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The 5-HT_2 receptor antagonist, pelanserin, inhibits α_1-adrenoceptor-mediated vasoconstriction in vitro

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Abstract

The antagonism by pelanserin (2,4(1*H*,3*H*)-quinazolinedione,3-[3-(4-phenyl-1-piperazinyl)-propyl]-HCl), a potent 5-HT_2 receptor antagonist, of α_1-adrenoceptor-mediated contractions of endothelium-denuded carotid, aorta, mesenteric and caudal arteries of both normotensive Wistar-Kyoto (WKY) and spontaneously hypertensive (SHR) rats was investigated. The selective α_1-adrenoceptor agonist methoxamine elicited concentration-dependent contractions in all four arterial rings, an effect which was competitively antagonized by pelanserin. pA_2 values for pelanserin were in the 7.67–8.11 range when evaluated against the α_1-adrenoceptor agonist in arteries from normotensive or hypertensive rats. These data support the conclusion that pelanserin displays α_1-adrenoceptor blocking properties. The ability of the 5-HT_2 receptor antagonist pelanserin to additionally block α_1-adrenoceptor-mediated constriction in different vessels of WKY and SHR may potentially contribute to its blood pressure lowering effects.

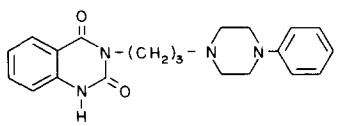
Keywords: Pelanserin; 5-HT receptor antagonist; Contraction; Methoxamine; Spontaneously hypertensive rat (SHR)

1. Introduction

Since the early 1960s there have been reports of interactions between α-adrenergic and serotonergic systems (Vane, 1960; Innes, 1962). In the past decade, more experimental evidence appeared indicating that some agonists (5-hydroxytryptamine, 5-HT), and antagonists (metitepine, ketanserin, cyproheptadine), of the 5-HT receptor subtypes are able to interact with α-adrenoceptors (Purdy et al., 1985, 1987; Van Nueten et al., 1981; Doods et al., 1988). It has been shown very recently that selective 5-HT_1 receptor agonists, like buspirone and 8-hydroxy-2-(di-*N*-propylamino)tetralin (8-OH-DPAT) (Castillo et al., 1993a), or ipsapirone (Eltze et al., 1991; Eltze and Boer, 1992; Castillo et al., 1993b), interact with α_{1A}-adrenoceptors in vascular tissue, suggesting similarities among these receptor systems that can help us to understand their role in modulating cell responses.

Hong et al. (1984, 1985) described an antihypertensive agent that interacts with 5-HT_2 receptors, pelanserin (also known as TR2515; 2,4(1*H*,3*H*)-quinazolinedione,3-[3-(4-phenyl-1-piperazinyl)-propyl]-HCl; Fig. 1), and which also shows α-adrenergic antagonism. Its antihypertensive effects have also been tested in renal hypertensive dogs (Flores-Murrieta et al., 1992). Recently, we were able to demonstrate that pelanserin blocks epinephrine-induced phosphatidylinositol turnover in rabbit aorta, but with a potency 1/100th that of prazosin (Villalobos-Molina et al., 1991).

Since pelanserin is a putative antihypertensive agent having some anti-α-adrenergic effects (Hong et al., 1984, 1985; Flores-Murrieta et al., 1992), it became



PELANSERIN (TR2515)

Fig. 1. Structural formula of pelanserin.

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important to study its actions at the adrenoceptor level in a well-characterized model: smooth muscle contraction. The present experiments were designed to assess if pelanserin affects the vascular reactivity of isolated arteries of normotensive (WKY) and spontaneously hypertensive (SHR) rats and the contraction induced by methoxamine, a selective α_1-adrenoceptor agonist.

2. Materials and methods

2.1. Determination of isometric tension changes

Male normotensive (WKY) and spontaneously hypertensive (SHR) rats of 6 months of age (±1 week) were reared in our animal facility and fed ad libitum on a standard diet (Purina, Mexico). The rats were anaesthetized with ether and the arteries were dissected. Carotid, aorta, mesenteric and caudal rings (3–4 mm in length) were placed in Krebs solution and freed of any connective tissue. Systolic blood pressure and cardiac frequency were measured in the conscious state by a tail-cuff method (average values for blood pressure were, 137 ± 3 mm Hg (WKY) and 179 ± 4 mm Hg (SHR); and for cardiac frequency, 357 ± 11 (WKY) and 398 ± 9 (SHR) beats/min, ($n = 6$)). Arterial rings were bathed in a 10 ml chamber, filled with Krebs solution (composition, in mM: NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 25, EDTA, 0.026; glucose, 11.1; at 37° C, pH 7.4 and bubbled with 95% O_2:5% CO_2). They were attached to the bottom of the chamber and to an isometric FT03 Grass force displacement transducer, connected to a 7D Grass polygraph. Arterial rings were subjected to an initial optimal tension of 3 g (carotid, aorta and mesenteric) or 2 g (caudal), as evaluated in preliminary experiments using increments in initial tension to reach the optimal. The tissue was challenged with methoxamine (3.1×10^{-6} M) and washed every 30 min for 2 h. Then, reproducible cumulative concentration-response curves for methoxamine (10^{-7} to 3.1×10^{-5} M) were obtained for each of the arteries. Pelanserin (10^{-8} to 10^{-7} M) was present 15 min before and throughout the experiment. In the present report endothelium-denuded arteries, obtained by rubbing the intima with a forceps, were used in order to avoid the possible role of endothelium-derived relaxing factor in the response (Furchgott and Zawadzki, 1980). In parallel experiments (not shown) we found that the presence of the endothelium did not affect the response to methoxamine nor the effect of pelanserin, when both WKY and SHR were compared. The absence of endothelium was determined by the lack of a relaxing response to acetylcholine (10^{-6} M).

In order to avoid fatigue of the arterial preparation, a 60 min recovery period was allowed between methoxamine curves. To rule out any change in tissue sensitivity to the agonist, due to the long duration of these experiments, parallel assays were done, i.e., control arteries in the presence of the agonist and arteries in the presence of the agonist plus the antagonist.

2.2. Analysis of data

Contraction is given in grams for an easy comparison among the experiments. Data are the means ± S.E.M. for six different animals for each group. The pD_2 values ($-\log EC_{50}$), calculated by nonlinear regression analysis, and the maximal response (E_{max}) were determined from individual concentration-response curves for methoxamine. The pA_2 and slope (m) values were calculated according to the method described by Arunlakshana and Schild (1959); pK_B values were calculated as described by Furchgott (1972). Statistical analysis was done by using the parallelism test (Tallarida and Murray, 1981) and Student's t-test.

2.3. Chemicals

Methoxamine was a generous gift of Burroughs Wellcome (NJ, USA); pelanserin was from our laboratory. Pelanserin was dissolved in distilled water. All other reagents were of analytical grade.

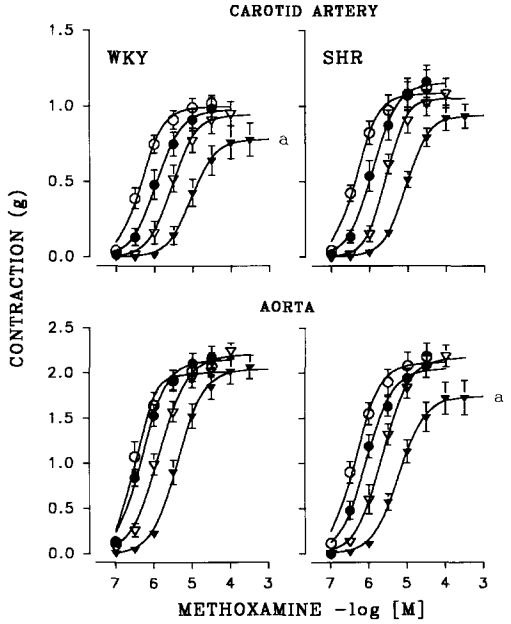

Fig. 2. Antagonism by pelanserin of methoxamine-induced contraction in carotid or aorta arteries from WKY and SHR rats. Carotid or aortic rings of 3–4 mm in length were exposed to none (○, control) or to different concentrations of pelanserin (○, 10^{-8} M; ▽, 3.1×10^{-8} M; ▼, 10^{-7} M), for 15 min before and throughout the experiment. Data represent the means ± S.E.M. for six different animals per group. [a] Maximal effect was significantly depressed ($P < 0.05$).

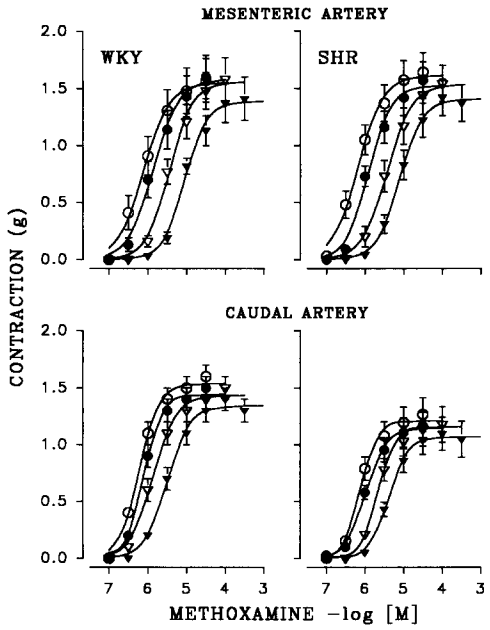

Fig. 3. Antagonism by pelanserin of methoxamine-induced contraction in mesenteric or caudal arteries from WKY and SHR rats. Mesenteric or caudal rings of 3–4 mm in length were exposed to none (○, control), or to different concentrations of pelanserin (○, 10^{-8} M; ▽, 3.1×10^{-8} M; ▼, 10^{-7} M), for 15 min before and throughout the experiment. Data represent the means ± S.E.M. for six different animals per group.

3. Results

Cumulative concentration-response curves for methoxamine (10^{-7} to 3.1×10^{-5} M; each concentration given until stabilization of the response) were obtained in the different blood vessels of both WKY and SHR rats. As observed in Figs. 2 and 3, the arteries responded to the α_1-adrenoceptor agonist with the same pattern, irrespective of the strain. The pD_2 ($-\log EC_{50}$) values were similar when WKY and SHR arteries were compared (Table 1). In general, the contractile response to methoxamine was greater in the SHR-derived arteries, except for the caudal artery where the response was lower than in the WKY (Table 1).

In the carotid artery, pelanserin caused a parallel shift to the right of the concentration-response curve for the α_1-adrenoceptor agonist in both strains of rats (Fig. 2, upper panels). The presence of pelanserin (10^{-7} M) depressed the maximal contraction induced by methoxamine in both types of arteries, being significant ($P < 0.05$) in the WKY artery (Fig. 2, upper left). The pA_2 and pK_B values varied between 8.09 and 8.20 among WKY and SHR; the Schild slope (m) values were significantly ($P < 0.05$) higher than 1 (Table 2).

When aortic rings were contracted by methoxamine, the presence of pelanserin caused a parallel shift to the right of the concentration-response curve for the artery (Fig. 2, lower panels). Pelanserin (10^{-7} M) significantly ($P < 0.05$) depressed the maximal contraction in aortae from SHR (Fig. 2, lower right). The pA_2 and pK_B values for this artery varied in the range of 7.67–8.02, the m values were also significantly ($P < 0.05$) higher than the theoretical value of 1 (Table 2).

In the mesenteric artery of both WKY and SHR pelanserin was able to produce a parallel shift to the right of the concentration-response curve for methoxamine (Fig. 3, upper panels). Pelanserin did not depress the maximal contraction evoked by methoxamine in this artery. The pA_2 and pK_B values varied between 7.81 and 8.11, and m was significantly ($P < 0.05$) higher than 1 in WKY but not in SHR (Table 2).

In the caudal artery, the 5-HT_2 receptor antagonist also caused a parallel shift to the right of the concentration-response curve to the α_1-adrenoceptor agonist (Fig. 3, lower panels). In this artery, pelanserin did not modify the maximal contractile effect of methoxamine. The pA_2 and pK_B values obtained in the caudal artery

Table 1
pD_2 and maximal effect (E_{max}) values for methoxamine in blood vessels of WKY and SHR rats

Artery	WKY		SHR	
	pD_2	E_{max} (g)	pD_2	E_{max} (g)
Carotid	6.31 ± 0.04	1.02 ± 0.05	6.34 ± 0.07	1.12 ± 0.12
Aorta	6.45 ± 0.05	2.07 ± 0.11	6.32 ± 0.05	2.18 ± 0.15
Mesenteric	6.07 ± 0.08	1.57 ± 0.19	6.17 ± 0.05	1.64 ± 0.17
Caudal	6.14 ± 0.02	1.60 ± 0.10	6.25 ± 0.03	1.27 ± 0.15

Data from Figs. 2 and 3. Each value is the mean ± S.E.M. for six different animals.

Table 2
pA_2, pK_B and slope (m) values for pelanserin in WKY and SHR arteries

Artery	WKY			SHR		
	pA_2	m	pK_B	pA_2	m	pK_B
Carotid	8.09 ± 0.05	1.53 ± 0.14 [a]	8.20 ± 0.10	8.11 ± 0.12	1.38 ± 0.14 [a]	8.09 ± 0.16
Aorta	7.67 ± 0.06	1.69 ± 0.12 [a]	7.85 ± 0.04	7.95 ± 0.10 [b]	1.44 ± 0.16 [a]	8.02 ± 0.12
Mesenteric	7.87 ± 0.07	1.35 ± 0.06 [a]	7.81 ± 0.09	8.11 ± 0.07	1.15 ± 0.09	8.07 ± 0.08
Caudal	7.75 ± 0.04	1.21 ± 0.10	7.76 ± 0.07	7.77 ± 0.08	1.24 ± 0.11	7.71 ± 0.11

Data from Figs. 2 and 3. Each value represents the mean ± S.E.M. for six different animals. [a] Different from 1, $P < 0.05$. [b] Different from WKY, $P < 0.05$.

varied between 7.71 and 7.77 for WKY and SHR, respectively, and in this artery the *m* values were not statistically different from 1 (Table 2).

4. Discussion

As has been reported previously (Villalobos-Molina et al., 1991), pelanserin antagonizes serotonin- and epinephrine-evoked contraction and phospholipid turnover in isolated rabbit aorta, suggesting that pelanserin interacts with 5-HT_2 receptors and with α_1-adrenoceptors. Some of these interactions also occur between 5-HT_{1A} receptor agonists which are antagonists at α_{1A}-adrenoceptors (Eltze et al., 1991; Castillo et al., 1993a,b), and between 5-HT_2 receptor antagonists which act as antagonists at α_1-adrenoceptors (Van Nueten et al., 1981; Hong et al., 1984, 1985; Villalobos-Molina et al., 1991).

The results presented in this report clearly demonstrate that pelanserin is able to antagonize α_1-adrenoceptor-evoked contractions of arteries from WKY and SHR rats.

Pelanserin antagonized the methoxamine-stimulated contraction in a concentration-related way in all four arteries tested, indicating that the 5-HT_2 receptor antagonist blocked the α_1-adrenoceptors present in these arteries. As observed, increasing concentrations of methoxamine overcame pelanserin-evoked antagonism (Figs. 2 and 3), indicating a competitive interaction except in some cases, when pelanserin (10^{-7} M) decreased the maximal contraction (carotid: WKY, and aorta: SHR), suggesting a non-competitive type of antagonism at higher concentrations of pelanserin.

The similarity of the pA_2 values obtained for pelanserin in the four different arteries, taken from either WKY or SHR, indicates that these arteries contain α_1-adrenoceptors that exhibit a similar affinity for pelanserin. Similar findings have been observed between the 5-HT_2 receptor antagonist, ketanserin, and α_1-adrenergic-induced contraction in rat caudal artery (Van Nueten et al., 1981) and in rabbit aorta (Villalobos-Molina et al., 1991).

The pA_2 values of pelanserin in the different arteries show that the 5-HT_2 antagonist has a relatively high affinity for α_1-adrenoceptors in these tissues, since by comparison with reported pA_2 values of 7.03 for 5-methyl-urapidil and 8.53 for WB 4101 (two selective α_{1A}-adrenoceptor antagonists) in rat aorta (Eltze and Boer, 1992), the affinity of pelanserin is between 4 and 8 times higher than that of 5-methyl-urapidil in this artery (pA_2 values: 7.67 and 7.95 for WKY and SHR, respectively, Table 2). In the study of Van Nueten et al. (1981), a pA_2 value of 7.74 for ketanserin was obtained when the caudal artery of the rat was contracted with norepinephrine, as compared to 7.75 in this report for pelanserin in the same artery (Table 2). In a previous study (Ibarra et al., unpublished results), we found that the antagonism by pelanserin of norepinephrine-induced contraction of the caudal artery (pA_2 values: 7.97 and 7.57 for WKY and SHR, respectively) was between 20 and 50 times less potent than when 5-hydroxytryptamine was the agonist (pA_2 values: 9.33 and 8.90 for WKY and SHR, respectively).

As observed, the slopes of the Schild plot values were steeper than 1, indicating that another action of the drug is involved in the responses (Furchgott, 1972; Tallarida et al., 1979). The present results may help explain the antihypertensive properties of pelanserin, supporting previous observations (Hong et al., 1984, 1985; Flores-Murrieta et al., 1992). In conclusion, our results suggest that pelanserin is a potent α_1-adrenoceptor antagonist and has the potential advantage of interacting with drugs that affect both 5-HT_2 and α_1-adrenoceptor systems.

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