1).

Interestingly, both the typical (pimozide and spiperone) and atypical (clozapine and risperidone) antipsychotic drugs antagonized the relaxing effects of 5-HT and 5-CT. Among these drugs, risperidone merits special comment as it shows more than 300 fold selectivity for the cloned 5-ht$_7$ receptor with respect to the cloned 5-ht$_6$ receptor (Roth *et al.*, 1994). Indeed, the affinity of risperidone for the relaxant 5-HT receptor (see pK_B values in Table 1) closely resembles that at the cloned 5-ht$_7$ receptor ($pK_i = 8.86$; Roth *et al.*, 1994). Similar results were obtained with spiperone, which shows more than 150 fold selectivity for the cloned 5-ht$_7$ receptor compared with the cloned 5-ht$_6$ receptor (pK_i values of 8 and 5.8, respectively; Roth *et al.*, 1994). These findings, in conjunction with the antagonist action of pimozide and clozapine at concentrations close to their reported pK_i values at the cloned 5-ht$_7$ receptor, strongly support the contention that the relaxant 5-HT receptor is similar to the cloned 5-ht$_7$ receptor subtype.

Effects of metergoline, mesulergine and lisuride on the relaxant effects of 5-CT

Metergoline, mesulergine and lisuride were selected as potential antagonists of 5-CT in the canine coronary artery smooth muscle because they show either high affinity or relative selectivity for the cloned 5-ht$_7$ receptor subtype (Ruat *et al.*, 1993; Shen *et al.*, 1993). Of special consideration is the fact that mesulergine, a drug displaying almost 100 fold selectivity for the rat cloned 5-ht$_7$ receptor ($pK_i = 7.7$; Shen *et al.*, 1993) with respect to the rat cloned 5-ht$_6$ receptor ($pK_i = 5.8$; Monsma *et al.*, 1993), competitively antagonized the relaxant effects of 5-CT. Significantly, the estimated affinity of mesulergine at the relaxant 5-HT receptor ($pK_B = 7.17$; Table 1) was only 3 fold

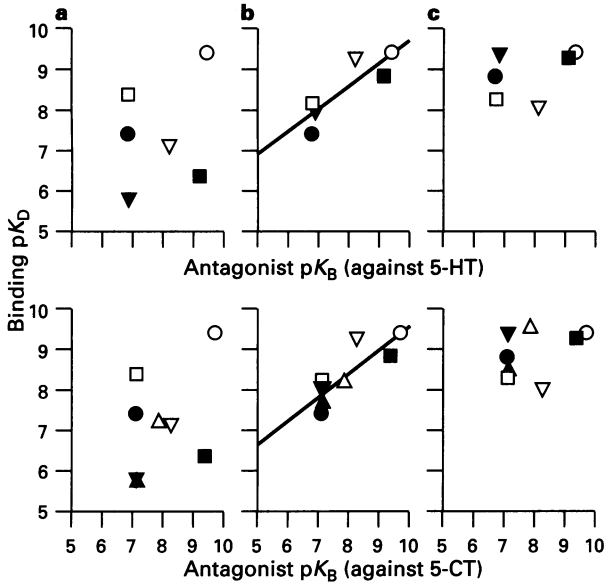

Figure 6 Correlations of antagonist affinity estimates (pK_B values against 5-HT and 5-CT) at the relaxant 5-HT receptor in the canine coronary artery smooth muscle and antagonist affinity estimates (pK_i values) at (a) 5-ht$_6$, (b) 5-ht$_7$ and (c) 5-HT$_{2A}$ binding sites: (○) methiothepin; (●) mianserin; (▽) pimozide; (▼) spiperone; (□) clozapine; (■) risperidone; (△) metergoline; and (▲) mesulergine. For correlation coefficients, see text.

lower than that at the rat cloned 5-ht$_7$ receptor (pK_i=7.68; Shen *et al.*, 1993). The estimated affinity of metergoline at the relaxant 5-HT receptor (pK_B=7.86; Table 1) was similar to that reported at the rat and human cloned 5-ht$_7$ receptor (pK_i=8.2; Bard *et al.*, 1993; Shen *et al.*, 1993).

Finally, though lisuride displays high affinity for both the cloned 5-ht$_6$ (pK_i=8.28; Monsma *et al.*, 1993) and 5-ht$_7$ receptors (pK_i values between 9.05 and 8.2; Ruat *et al.*, 1993; Shen *et al.*, 1993), its ability to antagonize strongly the 5-CT-induced relaxation at a low concentration (3 nM) is in keeping with the involvement of a 5-ht$_7$-like receptor. No affinity of lisuride at the relaxant 5-HT receptor could be estimated due to its noncompetitive interaction.

Correlation of the relaxant 5-HT receptor in the canine coronary artery smooth muscle with the cloned 5-ht$_7$ receptor subtype

In an attempt to establish the possible similarity of the relaxant 5-HT receptor in the canine coronary artery smooth muscle with some of the new members of the 5-HT receptor family, the functional affinities (pK_B values) of the antagonist drugs used in the present study against 5-HT and/or 5-CT (Table 1) were compared with the binding affinities reported for these drugs at the cloned 5-ht$_6$ and 5-ht$_7$ subtypes (Figure 6a and b, respectively). In view that these 5-HT receptor antagonists as well as the antipsychotic drugs display very high affinity for 5-HT$_{2A}$ binding sites (Leysen, 1985; Leysen *et al.*, 1988; Meltzer *et al.*, 1989; Roth *et al.*, 1991), correlations with this receptor were also performed (Figure 6c).

Significant correlations were found only with 5-ht$_7$ binding sites (r=0.87, P=0.024 and r=0.85, P=0.036, against 5-HT and 5-CT, respectively). The correlations with 5-ht$_6$ (r=0.28, against 5-HT) and 5-HT$_{2A}$ (r=0.44, against 5-HT) receptors were not only lower than those obtained with the 5-ht$_7$ subtype, but also a theoretical straight line connecting the correlation points (see Figure 6a and 6c) could be rejected (P>0.05). Other receptors for which the antagonists under study display high affinity are the 5-HT$_{2C}$, 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). Comparisons with these binding sites yielded no significant correlations (P>0.05; not shown).

In conclusion, it is suggested that the relaxant effect of 5-HT in the smooth muscle of the canine coronary artery is mediated by a receptor similar to the cloned 5-ht$_7$ subtype. Significantly, the pharmacological profile of this relaxant 5-HT receptor closely matches that previously described for a smooth muscle relaxant 5-HT$_1$-like receptor in other vascular preparations (see Martin, 1994). The above suggestion will be either strengthened or weakened once complete pharmacological data at the cloned 5-ht$_{5A}$ and 5-HT$_{5B}$ receptors become available. Although species variations should not be dismissed, the present finding may support previous observations demonstrating the expression of a messenger RNA coding for the 5-ht$_7$ receptor in the human coronary artery (Bard *et al.*, 1993). Thus, the 5-ht$_7$ receptor may play a role in the local regulation of the coronary artery blood flow. This highlights the possibility of developing selective 5-ht$_7$ receptor drugs as a novel therapeutic strategy in the treatment of some heart diseases.

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