

## Effects of Sarpogrelate, a Novel 5-HT<sub>2</sub> Antagonist, on 5-HT-Induced Endothelium-Dependent Relaxations in Porcine Coronary Artery

Mamunur Rashid<sup>1</sup>, Mikio Nakazawa<sup>2</sup> and Takafumi Nagatomo<sup>1,\*</sup>

<sup>1</sup>*Department of Pharmacology, Niigata College of Pharmacy, 5-13-2 Kamishinei-cho, Niigata 950-2081, Japan*

<sup>2</sup>*Department of Medical Technology, School of Health Sciences, Faculty of Medicine, Niigata University, Niigata 951-8518, Japan*

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**ABSTRACT**—The aim of the present study was to examine the effects of sarpogrelate, a 5-HT<sub>2</sub> antagonist, on 5-HT-induced endothelium-dependent relaxation in isolated porcine coronary artery preincubated with ketanserin ( $3 \times 10^{-6}$  M) and precontracted by U 46619 ( $5 \times 10^{-9}$  M) and compare its effects with other 5-HT<sub>2</sub> antagonists such as ritanserin and cyproheptadine. The investigation showed that sarpogrelate ( $10^{-7}$ – $10^{-5}$  M) had a weak antagonistic effect on 5-HT-induced relaxation and its effect was weaker than that of ritanserin ( $10^{-9}$ – $10^{-7}$  M) and cyproheptadine ( $10^{-8}$ – $10^{-6}$  M). The rank order of the antagonistic effects was: ritanserin > cyproheptadine > sarpogrelate. The study also showed that both sarpogrelate and ritanserin had no inhibitory effect on bradykinin-induced relaxation. In our previous study, we investigated the binding affinity of sarpogrelate, ritanserin and cyproheptadine to the 5-HT<sub>2A</sub>-receptor in rabbit cerebral cortex membranes and the pK<sub>i</sub> values found were 7.22, 8.98 and 7.54, respectively (M. Rashid et al., *Jpn J Pharmacol* **87**, 189–194, 2001). Rank order of the calculated ratio of concentration of pA<sub>2</sub> or pD'<sub>2</sub> vs K<sub>i</sub> was: sarpogrelate > ritanserin > cyproheptadine. Thus, these findings suggest that sarpogrelate has the lowest antagonistic effect on 5-HT-induced endothelium-dependent relaxation and the highest selectivity towards 5-HT<sub>2A</sub> receptor and might also be the safest drug with respect to its clinical implications in comparison with ritanserin and cyproheptadine.

**Keywords:** Sarpogrelate, Porcine coronary artery, Endothelium-dependent relaxation, 5-HT receptor

Recent studies have revealed that serotonin (5-HT) has produced both contractions and a relaxation response in the vascular smooth muscles (1–4). Multiple 5-HT receptors are involved in mediating these effects. 5-HT-induced vasoconstrictions of arteries are mainly mediated by 5-HT<sub>2A</sub> receptor subtype (5). 5-HT causes both endothelium-dependent and endothelium-independent relaxation of a number of isolated blood vessels in a variety of animals. In porcine coronary and pulmonary artery, 5-HT causes endothelium-dependent relaxation responses. So far, it has been reported that endothelium-dependent relaxant effects of 5-HT in pig coronary and pulmonary artery are mediated by 5-HT<sub>1</sub>-like and/or 5-HT<sub>2B</sub> receptors (6–9).

Sarpogrelate has been demonstrated to be selective and to show high affinity for the 5-HT<sub>2A</sub> receptor subtype, since it lacks significant 5-HT<sub>1</sub>, 5-HT<sub>3</sub>, 5-HT<sub>4</sub>;  $\alpha_1$ -,  $\alpha_2$ - and  $\beta$ -adrenoreceptor; histamine H<sub>1</sub> and H<sub>2</sub>; and muscarinic M<sub>3</sub> antagonistic activity (10–12). Sarpogrelate was introduced

as a therapeutic agent for the treatment of ischemic diseases associated with thrombosis (13). Sarpogrelate inhibits thrombus formation (14, 15) and suppresses platelet aggregation (16, 17). It inhibits 5-HT induced coronary artery spasm (18) and contraction of coronary artery in the porcine model mediated by 5-HT and  $\alpha$ -methylserotonin (19) and also inhibits vascular smooth muscle cell proliferation (20). All of these pathophysiological effects are mediated by the 5-HT<sub>2A</sub> subtype. Previously we reported that sarpogrelate inhibited the contraction response induced by 5-HT in pig coronary artery mediated by the 5-HT<sub>2A</sub> subtype (19).

The aim of the present study was to investigate the effects of sarpogrelate on 5-HT-induced endothelium-dependent relaxation in porcine coronary artery and compare its antagonistic activity with other 5-HT<sub>2</sub> antagonists such as ritanserin and cyproheptadine. This study would also establish the comparative selectivity towards 5-HT<sub>2A</sub> among these three antagonists.

\*Corresponding author. FAX: +81-25-268-1280

E-mail: nagatomo@niigata-pharm.ac.jp

## MATERIALS AND METHODS

### *Experimental protocol*

Fresh pig hearts were obtained from a local slaughterhouse within 10 min after death and transported to the laboratory immersed in ice-cold Krebs-Henseleit solution of the following composition: 118.4 mM NaCl, 4.7 mM KCl, 1.2 mM  $\text{MgSO}_4$ , 1.2 mM  $\text{KH}_2\text{PO}_4$ , 25.0 mM  $\text{NaHCO}_3$ , 2.5 mM  $\text{CaCl}_2$ , 0.026 mM  $\text{Na}_2\text{EDTA}$  and 11.1 mM dextrose. The first branch of the left anterior descending coronary artery was dissected and cleaned of all surrounding fat and connective 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. The bottom hook in each preparation was fixed, whereas the top hook was attached to a force transducer (T7-30-240; Orientec, Tokyo) mounted on a movable platform, which allowed adjustment of resting tension. Tension changes were recorded on a recorder (Rikadenki; Rikadenki Kogyo Co., Ltd., Tokyo). Each ring was suspended in a 10-ml organ bath and immersed in Krebs-Henseleit solution maintained at 37°C and bubbled continuously with 95%  $\text{O}_2$  and 5%  $\text{CO}_2$ . The ring was allowed to equilibrate for 60–90 min with an optimal resting tension of 1.5 g before all experiments. The responsiveness of each preparation was evaluated by  $3 \times 10^{-2}$  M KCl-induced contraction. The contractile response to KCl was allowed to reach a plateau and the existence of endothelium was confirmed by the presence of relaxation in response to bradykinin ( $10^{-7}$  M).

At first, the concentration-contraction response curve to the thromboxane  $\text{A}_2$  receptor agonist U 46619 was constructed in endothelium intact rings, by cumulative addition of the drug. Each concentration was added when a maximal response to the previous concentration had been reached. To determine the optimal concentration of ketanserin for observing the 5-HT-induced relaxation response, effects of three concentrations of ketanserin ( $3 \times 10^{-7}$ ,  $10^{-6}$  and  $3 \times 10^{-6}$  M) on responses to 5-HT in endothelium intact coronary artery precontracted with U 46619 were investigated. Ketanserin was added to the bath 20 min before U 46619 ( $5 \times 10^{-9}$  M, a concentration producing around 50% of the maximal effect). From these investigations,  $3 \times 10^{-6}$  M of ketanserin was used to study the effects of three 5-HT $_2$  antagonists on the endothelium-dependent relaxation induced by 5-HT. After a steady state contraction produced by U 46619, 5-HT or bradykinin was added cumulatively. Ketanserin was not added before the addition of U 46619 in the case of the bradykinin-induced concentration-dependent relaxation response. The effect of 5-HT and bradykinin were very rapid and transient; therefore, each concentration was added immediately after the previous one had produced its maximal effect. Bradykinin ( $10^{-7}$  M) was

added at the end of the addition of the last concentration of 5-HT to examine the functional integrity of the endothelium corresponding to a maximal relaxation.

In the experiments with 5-HT $_2$  antagonists such as sarpogrelate, ritanserin, and cyproheptadine, drugs were added at 20 min before the contraction produced by U 46619. Two concentration response curves were obtained in each preparation, one was for the control and the other was for the antagonist-treated preparation.

### *Data analyses*

Force responses were calculated as total developed tension minus resting tension immediately before addition of each agonist. The maximum relaxation response produced by 5-HT and bradykinin without antagonists treatment was taken as 100% relaxation response (control). Relaxation elicited by 5-HT and bradykinin with antagonists treatment was expressed as a percentage of the maximum relaxation response produced by 5-HT and bradykinin before the treatment of antagonists (control), respectively. Results are presented as the mean  $\pm$  S.E.M. of experiments. Statistical significance of the data was evaluated by Student's *t*-test for comparison of two groups and one-way ANOVA followed by Tukey's test for comparison of more than three groups. The agonist  $\text{EC}_{50}$  value of the concentration response curve was calculated by nonlinear analysis using the Sigma Plot Program (Jandel Scientific, San Rafael, CA, USA). Antagonists  $\text{pA}_2$  and  $\text{pD}'_2$  value were calculated using the equation of Van Rossum (21):  $\text{pA}_2 = \text{pA}_x + \log(X - 1)$ , where  $\text{pA}_x$  is the negative logarithm of antagonists concentration and  $X$  is the ratio of  $\text{EC}_{50}$  values of the agonist with/without antagonists;  $\text{pD}'_2 = \text{pD}'_x + \log(X - 1)$ , where  $\text{pD}'_x$  is the negative logarithm of antagonists concentration and  $X$  is the ratio of maximal effects of the agonist in the presence and absence of the antagonists.

### *Drugs used*

The following drugs were used: 5-HT (serotonin-creatinine sulfate; Toray Industries Inc., Tokyo), bradykinin and U 46619 (9,11-dideoxy-11 $\alpha$ ,9 $\alpha$ -epoxy-methano-prostaglandin  $\text{F}_{2\alpha}$ ; Wako Pure Chemical Industries, Ltd., Osaka), sarpogrelate (Mitsubishi Chemical Corporation, Tokyo), ketanserin and ritanserin (Sigma-RBI, St. Louis, MO, USA) and cyproheptadine (Sigma Chemical Co., St. Louis, MO, USA).

## RESULTS

### *Effects of ketanserin on responses to 5-HT in arterial rings precontracted with U 46619*

U 46619 produced a concentration-dependent response of contraction with a mean  $\text{EC}_{50}$  value of  $8.34 \times 10^{-9}$  M

( $n = 4$ ). Porcine coronary arteries with intact endothelium were contracted by  $5 \times 10^{-9}$  M of U 46619, a concentration that caused a steady state and sustained contraction (around 50% of the maximum contraction). The difference among the contractile responses to U 46619 in the individual series of experiments did not vary significantly. Without ketanserin, a small magnitude of relaxation response was observed, which was then abolished and followed by a contraction at high concentrations of 5-HT. With the pretreatment of ketanserin ( $3 \times 10^{-7}$  to  $3 \times 10^{-6}$  M), 5-HT causes relaxation responses in a concentration-dependent manner ( $10^{-9}$  to  $3 \times 10^{-6}$  M) in U 46619 contracted porcine coronary artery with intact endothelium (Fig. 1). In this study, all the experiments were performed in the presence of  $3 \times 10^{-6}$  M ketanserin that produced the maximum relaxation response induced by 5-HT, except for the bradykinin-induced relaxation response.

#### Effects of receptor antagonists on 5-HT-induced relaxation

5-HT elicited concentration-dependent relaxations in endothelium-intact porcine coronary artery preincubated with ketanserin ( $3 \times 10^{-6}$  M) and precontracted by U 46619 ( $5 \times 10^{-9}$  M). Representative recordings of 5-HT-induced relaxation in porcine coronary artery with endothelium and the effect of sarpogrelate on the relaxation response produced by 5-HT of the same preparation are shown in Fig. 2: a and b. Figure 3 shows the inhibitory effects of sarpogrelate, ritanserin and cyproheptadine on the concentration response curve of 5-HT. Sarpogrelate, ritanserin and cyproheptadine caused parallel rightward shifts of the concentration-relaxation curve of 5-HT with no significant effect on the maximum response at concentrations of  $10^{-7}$  and  $10^{-6}$  M,  $10^{-9}$  and  $10^{-8}$  M, and  $10^{-8}$  M, respectively (Fig. 3: a, b and

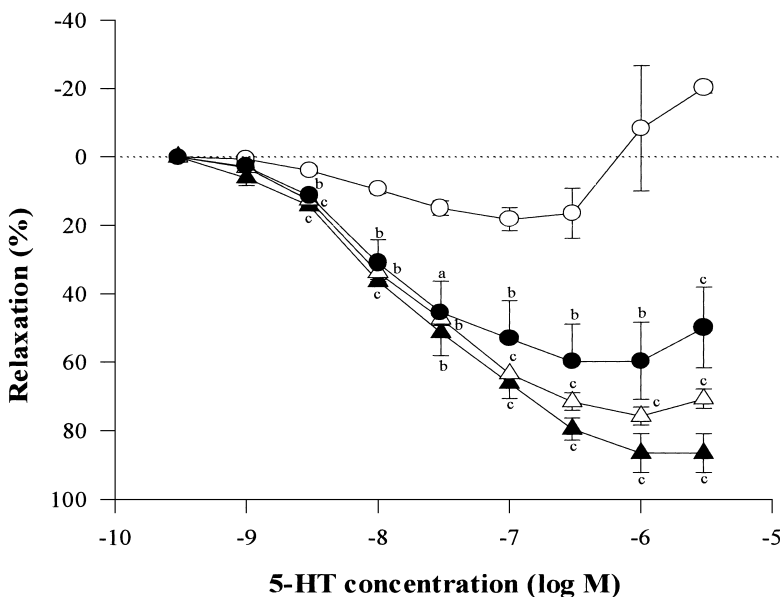
c), and the mean  $pA_2$  values were 6.67 and 6.17, 9.21 and 8.12, and 8.07, respectively (Table 1). Sarpogrelate at a concentration of  $10^{-5}$  M caused rightward shifts with an inhibition of the maximum response (mean  $pD'_2$  value = 4.79). Ritanserin only at the concentration of  $10^{-7}$  M and cyproheptadine at  $10^{-7}$  and  $10^{-6}$  M concentrations caused rightward shifts with an inhibition of the maximum relaxation effect induced by 5-HT; and mean  $pD'_2$  values were 6.99, 6.66 and 6.27, respectively (Table 1).

#### Effects of receptor antagonists on bradykinin-induced relaxation

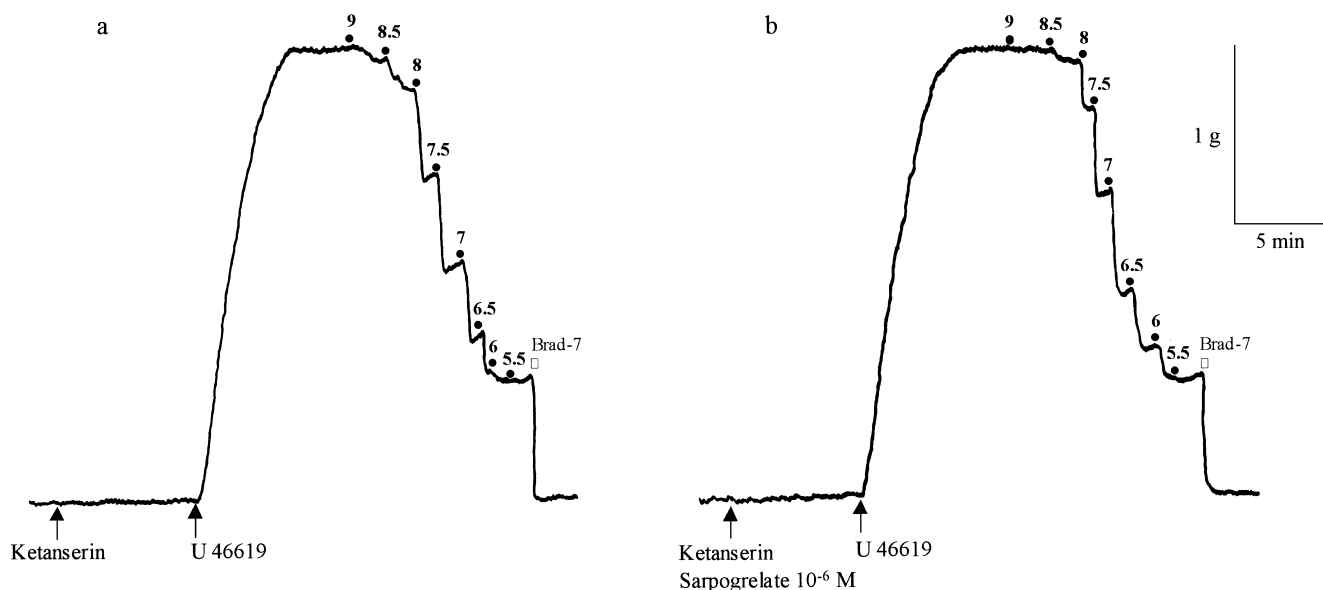
Figure 4 shows the effects of sarpogrelate, ritanserin, and cyproheptadine on the concentration-relaxation response curve of bradykinin. Both sarpogrelate ( $10^{-7}$ – $10^{-5}$  M) and ritanserin ( $10^{-9}$ – $10^{-7}$  M) did not inhibit bradykinin induced concentration-dependent relaxation of porcine coronary artery with endothelium (Fig. 4: a and b), whereas only  $10^{-6}$  M cyproheptadine inhibited the bradykinin induced relaxation with an inhibition of the maximum response (Fig. 4c).

#### DISCUSSION

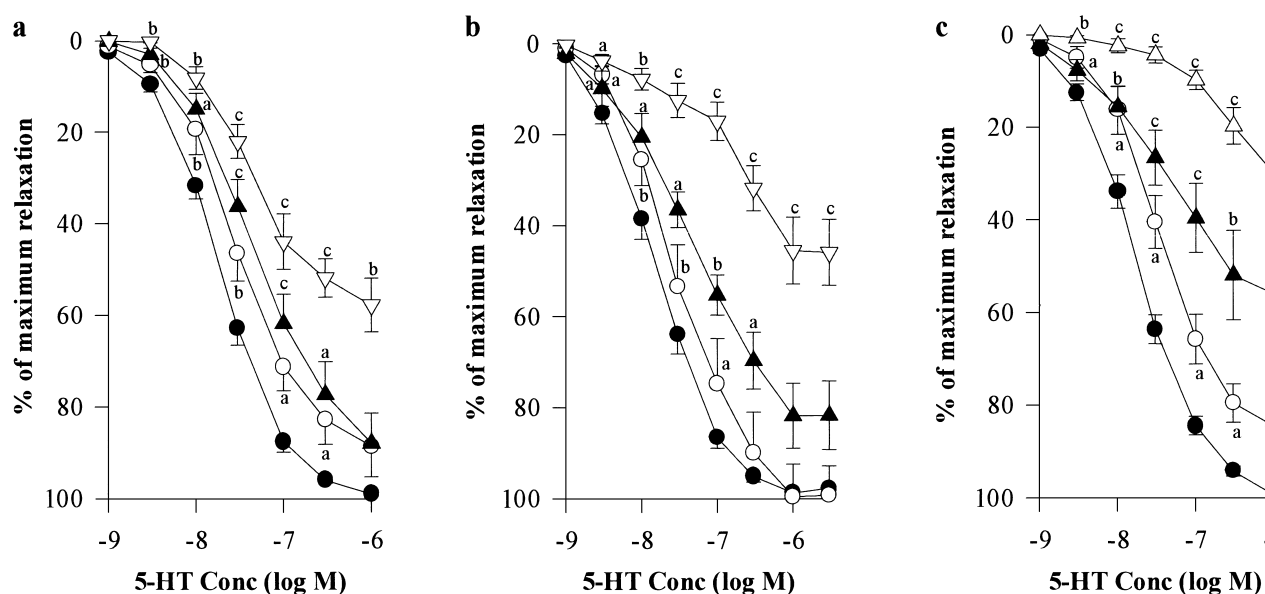
The investigations revealed that with the pretreatment of ketanserin, 5-HT caused relaxation in U 46619-precontracted pig coronary artery with intact endothelium. Without ketanserin, this relaxation response was abolished, and instead, a contraction was produced at a high 5-HT concentration (Fig. 1.). It also further suggests that ketanserin blocks the contractile response of 5-HT probably mediated by the 5-HT<sub>2A</sub> receptor, which in turn can conceivably result in the relaxant effect (6, 7). The previous studies have



**Fig. 1.** Effects of ketanserin on responses to 5-HT in porcine coronary artery with endothelium precontracted with U 46619. Ketanserin was added 20 min before contraction produced by U 46619 ( $5 \times 10^{-9}$  M). The responses occurred by cumulative addition of 5-HT with ketanserin (closed circles =  $3 \times 10^{-7}$  M; open triangles =  $1 \times 10^{-6}$  M; closed triangles =  $3 \times 10^{-6}$  M) or without ketanserin (open circles) were expressed as a percentage of the maximum contraction response produced by U 46619 ( $5 \times 10^{-9}$  M). Each point represents the mean response  $\pm$  S.E.M. of 4 arterial rings. <sup>a</sup> $P < 0.05$ , <sup>b</sup> $P < 0.01$  and <sup>c</sup> $P < 0.001$  vs without ketanserin.



**Fig. 2.** Typical recordings of 5-HT-induced relaxation in endothelium-intact porcine coronary artery preincubated with ketanserin and precontracted by U 46619 (a) and the effect of sarpogrelate on 5-HT-induced relaxation responses of the same preparation (b). The number on the trace represents negative logarithm concentrations of 5-HT. The starting point of each relaxation time course is at the addition of 5-HT (indicated by a dot). Brad-7 (open square): bradykinin  $10^{-7}$  M.



**Fig. 3.** Effect of 5-HT<sub>2</sub> antagonists on 5-HT-induced concentration-dependent relaxation in porcine coronary artery with endothelium. a: Sarpogrelate (closed circles = control,  $n = 8$ ; open circles =  $10^{-7}$  M,  $n = 8$ ; closed triangles =  $10^{-6}$  M,  $n = 8$ ; open triangles =  $10^{-5}$  M,  $n = 6$ ). b: Ritanserin (closed circles = control,  $n = 8$ ; open circles =  $10^{-9}$  M,  $n = 6$ ; closed triangles =  $10^{-8}$  M,  $n = 6$ ; open triangles =  $10^{-7}$  M,  $n = 8$ ). c: Cyproheptadine (closed circles = control,  $n = 10$ ; open circles =  $10^{-8}$  M,  $n = 5$ ; closed triangles =  $10^{-7}$  M,  $n = 8$ ; open triangles =  $10^{-6}$  M,  $n = 10$ ). Ketanserin ( $3 \times 10^{-6}$  M) was added 20 min before the contraction produced by U 46619. The relaxation responses occurred by cumulative addition of 5-HT with antagonists treatment were expressed as a percentage of the maximum relaxation response of 5-HT before antagonists. Each point represents the mean response  $\pm$  S.E.M. <sup>a</sup> $P < 0.05$ , <sup>b</sup> $P < 0.01$  and <sup>c</sup> $P < 0.001$  vs control.

shown that the relaxations induced by 5-HT in the arteries including porcine coronary arteries were mediated by activation of 5-HT receptors localized on the endothelial cells

(6, 7, 22–25). So far it has been reported that endothelium-dependent relaxation response involves two types of receptor: 5-HT<sub>1</sub>-like and 5-HT<sub>2B</sub> receptors (7–9). The relaxant

**Table 1.**  $pA_2$  and  $pD'_2$  values of 5-HT<sub>2</sub> antagonists for 5-HT-induced endothelium-dependent relaxation in porcine coronary artery

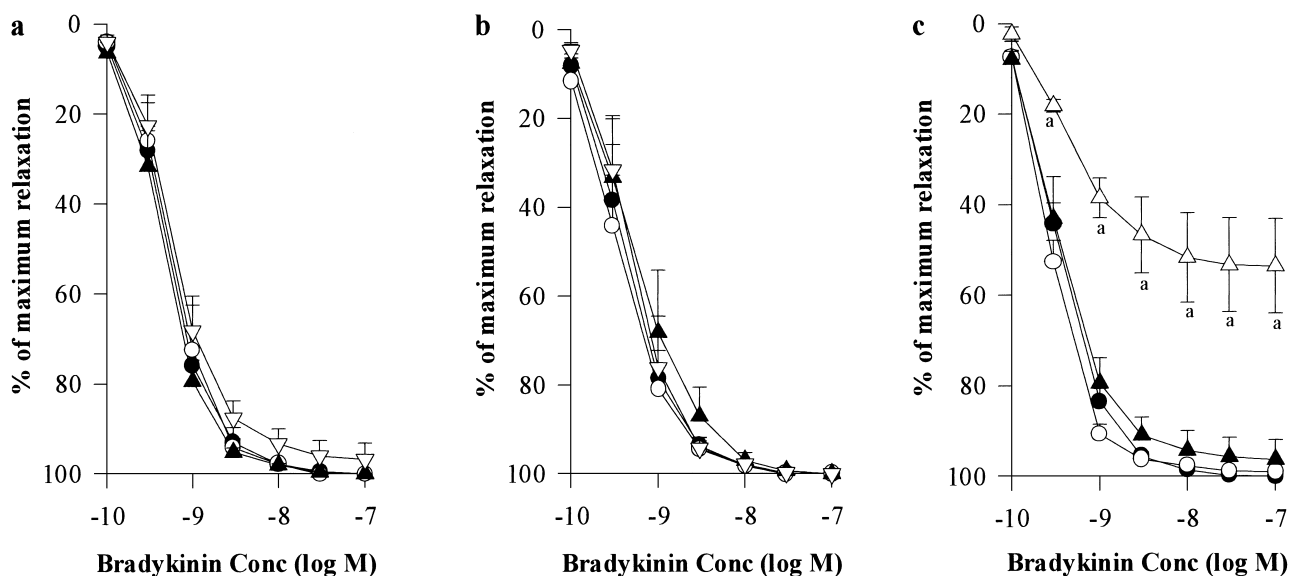
Antagonists	Conc. (M)	$pA_2$ value	(n)	$pD'_2$ value	(n)
Sarpogrelate	$10^{-7}$	$6.67 \pm 0.09$	(8)	—	
	$10^{-6}$	$6.17 \pm 0.13$	(8)	—	
	$10^{-5}$	—		$4.79 \pm 0.12$	(6)
Ritanserin	$10^{-9}$	$9.21 \pm 0.11^{a,b}$	(6)	—	
	$10^{-8}$	$8.12 \pm 0.21^{a,b}$	(6)	—	
	$10^{-7}$	—		$6.99 \pm 0.17^c$	(8)
Cyproheptadine	$10^{-8}$	$8.07 \pm 0.19^{a,b}$	(5)	—	
	$10^{-7}$	—		$6.66 \pm 0.31^c$	(8)
	$10^{-6}$	—		$6.27 \pm 0.12^c$	(10)

Values are the mean  $\pm$  S.E.M. The number in the parentheses indicates the number of experiments. <sup>a</sup> $P < 0.01$  vs  $10^{-7}$  M sarpogrelate, <sup>b</sup> $P < 0.01$  vs  $10^{-6}$  M sarpogrelate, <sup>c</sup> $P < 0.01$  vs  $10^{-5}$  M sarpogrelate.

response induced by 5-HT in porcine coronary artery with intact endothelium is due to release of NO (26).

In the present study, we investigated the effects of sarpogrelate on endothelium-dependent relaxation response induced by 5-HT in porcine coronary artery and compared it with other 5-HT<sub>2</sub> antagonists such as ritanserin and cyproheptadine. Sarpogrelate at concentrations of  $10^{-7}$  and  $10^{-6}$  M, ritanserin at  $10^{-9}$  and  $10^{-8}$  M, and cyproheptadine

at  $10^{-8}$  M induced parallel rightward shifts of the concentration-relaxation curve of 5-HT without significant effect on the maximum response (Fig. 3: a, b and c); and the mean  $pA_2$  values were 6.67 and 6.17, 9.21 and 8.12, and 8.07, respectively. Sarpogrelate at the concentration of  $10^{-5}$  M caused a rightward shift with an inhibition of the maximum response with a mean  $pD'_2$  value of 4.79. Ritanserin at  $10^{-7}$  M and cyproheptadine at  $10^{-7}$  and  $10^{-6}$  M caused rightward shifts with an inhibition of the maximum relaxation effect induced by 5-HT (Fig. 3: b and c); the mean  $pD'_2$  values were 6.99 for  $10^{-7}$  M ritanserin and 6.66 and 6.27 for  $10^{-7}$  and  $10^{-6}$  M cyproheptadine, respectively. The study demonstrated that sarpogrelate had a weak antagonistic effect on the 5-HT-induced relaxation in porcine coronary artery with intact endothelium. On the other hand, ritanserin had the highest antagonistic effect on such relaxation among these three antagonists and the antagonistic effect of cyproheptadine is also higher than that of sarpogrelate. All of these antagonists acted in a noncompetitive manner at a higher concentration (Fig. 3). Based on radioligand binding assay and functional experiments, it has been found that sarpogrelate has high affinity and selectivity towards the 5-HT<sub>2A</sub> receptor, but not towards 5-HT<sub>1</sub>, 5-HT<sub>3</sub>, 5-HT<sub>4</sub> and other receptors (10–12). The current study also showed that sarpogrelate had weak antagonistic effect on 5-HT-induced endothelium-dependent relaxation mediated by 5-HT<sub>1</sub>-like and/or 5-HT<sub>2B</sub> receptors. On the



**Fig. 4.** Effect of 5-HT<sub>2</sub> antagonists on bradykinin-induced concentration-dependent relaxation in porcine coronary artery with endothelium. a: Sarpogrelate (closed circles = control; open circles =  $10^{-7}$  M; closed triangles =  $10^{-6}$  M; open triangles =  $10^{-5}$  M). b: Ritanserin (closed circles = control; open circles =  $10^{-9}$  M; closed triangles =  $10^{-8}$  M; open triangles =  $10^{-7}$  M). c: Cyproheptadine (closed circles = control; open circles =  $10^{-8}$  M; closed triangles =  $10^{-7}$  M; open triangles =  $10^{-6}$  M). The relaxation responses occurred by cumulative addition of bradykinin with antagonists treatments were expressed as a percentage of the maximum relaxation response of bradykinin before antagonists. Each point represents the mean response  $\pm$  S.E.M. of 4 to 6 arterial rings. <sup>a</sup> $P < 0.05$  vs control.

other hand, ritanserin is more selective and has higher affinity towards the 5-HT<sub>2A</sub>-receptor subtype than the 5-HT<sub>2B</sub>- and 5-HT<sub>2C</sub>-receptor subtypes (27), although its affinity to 5-HT<sub>2B</sub>- and 5-HT<sub>2C</sub>-receptor subtypes is higher than that of cyproheptadine and sarpogrelate. Cyproheptadine is a 5-HT<sub>2</sub> antagonist (28) as well as Ca<sup>2+</sup> channel antagonist (29), and its affinity towards 5-HT<sub>2A</sub> receptors is slightly higher than its affinities for the 5-HT<sub>2B</sub> and 5-HT<sub>2C</sub> receptor.

This study also revealed that both sarpogrelate (10<sup>-7</sup> – 10<sup>-5</sup> M) and ritanserin (10<sup>-9</sup> – 10<sup>-7</sup> M) did not inhibit the bradykinin-induced relaxation response in U 46619-pre-contracted porcine coronary artery with endothelium, but the same concentrations of these antagonists inhibited the relaxation induced by 5-HT. Bradykinin stimulates endothelial cells in porcine coronary artery to release NO and EDHF (endothelium-derived hyperpolarizing factor), which cause relaxations (30). The result indicated that both sarpogrelate and ritanserin could compete at the serotonin receptors existing in the endothelium of porcine coronary artery. In contrast, cyproheptadine at the concentration of 10<sup>-6</sup> M inhibited bradykinin-induced relaxation with an inhibition of the maximum response. It was also found (data were not shown) in our preliminary experiments that all the receptor antagonists did not affect the contractile responses of U 46619, except only cyproheptadine at the concentration of 10<sup>-6</sup> M, which had some inhibitory effect on the contractile response of U 46619. Thus, 10<sup>-6</sup> M cyproheptadine may have a non-specific inhibitory effect on the endothelium and/or arterial smooth muscle.

Previously, radioligand binding assays indicated that ritanserin had higher binding affinity (mean pK<sub>i</sub> = 8.98) towards 5-HT<sub>2A</sub> receptors in the rabbit cerebral cortex membrane than cyproheptadine (mean pK<sub>i</sub> = 7.54) and sarpogrelate (mean pK<sub>i</sub> = 7.22) (31). We calculated the ratio of the concentration of pA<sub>2</sub> or pD'<sub>2</sub> vs K<sub>i</sub> to evaluate the selectivity of these antagonists towards 5-HT<sub>2A</sub> receptor (Table 2) and it shows that the ratios are higher for sarpogrelate than those for ritanserin and cyproheptadine. The higher ratio means higher selectivity towards the 5-HT<sub>2A</sub> receptor than 5-HT<sub>1</sub>-like or 5-HT<sub>2B</sub> receptors, because higher concentration of the antagonists will be required for the antagonistic effect of relaxation response induced by 5-HT that is mediated by 5-HT<sub>1</sub>-like and 5-HT<sub>2B</sub> receptors than that for the blocking effect of 5-HT<sub>2A</sub> receptor in the rabbit cerebral cortex membrane.

There is a high physiological significance for the 5-HT induced endothelium-dependent relaxation. It is reported that the endothelium derived relaxing factor may protect the coronary artery wall from the direct vasoconstrictor action of 5-HT on the artery wall (32, 33) and loss or injury of the endothelium may reverse the vasodilator action of 5-HT to vasoconstriction (2, 34, 35). Sarpogrelate, a 5-HT<sub>2A</sub> antagonist, was introduced as a therapeutic agent for

**Table 2.** Calculated ratio of pA<sub>2</sub> vs K<sub>i</sub> and pD'<sub>2</sub> vs K<sub>i</sub> of 5-HT<sub>2</sub> antagonists

Antagonists	Conc. (M)	pA <sub>2</sub> vs K <sub>i</sub>	pD' <sub>2</sub> vs K <sub>i</sub>
Sarpogrelate	10 <sup>-7</sup>	3.55	—
	10 <sup>-6</sup>	11.22	—
	10 <sup>-5</sup>	—	269.15
Ritanserin	10 <sup>-9</sup>	0.59	—
	10 <sup>-8</sup>	7.24	—
	10 <sup>-7</sup>	—	97.72
Cyproheptadine	10 <sup>-8</sup>	0.30	—
	10 <sup>-7</sup>	—	7.59
	10 <sup>-6</sup>	—	18.62

Ratio of pA<sub>2</sub> vs K<sub>i</sub> and pD'<sub>2</sub> vs K<sub>i</sub> were calculated from the concentration of pA<sub>2</sub> or pD'<sub>2</sub> value vs K<sub>i</sub>. pA<sub>2</sub> and pD'<sub>2</sub> values were obtained from the present study. K<sub>i</sub> values were obtained from our previous study (31) using rabbit cerebral cortex as the membrane fraction and [<sup>3</sup>H]ketanserin as the radioligand.

the treatment of ischemic diseases associated with thrombosis (13). Most recently, Sharma et al. (36) suggested that sarpogrelate could be used as a therapeutic agent to inhibit serotonin-induced neointimal hyperplasia and improve the success rate of coronary artery bypass grafts. From this investigation, it is observed that slightly higher concentration of sarpogrelate is required for the inhibition of the relaxation response in comparison with ritanserin and cyproheptadine. The ratio of pA<sub>2</sub> or pD'<sub>2</sub> vs K<sub>i</sub> is a measure of the selectivity of these compounds to 5-HT<sub>2A</sub>. The value for sarpogrelate was higher than those of ritanserin and cyproheptadine. This result suggests that sarpogrelate may be the safer drug with respect to its clinical implications.

In conclusion, 1) sarpogrelate has the lowest antagonistic effect on the endothelium-dependent relaxation response induced by 5-HT that is mediated by 5-HT<sub>1</sub>-like and/or 5-HT<sub>2B</sub> receptors, compared with ritanserin and cyproheptadine, and the rank order for this antagonistic effect is: ritanserin > cyproheptadine > sarpogrelate. 2) The rank order of the calculated ratio of concentration of pA<sub>2</sub> or pD'<sub>2</sub> vs K<sub>i</sub> is sarpogrelate > ritanserin > cyproheptadine. Therefore, our results suggest that sarpogrelate has the highest selectivity towards the 5-HT<sub>2A</sub> receptor and might also be the safest drug in comparison with ritanserin and cyproheptadine.

#### Acknowledgment

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