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Insurmountable antagonism of AT-1015, a 5-HT_2 antagonist, on serotonin-induced endothelium-dependent relaxation in porcine coronary artery

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Abstract

The purpose of this study was to examine the inhibitory effects of AT-1015, a newly synthesized 5-HT_2 receptor antagonist, on serotonin-induced endothelium-dependent relaxation in U 46619 (5×10^{-9} M)-precontracted porcine coronary artery pre-incubated with ketanserin (3×10^{-6} M), and then compare its effects with another potent 5-HT_2 antagonist, ritanserin. The investigation showed that AT-1015 (10^{-8}–10^{-6} M) caused rightward shift with significant inhibition of maximum relaxation response induced by serotonin in porcine coronary artery with endothelium. Ritanserin caused a rightward shift of serotonin-induced relaxation without decreasing maximum response at 10^{-9} and 10^{-8} M, but it inhibited the maximum relaxation response at 10^{-7} M. The study showed that AT-1015 and ritanserin had no inhibitory effect on bradykinin-induced relaxation in porcine coronary artery with endothelium. Thus, these findings suggested that AT-1015 at concentrations of 10^{-8}–10^{-6} M caused noncompetitive blockade of serotonin-induced endothelium-dependent relaxation in porcine coronary artery. The antagonistic effects of AT-1015 on serotonin-induced relaxation were different from that of ritanserin, except at 10^{-7} M ritanserin. The variation of inhibitory effects between these two 5-HT_2 antagonists may be due to the different chemical structure and/or interaction sites at the receptor.

Introduction

Serotonin (5-hydroxytryptamine, 5-HT) induces both endothelium-dependent and endothelium-independent relaxation of a number of isolated blood vessels in a variety of animals. Serotonin causes endothelium-dependent relaxation responses in porcine coronary and pulmonary artery. It has been reported that endothelium-dependent relaxant effects of serotonin in pig coronary and pulmonary artery were mediated by 5-HT_1-like and 5-HT_{2B} receptors, respectively (Cocks & Angus 1983; Molderings et al 1989; Schoeffter & Hoyer 1990; Glusa & Pertz 2000). On the other hand, serotonin-induced constriction in human, monkey and dog coronary arteries was mediated mainly by 5-HT_{2A} receptor and also by 5-HT_1-like subtypes (Toda & Okamura 1990).

AT-1015 is a potent 5-HT_{2A}-receptor antagonist and it reduced the maximum contraction response induced by serotonin in rat aorta. It selectively inhibited in-vitro 5-HT_{2A} receptor mediated platelet aggregation and prevented the laurate-induced peripheral vascular lesion in rats (Kihara et al 2000). Previously, it was found that AT-1015 was a strong noncompetitive 5-HT_2 antagonist and it inhibited the maximum contraction response induced by serotonin in pig coronary artery without endothelium (Gong et al 2000). In addition, it was observed that AT-1015 had high binding affinity to 5-HT_2 receptors in rabbit cerebral cortex membranes (Rashid et al 2001) and it dissociated slowly from 5-HT_2 receptors in rabbit cerebral cortex membranes (Rashid et al 2001, 2002a). More recently, it was reported that AT-1015 was a potent and long acting antithrombic agent with a low risk of bleeding time prolongation in a photochemically-induced arterial thrombosis model in the rat femoral artery (Kihara et al 2001).

We have investigated the effects of AT-1015 (Figure 1) on serotonin-induced endothelium-dependent relaxation in porcine coronary artery mediated by

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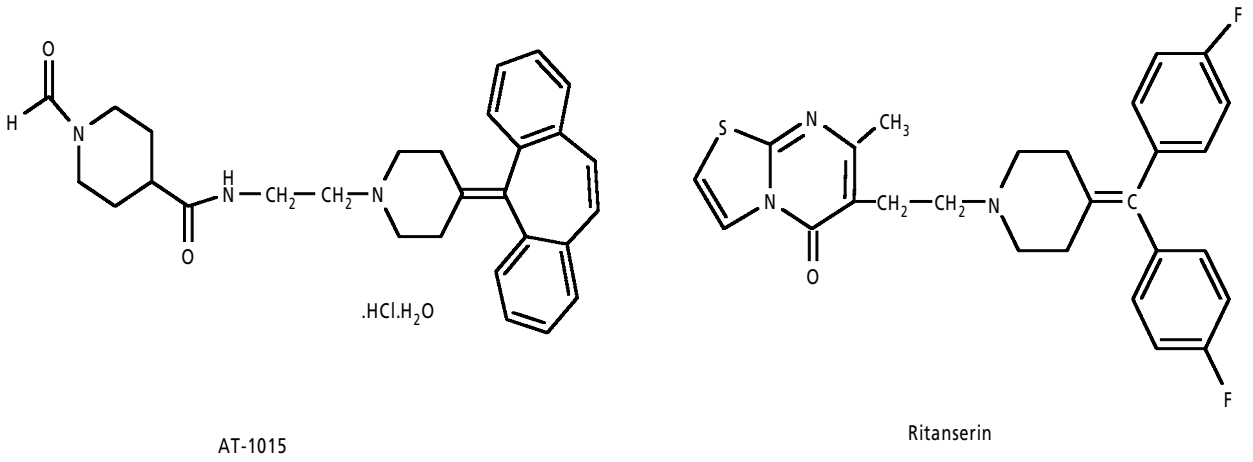

Figure 1 Chemical structures of AT-1015 and ritanserin.

5-HT_1-like receptor and have compared its antagonistic activity with another potent and long-acting 5-HT_2 antagonist, ritanserin (Figure 1).

Materials and Methods

Experimental protocol

Relaxation-response study in porcine coronary artery was determined using the method described by Rashid et al (2002b). In brief, the first branch of the left anterior descending coronary artery was dissected and removed from the surrounding tissue, and was cut into rings of 2–3 mm in length. Care was taken to keep the endothelium of the artery intact. Vascular rings were mounted on two stainless steel hooks inserted through the lumen of the ring. Each ring was suspended in a 10-mL organ bath and immersed in Krebs–Henseleit buffer (in mM: NaCl 118.4, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25.0, $CaCl_2$ 2.5, Na_2EDTA 0.026 and dextrose 11.1) maintained at 37 °C and bubbled continuously with 95% O_2 and 5% CO_2. The ring was allowed to equilibrate for 60–90 min with an optimal resting tension of 1.5 g before all experiments. Ketanserin (3×10^{-6} M) was added to the bath 20 min before addition of U 46619 (5×10^{-9} M, a concentration producing approximately 50% of the maximal effect). With the pretreatment of ketanserin (3×10^{-6} M), serotonin produces maximum relaxation response. Ketanserin was used to block the contractile response of serotonin probably mediated by 5-HT_{2A} receptor, which in turn can conceivably result in the relaxant effect. After a steady-state contraction produced by U 46619, serotonin or bradykinin was added cumulatively. Ketanserin was not added before addition of U 46619 in the case of bradykinin-induced concentration-dependent relaxation response. Bradykinin (10^{-7} M) was added at the end of the addition of the last concentration of serotonin to examine the functional integrity of the endothelium corresponding to a maximal relaxation.

In the experiments with the 5-HT_2 antagonists AT-1015 and ritanserin, drugs were added 20 min before the contraction produced by U 46619. Two concentration–response curves were obtained in each preparation, one for the control and the second for the antagonist-treated preparations.

Data analysis

The data of force responses were calculated as total developed tension minus resting tension immediately before addition of each agonist. Relaxation elicited by serotonin and bradykinin with antagonist treatment was expressed as a percentage of the maximum relaxation response (control) produced by serotonin and bradykinin, respectively. Results are presented as mean ± s.e.m. of experiments. Statistical significance of the data was evaluated using Student's *t*-test for comparison of two groups and one-way analysis of variance followed by Tukey's test for comparison of more than three groups. The agonist EC50 values of the concentration–response curve were calculated by non-linear analysis using the Sigma Plot Program (Jandel Scientific, San Rafael, CA). Antagonist pA_2 and pD'_2 values were calculated using the equation of Van Rossum et al (1963): $pA_2 = pA_x + \log (X - 1)$, where pA_x is the negative logarithm of antagonist concentration and X is the ratio of EC50 values of the agonist with/without antagonist; $pD'_2 = pD'_x + \log (X - 1)$, where pD'_x is the negative logarithm of antagonist concentration and X is the ratio of maximal effects of the agonist in the presence and absence of the antagonist.

Drugs used

Serotonin (serotonin-creatinine sulfate) was from Toray Industries Inc., Tokyo, Japan. Bradykinin and U 46619 (9,11-dideoxy-11 α, 9α-epoxy-methano-prostaglandin $F_{2\alpha}$) were from Wako Pure Chemical Industries Ltd, Osaka,

Japan. AT-1015 (N-[2-[4-(5H-dibenzo[a,d]cyclohepten-5-ylidene)-piperidino]ethyl]-1-formyl- 4-piperidinecarboxamide monohydrochloride monohydrate) was donated by Ajinomoto Co. Ltd, Tokyo, Japan. Ketanserin and ritanserin were from Sigma-RBI, St Louis, MO.

Results

Effects of AT-1015 and ritanserin on serotonin-induced relaxation

The inhibitory effects of AT-1015 and ritanserin on the relaxation-response curve of serotonin in porcine coronary artery with endothelium pre-incubated with ketanserin (3×10^{-6} M) and precontracted by U 46619 (5×10^{-9} M) are shown in Figure 2. AT-1015 caused a rightward shift on the concentration–relaxation curve of serotonin with significant inhibitory effects on maximum relaxation response at 10^{-8}–10^{-6} M (Figure 2A). Ritanserin at 10^{-9} and 10^{-8} M induced rightward shift without decreasing maximum relaxation response, whereas at 10^{-7} M caused rightward shift with significant inhibition of maximum response (Figure 2B). As shown in Table 1, mean pD'_2 values of AT-1015 at 10^{-8}, 10^{-7} and 10^{-6} M were 7.34, 6.54 and 5.84, respectively. This antagonism was noncompetitive, as the slope value (0.57) of the Schild plot was significantly different from unity (figure not shown). Mean pA_2 values of ritanserin at 10^{-9} and 10^{-8} M were 9.17 and 8.08, respectively, and the mean pD'_2 value was 6.89 at 10^{-7} M (Table 1) and the slope value (0.85) of the Schild plot was not significantly different from unity.

Effects of AT-1015 and ritanserin on bradykinin-induced relaxation

Figure 3 shows the effects of AT-1015 and ritanserin on the bradykinin-induced relaxation responses in porcine coronary artery with endothelium. AT-1015 (10^{-8}–10^{-6} M) and ritanserin (10^{-9}–10^{-7} M) did not inhibit bradykinin induced concentration-dependent relaxation of porcine coronary artery with endothelium.

Discussion

It had been previously reported that serotonin induced endothelium-dependent relaxation in U 46619-precontracted pig coronary artery with ketanserin pretreatment. In this study, we have used the same experimental protocol as in our previous study (Rashid et al 2002b). Ketanserin has high affinity to 5-HT_2 receptors and it has affinity to α_1-adrenergic receptor and it inhibits the vasoconstriction mediated by those receptors. Ritanserin is a very potent and long acting 5-HT_2 antagonist (Leysen et al 1985) and its selectivity towards 5-HT_{2A}–receptor subtype is higher than the 5-HT_{2B}- and 5-HT_{2C}-receptor subtypes (Bonhaus et al 1995). In this study, we have explored the inhibitory effects of AT-1015 on serotonin- and bradykinin-induced relaxations in porcine coronary

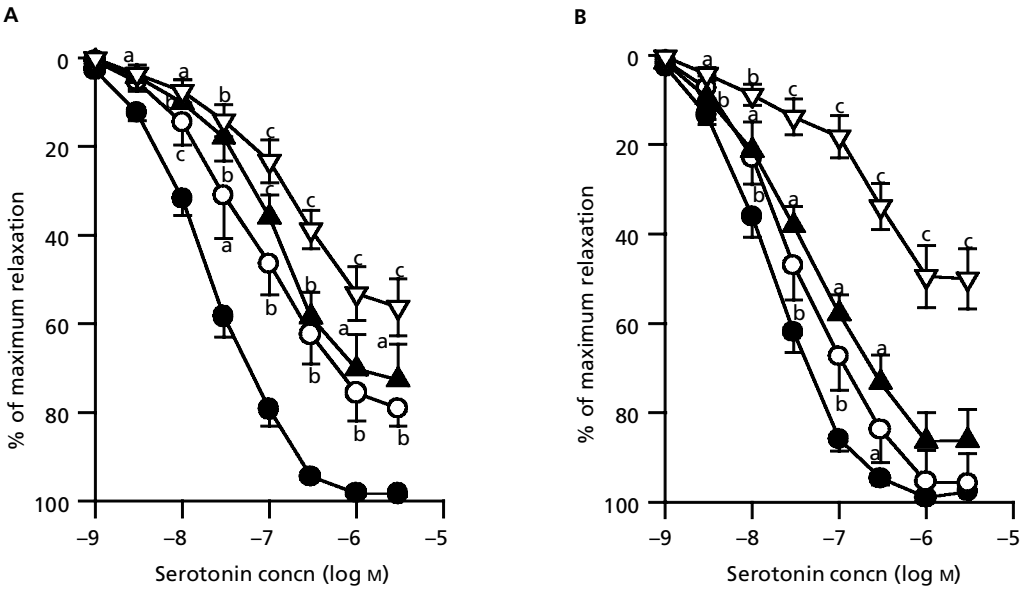

Figure 2 Effect of AT-1015 and ritanserin on serotonin-induced concentration-dependent relaxation in porcine coronary artery with endothelium. A. AT-1015 (● control, n = 8; ○ 10^{-8} M, n = 6; ▲ 10^{-7} M, n = 6; ▽ 10^{-6} M, n = 8). B. Ritanserin (● control, n = 7; ○ 10^{-9} M, n = 5; ▲ 10^{-8} M, n = 5; ▽ 10^{-7} M, n = 7). All concentration–response curves were carried out in the presence of ketanserin (3×10^{-6} M). The relaxation responses occurred by cumulative addition of serotonin with antagonist treatment and were expressed as a percentage of the maximum relaxation response of serotonin in the absence of antagonist. Each point represents the mean response ± s.e.m. [a]$P < 0.05$, [b]$P < 0.01$ and [c]$P < 0.001$ compared with control.

Table 1 pA_2 and pD'_2 values of AT-1015 and ritanserin for serotonin-induced endothelium-dependent relaxation in porcine coronary artery.

Antagonists	Concn (M)	pA_2 value (n)	pD'_2 value (n)
AT-1015	10^{-8}	—	7.34 ± 0.12 (6)
	10^{-7}	—	6.54 ± 0.16 (6)
	10^{-6}	—	5.84 ± 0.14 (8)**
Ritanserin	10^{-9}	9.17 ± 0.13 (5)	—
	10^{-8}	8.08 ± 0.26 (5)	—
	10^{-7}	—	6.89 ± 0.15 (7)

Values are mean ± s.e.m. The number in the parenthesis indicates the number of experiments. $^{**}P < 0.01$ compared with ritanserin 10^{-7} M.

artery with endothelium and have compared its effects with ritanserin.

AT-1015 at 10^{-8}–10^{-6} M caused a rightward shift with significant inhibition of maximum relaxation response induced by serotonin in porcine coronary artery with endothelium (Figure 2A). This suggested that AT-1015 might inhibit the relaxation response induced by serotonin that was mediated mainly by receptors resistant to ketanserin, as all the experiments were carried out in the presence of ketanserin. On the other hand, ritanserin shifted the serotonin concentration–response curve to the right without significant effect on maximum response at 10^{-9} and 10^{-8} M, but at 10^{-7} M it shifted the concentration-response curve of serotonin to the right with decreasing maximum response (Figure 2B). The antagonistic potency (mean pD'_2 value) of AT-1015 on serotonin-induced relaxation were 7.34, 6.54 and 5.84 at 10^{-8}, 10^{-7} and 10^{-6} M, respectively, and the slope value (0.57) of the Schild plot analysis depicted the noncompetitive antagonism of AT-1015 of the relaxation–response curve to serotonin. The results indicated that AT-1015 acted as a noncompetitive or insurmountable antagonist at all the concentrations used in this study, whereas ritanserin acted as both competitive and noncompetitive antagonist depending on the concentration used, and at 10^{-7} M it acted as a noncompetitive or insurmountable antagonist. Therefore, the antagonistic effect of AT-1015 on serotonin-induced relaxation was different compared with ritanserin, except at 10^{-7} M ritanserin.

This variation of the inhibitory effects between AT-1015 and ritanserin gives rise to the question as to why these two antagonists do not act in the same way, although both drugs slowly dissociate from the 5-HT_2 receptor sites. De Chaffoy De Courcelles et al (1986) reported that only slowly reversible binding could explain the apparent noncompetitive inhibition of the biochemical effects. Several hypothetical mechanisms have been proposed to explain the molecular basis for insurmountable antagonism. Recently, we reported (Rashid et al 2001) that AT-1015 was a slowly dissociating 5-HT_2 antagonist. AT-1015 might have allosteric binding sites in the receptor,

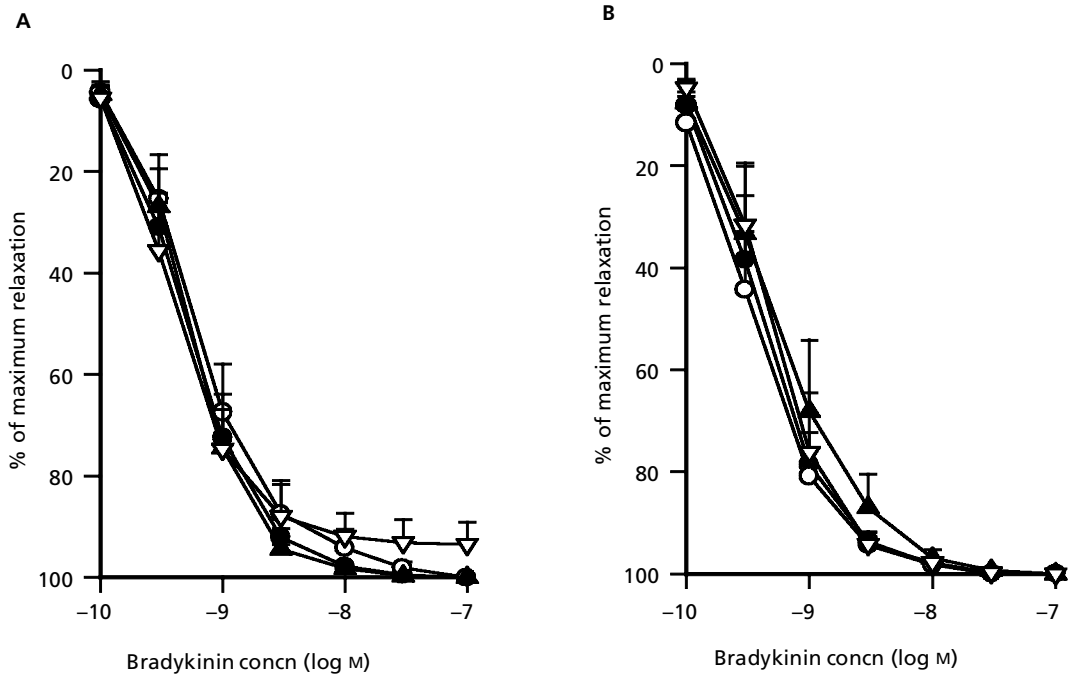

Figure 3 Effect of AT-1015 and ritanserin on bradykinin-induced concentration-dependent relaxation in porcine coronary artery with endothelium. A. AT-1015 (● control; ○ 10^{-8} M; ▲ 10^{-7} M; Δ = 10^{-6} M). B. Ritanserin (● control; ○ 10^{-9} M; ▲ 10^{-8} M; ▽ = 10^{-7} M). The relaxation responses occurred by cumulative addition of bradykinin with antagonist treatment and were expressed as a percentage of the maximum relaxation response of bradykinin in the absence of antagonist. Each point represents the mean response ± s.e.m. of four to six arterial rings.

which could be responsible for insurmountable antagonism although further studies are required to clarify the exact mechanism of this phenomenon (Rashid et al 2002a). In contrast, ritanserin also slowly dissociates from 5-HT_2 receptors (Leysen et al 1985), and so far, allosteric modulation of ritanserin in the receptor sites has not yet been reported. On the other hand, the difference in these main pharmacological profiles between AT-1015 and ritanserin may be due to different chemical structures. The basic chemical structure of AT-1015 and ritanserin is similar, that is, ethylpiperidine. However, they possess two different hydrophobic residues at two positions in their basic structures (Figure 1). Thus, we may predict that these four different groups may affect the 5-HT_2 blocking potencies and/or different affinities to serotonin receptor subtypes for contractile and relaxation of coronary arteries. However, further studies are required to clarify the exact reasons for this variation.

This study revealed that AT-1015 (10^{-8}–10^{-6} M) and ritanserin (10^{-9}–10^{-7} M) did not inhibit the bradykinin-induced relaxation response in U 46619-precontracted porcine coronary artery with endothelium (Figure 3A, B), but the same concentrations of these antagonists could compete against serotonin-induced relaxation response. Serotonin stimulates serotonin receptors in endothelial cell to release NO that causes relaxation (Matsumoto et al 1993). On the other hand, bradykinin stimulates endothelial cell to release NO and EDHF (endothelium-derived hyperpolarizing factor) that causes relaxation (Kilpatrick & Cocks 1994). This suggested that AT-1015 and ritanserin could compete only at the serotonin receptors existing in the endothelium of porcine coronary artery.

Previously (Rashid et al 2002b), it was observed that sarpogrelate competitively inhibited 5-HT-induced endothelium-dependent relaxation mediated by 5-HT_1-like receptors in porcine coronary artery and its effect was weaker than that of other 5-HT_2 receptor selective antagonists, such as ritanserin and cyproheptadine. In this study, AT-1015 (10^{-8}–10^{-6} M) noncompetitively inhibited serotonin-induced relaxation response and its antagonistic effects were different from that of ritanserin, except at 10^{-7} M ritanserin. The results suggested that AT-1015 at slightly higher concentrations would be required for the inhibition of relaxation response in comparison with its antagonistic effect on contraction response (pK_B value 9.04) in our previously published report (Gong et al 2000). Therefore, these findings will be helpful for further research as well as for its clinical implications.

In conclusion, AT-1015 (10^{-8}–10^{-6} M) caused noncompetitive blockade of serotonin-induced relaxation in porcine coronary artery with endothelium and its effects were different from that of ritanserin.

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