ELSEVIER

European Journal of Pharmacology 300 (1996) 109–112

ejp

Short communication

# GR127935 is a potent antagonist of the 5-$HT_1$-like receptor mediating contraction in the canine coronary artery

José A. Terrón *

*Sección de Terapéutica Experimental, Departamento de Farmacología y Toxicología, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Apdo. Postal 22026, 14000 México, D.F., Mexico*

Received 27 July 1995; revised 8 January 1996; accepted 12 January 1996

**Abstract**

The effects of the recently developed 5-$HT_{1D}$ receptor antagonist, GR127935 (*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), and those of the preferential human 5-$HT_{1D\alpha}$ receptor antagonist, ketanserin, on the isometric contraction induced by 5-hydroxytryptamine (5-HT) and sumatriptan in endothelium-denuded ring segments of canine coronary artery were analyzed. Sumatriptan mimicked 5-HT with lower potency but similar efficacy. GR127935 (1, 3 and 10 nM) concentration dependently antagonized the contractions elicited by both agonists; only the 5-HT maximum was reduced. Ketanserin and mianserin (both at 1 $\mu$M) were inactive. These data strongly suggest that a 5-$HT_{1D}$ receptor mediates contraction in the dog coronary artery. The possibility that this 5-$HT_{1D}$ receptor resembles the cloned human 5-$HT_{1D\beta}$ subtype is discussed.

*Keywords:* Coronary artery, canine; Contraction; GR127935; 5-HT (5-hydroxytryptamine, serotonin); 5-$HT_{1D}$ receptor; Sumatriptan

## 1. Introduction

Several attempts to characterize the receptor subtype(s) mediating the contraction induced by serotonin (5-hydroxytryptamine; 5-HT) in the canine coronary artery have led to discrepant conclusions. Nevertheless, recent molecular biology studies have identified a base pair sequence closely resembling that of the 5-$HT_{1D}$ receptor in the canine coronary artery and saphenous vein (Cushing et al., 1994). These findings, along with pharmacological data, have led to the suggestion that 5-$HT_{1D}$-like receptors may mediate contraction in both tissues (Cushing and Cohen, 1992a; Cushing et al., 1994). Unequivocal evidence supporting the involvement of 5-$HT_{1D}$ receptors, however, should be provided by the use of a potent and selective 5-$HT_{1D}$ receptor antagonist such as the recently described drug, GR127935 (*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; Skingle et al., 1993).

The present study investigated the effects of GR127935 on the contractions produced by 5-HT and the 5-$HT_{1D}$ receptor agonist, sumatriptan (Schoeffter and Hoyer, 1989), in the dog coronary artery. The effects of ketanserin, a preferential antagonist at the cloned human 5-$HT_{1D\alpha}$ (Weinshank et al., 1991), but not at the cloned dog 5-$HT_{1D\alpha}$ (Branchek et al., 1995) receptor were also analyzed and compared with those of the 5-$HT_2$ drug with low affinity for 5-$HT_{1D}$ receptors, mianserin (Hoyer and Schoeffter, 1991).

The results strongly suggest that the contraction induced by 5-HT in the dog coronary artery is primarily mediated by a 5-$HT_{1D}$ receptor. The rank order of potency for 5-HT and sumatriptan and the estimated affinity of GR127935 at this vascular 5-$HT_{1D}$ receptor suggest that it resembles the cloned 5-$HT_{1D\beta}$ subtype.

## 2. Materials and methods

### 2.1. Tissue preparation

Circumflex coronary arteries were obtained from mongrel dogs (15–25 kg) and placed in Krebs bicarbonate solution. Ring segments (4 mm long) with mechanically disrupted endothelium were mounted in 10 ml organ chambers filled with Krebs bicarbonate solution bubbled with 95% $O_2$ and 5% $CO_2$ (37°C, pH 7.4). Tissues were gradually stretched over a 90 min period to an optimal tension of 10 g. Two or three contractions in response to

* Tel.: (+52) (5) 675 9341; fax: (+52) (5) 675 9168.


*PII* S0014-2999(96)00041-6

potassium chloride (KCl; 20 mM) followed by a submaximum response to KCl 40 mM were obtained (Houston and Vanhoutte, 1988). The absence of functional endothelium was verified by addition of acetylcholine (1 $\mu$M) under precontraction with prostaglandin $F_{2\alpha}$ (2 $\mu$M). All experiments with 5-HT were conducted in the presence of desipramine (1 $\mu$M) and deoxycorticosterone (10 $\mu$M).

### 2.2. Experimental protocol

Cumulative concentration-response curves for either 5-HT (1 nM to 1 $\mu$M) or sumatriptan (1 nM to 1 mM) were obtained in rings preincubated for 1 h with vehicle, GR127935 (1, 3 and 10 nM), ketanserin (1 $\mu$M) or mianserin (1 $\mu$M). Each concentration of agonist (spaced by a factor of $10^{1/2}$) was added only after the maximum response to the previous concentration had been attained.

### 2.3. Data presentation and statistical evaluation

All data in the text and figures are expressed as the means ± S.E.M., where $n$ represents the number of dogs from which the vessels were taken. No more than one tissue was used from each animal for any given treatment. Changes in tension are expressed as percentages of the contraction elicited by KCl (40 mM). Comparisons between vehicle- and antagonist-treated rings obtained from the same animal were made in separate tissues and no tissue was used to generate more than one agonist concentration-response curve. The $pD_2$ values ($-\text{Log } EC_{50}$, calculated by nonlinear regression analysis) and the maximum response ($E_{max}$) were determined from individual concentration-response curves. Significant differences between mean values of vehicle- and antagonist-treated tissues were determined as appropriate with $t$-test or one-way analysis of variance followed by Dunnett's test with an $\alpha$ value of 0.05. A $pA_2$ value was calculated by linear regression analysis according to Tallarida et al. (1979). Since the slope did not differ significantly from unity, the $pA_2$ and its error were estimated by constraining the slope to unity.

### 2.4. Drugs

5-Hydroxytryptamine creatinine sulphate, deoxycorticosterone and prostaglandin $F_{2\alpha}$ (Sigma Chemical Company, St. Louis, MO, USA); acetylcholine chloride and desipramine hydrochloride (Research Biochemicals Int., Natick, MA, USA); sumatriptan succinate and GR127935 hydrochloride monohydrate (gift: Glaxo Group Research, Ware, UK); ketanserin tartrate (gift: Janssen Pharmaceutica, Beerse, Belgium); and mianserin hydrochloride (gift: Organon de México, Mexico City, Mexico). The compounds were dissolved in distilled water with the exception of deoxycorticosterone (5% propylene glycol). The vehicles had no effect on baseline tension or agonist-induced responses.

## 3. Results

### 3.1. Initial effects of 5-HT and sumatriptan

5-HT and sumatriptan produced concentration-dependent contractions of the canine coronary artery (Figs. 1 and 2). 5-HT ($pD_2 = 7.7 \pm 0.07$; $n = 9$) was almost 1 log unit more potent than sumatriptan ($pD_2 = 6.9 \pm 0.11$; $n = 9$). Both agonists had similar maximum effects (the corresponding $E_{max}$ values expressed as percentage of the contraction produced by KCl 40 mM were 33 ± 7 and 32 ± 5 for 5-HT and sumatriptan, respectively). In four of nine experiments, the contraction elicited by sumatriptan showed a second phase at concentrations above 10 $\mu$M (not shown).

It should be noted that in previous experiments ($n = 3$) the addition of desipramine (1 $\mu$M) and deoxycorticosterone (10 $\mu$M) did not modify 5-HT- and sumatriptan-induced responses (not shown).

### 3.2. Effects of GR127935 on agonist-induced contractions

As depicted in Fig. 1, low concentrations of GR127935 (1–10 nM) produced a concentration-dependent blockade of the contraction elicited by 5-HT and sumatriptan; only in the case of 5-HT was the $E_{max}$ significantly ($P < 0.05$) reduced. In contrast, GR127935 caused a roughly parallel rightward shift of the concentration-response curve for

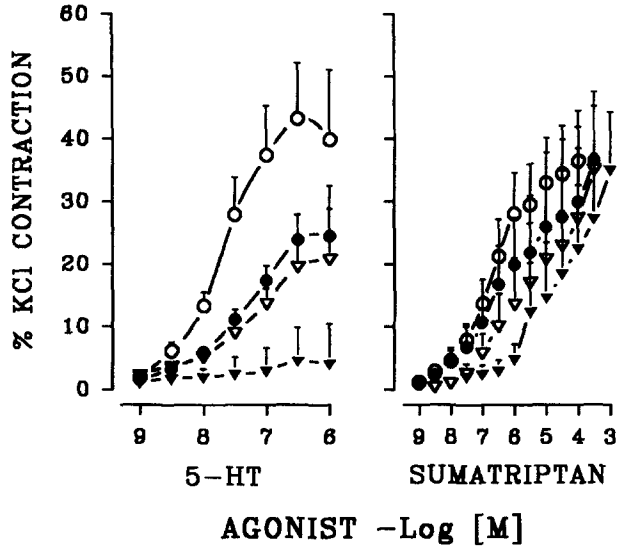


Fig. 1. The effects of GR127935 (○, 0; ●, 1; ▽, 3; and ▼, 10 nM) on cumulative concentration-response curves for 5-HT or sumatriptan in canine coronary artery rings without endothelium taken from the same animal. Changes in tension are expressed as a percentage of the maximum response of each ring to KCl (40 mM). Note that only in the case of 5-HT-induced responses did GR127935 reduce the maximum contraction. Contractions elicited by KCl in control and GR127935-treated segments were 9.6 ± 0.7, 9.1 ± 0.8, 9.6 ± 1.5 and 9.7 ± 0.8 g for rings contracted with 5-HT, and 9 ± 0.6, 9 ± 1, 9 ± 0.9 and 8.3 ± 0.6 g for rings contracted with sumatriptan. Points are the means and vertical bars denote the S.E.M. of 5 observations.

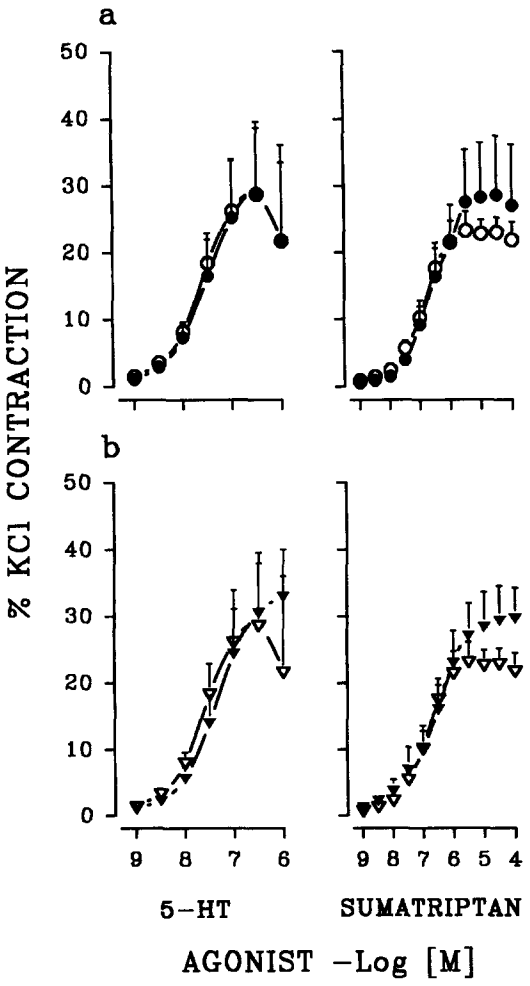


Fig. 2. The effects of (a) ketanserin (○, 0; and ●, 1 μM) or (b) mianserin (▽, 0; and ▼, 1 μM) on cumulative concentration-response curves for 5-HT or sumatriptan in canine coronary artery rings without endothelium taken from the same animal. Changes in tension are expressed as a percentage of the maximum response of each ring to KCl (40 mM). Neither blocker modified agonist-induced contractions. The control curve is the same as that for each agonist. Contractions elicited by KCl in control, ketanserin- and mianserin-treated rings were 5.7 ± 0.7, 7 ± 0.7 and 5.2 ± 0.9 g respectively for rings contracted with 5-HT, and 5.3 ± 0.2, 6.3 ± 0.2 and 5.4 ± 0.9 g for rings contracted with sumatriptan. Points are the means and vertical bars denote the S.E.M. of 4 observations.

sumatriptan without significantly changing $E_{max}$ (Fig. 1). Notably, the blockade of sumatriptan-induced contraction by GR127935 could be overcome only when very high concentrations of the agonist (up to 1 mM) were used. Under these experimental conditions, the estimated $pA_2$ for GR127935 (see section 2.3) was 10.3 ± 0.3 ($n = 5$).

### 3.3. Effects of ketanserin and mianserin on agonist-induced contractions

Unlike the effects of GR127935, a high concentration (1 μM) of either ketanserin or mianserin failed to modify the contractile responses to 5-HT and sumatriptan (Fig. 2). Instead, both blockers tended to increase – though not significantly – the agonist-induced contractions.

## 4. Discussion

The main finding of the present study is that GR127935 behaves as a potent antagonist of both 5-HT and sumatriptan in the canine coronary artery. Although the activity of sumatriptan and other 5-HT receptor agonists is consistent with stimulation of $5\text{-HT}_{1D}$-like receptors (present results; Cushing and Cohen, 1992a), which are indeed expressed in the dog coronary artery (Cushing et al., 1994), the high antagonist potency of GR127935 represents clear-cut evidence that $5\text{-HT}_{1D}$ receptors are involved in the contractile response.

Consistent with its high affinity at $5\text{-HT}_{1D}$ binding sites ($pK_i = 8.5–9.9$; Skingle et al., 1993), GR127935 in nanomolar concentrations (1–10 nM) strongly and concentration dependently antagonized 5-HT- and sumatriptan-induced contractions of the canine coronary artery ($pA_2 = 10.3 \pm 0.3$ against sumatriptan). The significant reduction in 5-HT $E_{max}$ most probably reflects the ability of 5-HT, but not of sumatriptan, to stimulate relaxant 5-HT receptors in this tissue (Houston and Vanhoutte, 1988; Cushing and Cohen, 1992b).

Interestingly, GR127935, at the same concentration range used in the present study, antagonized sumatriptan-induced contractions in the dog basilar artery, with a reduced $E_{max}$ (Skingle et al., 1993). The explanation for the discrepancy in the effects of GR127935 on the $E_{max}$ of sumatriptan in the canine basilar and coronary arteries remains to be determined. Conceivably, the biphasic pattern of the sumatriptan-induced responses observed in some preparations and/or the very high concentrations of this agonist required to surmount the effect of GR127935 in the coronary artery (present data) might suggest that a mechanism unrelated to $5\text{-HT}_{1D}$ receptors is involved. In any case, the high antagonist potency of GR127935 in both preparations may suggest that a common receptor site mediates the contractile response.

It is now known that two structurally dissimilar $5\text{-HT}_{1D}$ subtypes ($5\text{-HT}_{1D\alpha}$ and $5\text{-HT}_{1D\beta}$) displaying indistinguishable pharmacology are encoded by the human genome (Hartig et al., 1992). Information as to which of these subtypes represents the above vascular $5\text{-HT}_{1D}$ receptor will not be provided until selective agonists and antagonists are identified. In this regard, original radioligand binding data showed that the $5\text{-HT}_2$ receptor antagonist, ketanserin, displays an about 70-fold selectivity for the human $5\text{-HT}_{1D\alpha}$ with respect to the $5\text{-HT}_{1D\beta}$ subtype (Weinshank et al., 1991). More recently, however, using cloned human and canine $5\text{-HT}_{1D\alpha}$ and $5\text{-HT}_{1D\beta}$ receptors, it was shown that ketanserin is unable to differentiate between the dog $5\text{-HT}_{1D\alpha}$ and $5\text{-HT}_{1D\beta}$ subtypes and that it has low affinity for them ($pK_i$ of 5.5 and 5.3, respectively; Branchek et al., 1995). Thus, the failure of ketanserin to antagonize 5-HT- and sumatriptan-induced contractions in the dog coronary artery is in keeping with its low affinity at the above dog $5\text{-HT}_{1D}$ subtypes.

It is worth mentioning that, at the canine 5-$HT_{1D}$ receptor encoded by a cDNA clone known as RDC4, which is the species homologue of the human 5-$HT_{1D\alpha}$ receptor (see Hartig et al., 1992), sumatriptan exhibits a higher affinity (more than 2-fold) than 5-HT (Zgombick et al., 1991). In contrast, at the contractile 5-HT receptor in the canine coronary artery 5-HT displays higher affinity (more than 6-fold; present results) than sumatriptan. This discrepancy in the affinity of 5-HT and sumatriptan at both receptor sites might suggest that the contractile 5-HT receptor in the dog coronary artery is similar to the cloned human 5-$HT_{1D\beta}$ receptor. In support of this possibility is the fact that the affinity of GR127935 at the contractile 5-HT receptor in the dog coronary artery ($pA_2 = 10.3 \pm 0.3$; present results) is closely similar to that reported at the cloned human 5-$HT_{1D\beta}$ subtype ($pK_i = 9.9$; Skingle et al., 1993).

In accordance with previous observations (Cushing and Cohen, 1992a), the involvement of 5-$HT_2$ receptors could be further excluded with mianserin, a potent 5-$HT_2$ antagonist with low affinity for 5-$HT_{1D}$ receptors (Hoyer and Schoeffter, 1991).

Significantly, recent evidence from functional and molecular biology studies has associated human cerebrovascular 5-$HT_{1D}$ receptors with the cloned 5-$HT_{1D\beta}$ subtype (Hamel et al., 1993a, b). This may imply that, according to the vascular theory of migraine in which an important component is represented by the distension of large intracranial vessels during the headache phase (see Humphrey and Feniuk, 1991), the vasoconstrictor effect of sumatriptan in intracranial vessels is mediated by the same receptor subtype (5-$HT_{1D\beta}$) involved in the constriction of the coronary artery (see Kaumann et al., 1994) and, consequently, that the beneficial and unwanted effects of the drug cannot be dissociated.

To conclude, this study confirms previous suggestions that a 5-$HT_{1D}$ receptor mediates contraction in the dog coronary artery. Definite characterization of this vascular receptor will await the identification of drugs that discriminate between the two cloned dog 5-$HT_{1D}$ receptors.

## Acknowledgements

The skilful technical assistance of Juan J. López-Guerrero (B.Sc.) is acknowledged. The author thanks the generosity of the companies supplying some of the drugs used in this work.

## References

Branchek, T.A., J.A. Bard, S.A. Kucharewicz, J.M. Zgombick, R.L. Weinshank and M.L. Cohen, 1995, Migraine-relationship to cloned canine and human 5-$HT_{1D}$ receptors, in: Experimental Headache Models, eds. J. Olesen and M.A. Moskowitz (Raven Press, New York) p. 125.

Cushing, D.J. and M.L. Cohen, 1992a, Comparison of the serotonin receptors that mediate smooth muscle contraction in the canine coronary artery, J. Pharmacol. Exp. Ther. 261, 856.

Cushing, D.J. and M.L. Cohen, 1992b, Serotonin-induced relaxation in canine coronary artery smooth muscle, J. Pharmacol. Exp. Ther. 263, 123.

Cushing, D.J., M. Baez, J.D. Kursar, K. Schenck and M.L. Cohen, 1994, Serotonin-induced contraction in the canine coronary artery and saphenous vein: role of a 5-$HT_{1D}$-like receptor, Life Sci. 54, 1671.

Hamel, E., L. Grégoire and B. Lau, 1993a, 5-$HT_1$ receptors mediating contraction in bovine cerebral arteries: a model for human cerebrovascular '5-$HT_{1D\beta}$' receptors, Eur. J. Pharmacol. 242, 75.

Hamel, E., E. Fan, D. Linville, V. Ting, J.G. Villemure and L.S. Chia, 1993b, Expression of mRNA for the serotonin 5-hydroxytryptamine$_{1D\beta}$ receptor subtype in human and bovine cerebral arteries, Mol. Pharmacol. 44, 242.

Hartig, P.R., T.A. Branchek and R.L. Weinshank, 1992, A subfamily of 5-$HT_{1D}$ receptor genes, Trends Pharmacol. Sci. 13, 152.

Houston, D.S. and P.M. Vanhoutte, 1988, Comparison of serotonergic receptor subtypes on the smooth muscle and endothelium of the canine coronary artery, J. Pharmacol. Exp. Ther. 244, 1.

Hoyer, D. and P. Schoeffter, 1991, 5-HT receptors: subtypes and second messengers, J. Receptor Res. 11, 197.

Humphrey, P.P.A. and W. Feniuk, 1991, Mode of action of the anti-migraine drug sumatriptan, Trends Pharmacol. Sci. 12, 444.

Kaumann A.J., M. Frenken, H. Posival and A.M. Brown, 1994, Variable participation of 5-$HT_1$-like receptors and 5-$HT_2$ receptors in serotonin-induced contraction of human isolated coronary arteries. 5-$HT_1$-like receptors resemble cloned 5-$HT_{1D\beta}$ receptors, Circulation 90, 1141.

Schoeffter, P. and D. Hoyer, 1989, How selective is GR43175? Interactions with functional 5-$HT_{1A}$, 5-$HT_{1B}$, 5-$HT_{1C}$ and 5-$HT_{1D}$ receptors, Naunyn-Schmied. Arch. Pharmacol. 340, 135.

Skingle, M., D.I.C. Scopes, W. Feniuk, H.P. Connor, M.C. Carter, J.W. Clitherow and M.B. Tyers, 1993, GR127935: a potent orally active 5-$HT_{1D}$ receptor antagonist, Br. J. Pharmacol. 110, 9P.

Tallarida, R.J., A. Cowan and M.W. Adler, 1979, $pA_2$ and receptor differentiation: a statistical analysis of competitive antagonism, Life Sci. 25, 637.

Weinshank, R.L., T. Branchek and P.R. Hartig, 1991, International Application Patent, International Publication Number WO '91/17174, 14-11-1991.

Zgombick, J.M., R.L. Weinshank, M. Macchi, L.E. Schechter, T.A. Branchek and P.R. Hartig, 1991, Expression and pharmacological characterization of a canine 5-hydroxytryptamine$_{1D}$ receptor subtype, Mol. Pharmacol. 40, 1036.