J. Pharm. Pharmacol. 1995, 47: 310–316
Received May 23, 1994
Accepted October 20, 1994

In-vitro Pharmacology of Sarpogrelate and the Enantiomers of its Major Metabolite: 5-HT_{2A} Receptor Specificity, Stereoselectivity and Modulation of Ritanserin-induced Depression of 5-HT Contractions in Rat Tail Artery

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Abstract

The new antiplatelet agent sarpogrelate (MCI-9042), its major metabolite (*R,S*)-1-[2-[2-(3-methoxyphenyl)ethyl]phenoxy]-3-(dimethylamino)-2-propanol ((*R,S*)-M-1) and the enantiomers of (*R,S*)-M-1 were studied as antagonists at 5-HT_{2A} receptors, 5-HT_1-like receptors, 5-HT_3 receptors, α_1-adrenoceptors, β-adrenoceptors, histamine H_1 receptors, histamine H_2 receptors and muscarinic M_3 receptors in various functional in-vitro assays.

Sarpogrelate, (*R,S*)-M-1, (*R*)-M-1 and (*S*)-M-1, respectively, were competitive antagonists of 5-hydroxytryptamine (5-HT) at 5-HT_{2A} receptors of rat tail artery with calculated pA_2 values of 8·53, 9·04, 9·00 and 8·81, respectively. Sarpogrelate lacked prominent 5-HT_1-like, 5-HT_3, β, H_1, H_2 and M_3 antagonist activity and weakly blocked α_1-adrenoceptors ($pK_B = 6{\cdot}30$). (*S*)-M-1 showed weak affinity for 5-HT_1-like receptors ($pK_B = 6{\cdot}30$), α_1- ($pK_B = 6{\cdot}80$) and β- ($pK_B = 6{\cdot}54$) adrenoceptors, while (*R*)-M-1 was a weak antagonist at histamine H_1 receptors ($pK_B = 6{\cdot}49$).

Stereoselectivity of M-1 enantiomers was low. (*R*)-M-1 showed 1·6-fold, 2·3-fold and 2·5-fold higher antagonist activity than (*S*)-M-1 for 5-HT_{2A}, H_1 and M_3 receptor, respectively. Affinity at β-adrenoceptors and 5-HT_1-like receptors was 5-fold and 3-fold higher for (*S*)-M-1 than for (*R*)-M-1.

The depression of the maximum effect of 5-HT-induced contractions of rat-tail artery which amounted to 58–72% in the presence of ritanserin (1 nM), was totally prevented after preincubation with sarpogrelate (1 μM) and (*R*)- and (*S*)-M-1 (30 and 300 nM), respectively, and partially prevented after preincubation with (*R*)- and (*S*)-M-1 (0·3–3 nM). (*R*)- and (*S*)-M-1 failed to differ in restoring the ritanserin-induced depression of the 5-HT maximum response.

It is concluded that sarpogrelate, its major metabolite (*R,S*)-M-1, and M-1 enantiomers are specific antagonists of 5-HT at 5-HT_{2A} receptors. The stereochemical configuration of the ligands does not seem to be crucial for binding to the 5-HT_{2A} receptor. Like ketanserin, sarpogrelate and M-1 enantiomers appear to be allosteric activators of the 5-HT_{2A} receptor system in rat tail artery.

Sarpogrelate (MCI-9042) {(*R, S*)-1-[2-[2-(3-methoxyphenyl)ethyl]phenoxy]-3-(dimethylamino)-2-propyl hydrogen succinate hydrochloride} is a new type of 5-HT_{2A} 5-HT-ergic-receptor antagonist which was recently introduced as a therapeutic agent for ischaemic diseases associated with thrombosis. Sarpogrelate inhibits in-vitro the collagen-induced platelet aggregation and in-vivo the thrombus formation in mice. The platelet anti-aggregatory effect of sarpogreglate has been explained by its 5-HT_{2A}-receptor blocking properties (Kikumoto et al 1990). Furthermore, sarpogrelate inhibits the 5-hydroxytryptamine (5-HT) release accompanied with collagen-induced platelet aggregation and also the secondary wave of aggregation induced by ADP and adrenaline (Hara et al 1991). These are properties which sarpogrelate shares with the classical 5-HT_{2A}-receptor antagonist ketanserin (De Clerck & Xhonneux 1985).

In rats, dogs, monkeys and man, oral sarpogrelate is first hydrolysed to (*R,S*)-M-1 (1-[2-[2-(3-methoxyphenyl)ethyl]phenoxy]-3-(dimethylamino)-2-propanol) (Komatsu et al 1991). Previous studies have shown that sarpogrelate and its major metabolite (*R,S*)-M-1 exhibit specificity toward 5-HT_{2A} receptors, since affinities for 5-HT_1 receptors, α_1-, α_2-, β-adrenoceptors and muscarinic receptors, based on radioligand binding studies, and for 5-HT_{2B} receptors and α_1-adrenoceptors, based on functional studies, were low (Hara et al 1991; Maruyama et al 1991).

The aim of the present study was to extend the affinity profile of sarpogrelate and (*R,S*)-M-1 in further functional in-vitro assays with special regard to stereoselectivity of the enantiomers of (*R,S*)-M-1, (*R*)-M-1 and (*S*)-M-1. Since sarpogrelate and its metabolites proved to be competitive antagonists of 5-HT at 5-HT_{2A} receptors of rat tail artery, further experiments were performed to examine whether sarpogrelate, (*R*)-M-1 or (*S*)-M-1 can prevent the depression of the 5-HT maximum response induced by the insurmountable antagonist ritanserin in this tissue by allosteric modulation of 5-HT_{2A} receptors (Frenken & Kaumann 1987). A preliminary account of this work has been presented at the 35th Spring Meeting of the Deutsche Gesellschaft für experimentelle und klinische Pharmakologie und Toxikologie, Mainz, Germany (Pertz et al 1994).

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Materials and Methods

Compounds

The following compounds were obtained as gifts: cimetidine (SmithKline Beecham, Welwyn, UK), dinoprost tromethamine ($PGF_{2\alpha}$; Upjohn, Heppenheim, Germany), doxazosin mesylate (Pfizer, Karlruhe, Germany), flesinoxan hydrochloride (Duphar, The Netherlands), ketanserin tartrate (Janssen, Beerse, Belgium), (*R,S*)-1-[2-[2-(3-methoxyphenyl) ethyl] phenoxy] - 3 - (dimethylamino) - 2 - propanol hydrochloride ((*R,S*)-M-1), (*R*)-1-[2-[2-(3-methoxyphenyl)ethyl]phenoxy]-3-(dimethylamino)-2-propanol hydrochloride ((*R*)-M-1), (*S*)-1-[2-[2-(3-methoxyphenyl)ethyl] phenoxy]-3-(dimethylamino)-2-propanol hydrochloride ((*S*)-M-1; Mitsubishi Kasei, Yokohama, Japan), methysergide hydrogen maleate (Sandoz, Switzerland), (*R*)-phenylephrine (Winthrop, Norderstedt, Germany), sarpogrelate hydrochloride (Mitsubishi Kasei, Yokohama, Japan), tropisetron hydrochloride (Sandoz, Basle, Switzerland). The following compounds were purchased: atropine sulphate (Merck, Darmstadt, Germany), carbachol (Sigma, St Louis, USA), cocaine hydrochloride (Merck, Darmstadt, Germany), histamine dihydrochloride (Merck, Darmstadt, Germany), 5-hydroxytryptamine creatinine sulphate (5-HT; Janssen, Beerse, Belgium), (*R,S*)-isoprenaline hydrochloride (Sigma, St Louis, USA), mepyramine hydrogen maleate (Sigma, St Louis, USA), methiothepin mesylate (RBI, Natick, USA), (*R,S*)-metoprolol tartrate (Sigma, St Louis, USA), 5-methoxytryptamine (5-MeOT; Aldrich, Steinheim, Germany), prazosin hydrochloride (RBI, Natick, USA), (*R,S*)-propranolol hydrochloride (Sigma, St Louis, USA), spiperone hydrochloride (RBI, Natick, USA).

Rat tail artery: 5-HT_{2A} receptors

Male Wistar rats (280–350 g) were killed by cervical dislocation. The ventral caudal artery was quickly dissected and cleared of all connective tissue. A stainless-steel wire (diam. 0·3 mm) was inserted into the artery to rub off the endothelium. Up to 20 cylindrical segments of 5–6 mm length were prepared from each artery and were horizontally suspended between two L-shaped stainless-steel hooks (diam. 0·15 mm) gently inserted into the lumen for the recording of contractile responses as previously described for the rat tail artery by Bradley et al (1985). Each preparation was mounted in a 20-mL organ bath containing modified Krebs–Henseleit solution of the following composition (mM): NaCl 118, KCl 4·7, $CaCl_2$ 2·5, $MgSO_4$ 1·2, KH_2PO_4 1·2, $NaHCO_3$ 25 and glucose 10. The solution was continuously gassed with 95% O_2–5% CO_2 and warmed to a constant temperature of 37°C. Preparations were connected to a force displacement transducer attached to a TSE 4711 transducer coupler and a Siemens C 1016 compensograph for continuous recording of isometric changes in tension. Resting force was adjusted to 5 mN at the beginning of each experiment. During an equilibration period of 120 min, preparations were stimulated once (after 60 min) with 5-HT (1 μM). Three cumulative concentration-effect curves for 5-HT were determined on each arterial segment at intervals of 60 min, when affinities for ketanserin, sarpogrelate, (*R,S*)-M-1, (*R*)-M-1 and (*S*)-M-1, respectively, were estimated. Antagonists were incubated for 30 min. The blocking effect of a given concentration of antagonist was the same whether determined in a second or a third concentration-effect curve for 5-HT. In experiments dealing with allosteric modulation of 5-HT_{2A} receptors, two cumulative concentration-effect curves for 5-HT were established at an interval of 3 h in the continued presence of cocaine (6 μM), prazosin (30 nM) and ascorbate (0·2 mM). Potential allosteric activators (sarpogrelate, (*R*)-M-1 and (*S*)-M-1) were incubated 150 min before determining the second cumulative concentration-effect curve for 5-HT. The deactivator (ritanserin) was incubated 30 min after the administration of the activator in the same experiment. In all experiments, at least two arterial segments served as control to estimate spontaneous changes in agonist sensitivity.

Rat aorta: α_1-adrenoceptors

The thoracic aorta was removed from rats used for studies at 5-HT_{2A} receptors in rat tail artery (see above). When cleared of connective tissue the aorta was cut into 6–12 rings of 4–6 mm length. Each cylindrical segment was rolled with a pair of tweezers to destroy the endothelium. The segments were horizontally suspended between two L-shaped stainless-steel holders (diam. 0·3 mm), using a method similar to that of Hooker et al (1977). The organs were isometrically mounted as described for rat tail artery experiments (see above). The bath fluid (modified Krebs–Henseleit solution) contained (*R, S*)-propranolol (1 μM) to block β-adrenoceptors. The applied resting force was 20 mN. During an equilibration period of 140 min the organs were stimulated twice with (*R*)-phenylephrine (100 nM). Two cumulative concentration-effect curves for (*R*)-phenylephrine were determined in the absence and presence of antagonist. Antagonists were incubated for 30 min. For doxazosin, a pA_2 value of $9{\cdot}35 \pm 0{\cdot}04$ (slope $1{\cdot}06 \pm 0{\cdot}04$ of Schild plot, $n = 34$) was obtained.

Guinea-pig iliac artery: 5-HT_1-like receptors

Guinea-pigs of either sex, 300–450 g, were stunned by a blow on the neck and bled. The abdominal aorta and the right and left common iliac arteries were removed and cleared of adhering connective tissue. Two or three cylindrical segments of 1–2 mm length from each iliac artery were horizontally suspended between two L-shaped stainless-steel hooks (diam. 0·15 mm) and isometrically mounted as described for rat tail artery experiments (see above). The bath fluid (modified Krebs–Henseleit solution with $CaCl_2$ 1·25 mM and glucose 11·5 mM) contained spiperone (0·3 μM), mepyramine (0·3 μM), cimetidine (30 μM) and cocaine (30 μM) to block 5-HT_{2A} receptors, histamine H_1 receptors, histamine H_2 receptors and neuronal uptake of 5-HT. The applied resting force was 5 mN. During an equilibration period of 4·5 h, the organs were stimulated after 100 min with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; 30 μM). Relaxation was achieved by subsequent addition of carbachol (10 μM). After 175 min the organs were moderately precontracted with an EC10–EC20 (50–500 nM) of $PGF_{2\alpha}$ and subsequently stimulated with 5-HT (0·3 μM). Two cumulative concentration-effect curves for 5-HT were determined at an interval of 80 min in the absence and presence of antagonist on organs precontracted with an EC10–EC20 of

$PGF_{2\alpha}$ as above. Antagonists were incubated for 45 min. For methiothepin and flesinoxan, respectively, pK_B values of 8·92 ± 0·09 (n = 4) and 6·03 ± 0·10 (n = 4), respectively, were obtained (Pertz 1993).

Guinea-pig ileum longitudinal muscle-myenteric plexus preparation: 5-HT_3 receptors

The ileum was removed from guinea-pigs used for studies at 5-HT_1-like receptors in the iliac artery. Strips of longitudinal muscle with adhering myenteric plexus, 2 cm in length and proximal to the ileocaecal junction, were prepared as previously described by Buchheit et al (1985), and mounted isometrically under a tension of approximately 7·5 mN in 20-mL organ baths filled with Tyrode solution of composition (mM): NaCl 137, KCl 2·7, $CaCl_2$ 1·8, $MgCl_2$ 1·0, NaH_2PO_4 0·4, $NaHCO_3$ 11·0 and glucose 5·6 containing choline (1 μM). The solution was gassed with 95% O_2–5% CO_2 and warmed to a constant temperature of 37°C. In all experiments, 5-methoxytryptamine (10 μM) was present for desensitization of 5-HT_4 receptors and methysergide (1 μM) to block 5-HT_1 and 5-HT_{2A} receptors (Craig et al 1990). After a stabilization period of 30 min the strips were stimulated three times with 5-HT (3 μM) during a period of 60 min to establish tissue viability. Two non-cumulative concentration-effect curves were constructed at an interval of 60 min on each strip by adding increasing concentrations of 5-HT to the organ bath (1–30 μM). Each concentration was left in contact with the tissue for 15–30 s followed by wash-out with at least 100 mL Tyrode solution. The next higher concentration of 5-HT was administered 15 min later. Antagonists were added 30 min before the second concentration-effect curve and were retained in the bath fluid during construction of the second curve. For tropisetron, a pA_2 value of 7·84 ± 0·08 (slope 1·00 ± 0·04 of Schild plot, n = 25) was obtained.

Guinea-pig ileum (whole segments): muscarinic M_3 receptors

The ileum was removed from guinea-pigs used for studies at 5-HT_1-like receptors in the iliac artery. Whole segments of guinea-pig ileum (1·5 cm in length) were mounted isotonically (preload 5 mN) in 20-mL organ baths filled with Tyrode solution (37°C, gassed with 95% O_2–5% CO_2). After a stabilization period of 20 min, the segments were stimulated three times with carbachol (1 μM) during a period of 45 min to establish a constant response. Four cumulative concentration–effect curves for carbachol were constructed at intervals of 15–20 min in the absence and presence of antagonist. Antagonists were incubated for 5–10 min. For atropine, a pA_2 value of 9·02 ± 0·06 (slope 1·05 ± 0·03 of Schild plot, n = 30) was obtained.

Guinea-pig ileum (whole segments): histamine H_1 receptors

The ileum was removed from guinea-pigs used for studies at 5-HT_1-like receptors in the iliac artery. Whole segments of ileum (1·5 cm in length) were mounted isotonically (preload 5 mN) in 20-mL organ baths filled with Tyrode solution containing atropine (0·1 μM). The solution was gassed with 95% O_2–5% CO_2 and warmed to a constant temperature of 37°C. During a stabilization period of ca 80 min the organs were stimulated three times with histamine (1 μM). Four cumulative concentration-effect curves for histamine were determined in the absence and presence of antagonist. Antagonists were incubated for 5 min. For mepyramine, a pA_2 value of 9·09 ± 0·09 (slope 0·97 ± 0·04 of Schild plot, n = 29) was determined.

Guinea-pig right atrium (spontaneously beating): histamine H_2 receptors

Guinea-pigs of either sex were stunned and exsanguinated. The heart was quickly removed and the right atrium set up isometrically in Krebs–Henseleit solution (32·5°C) under a resting force of 5 mN similar to the method described by Black et al (1972). After 30 min of continuous washing and an equilibration period of 15 min, two concentration-frequency curves for histamine (0·01–10 μM) were determined in the absence and presence of antagonist (incubation 30 min) at an interval of 80 min. (*R,S*)-Metoprolol (300 nM) was present in the organ bath throughout the experiment. For cimetidine, a pA_2 value of 6·30 ± 0·10 (slope 0·80 ± 0·04 of Schild plot, n = 16) was obtained.

Guinea-pig right atrium (spontaneously beating): β-adrenoceptors

Isolated atria were set up as described for experiments on H_2 receptors (in the absence of (*R,S*)-metoprolol). After 30 min of continuous washing and an equilibration period of 30 min, two concentration-frequency curves for (*R,S*)-isoprenaline (0·1–100 nM) were determined in the absence and presence of antagonist (incubation 30 min) at an interval of 90 min. For (*R,S*)-metoprolol, a pA_2 value of 8·00 ± 0·11 (slope 0·91 ± 0·04 of Schild plot, n = 20) was obtained.

Expression of results

Results are given as means ± s.e.; pK_B values were calculated from the equation:

$$pK_B = pA_x + \log(CR - 1) \quad (1)$$

where pA_x is the negative logarithm of the concentration of antagonist used and CR (concentration ratio) is the ratio of agonist EC50 measured in the presence of antagonist over that measured in the absence of antagonist (Furchgott 1972). Slopes ± s.d. of the regression lines and pA_2 values were estimated according to the method of Arunlakshana & Schild (1959), using three different concentrations of antagonist over 2 log units. Non-competititve antagonists were characterized by their pD_2' values according to the equation:

$$pD_2' = pA_x + \log(100/E_{max} - 1) \quad (2)$$

where pA_x is the negative logarithm of the concentration of antagonist used and E_{max} is the maximum response (%) in the presence of antagonist (van Rossum 1963). Student's *t*-test (two-tailed) was used to assess the significance between two mean values, with $P < 0.05$ being considered as significant. When one control group was compared with more than one group of treatments, a one-way analysis of variance was performed.

Results

Antagonist activity and specificity of sarpogrelate, (R,S)-M-1, (R)-M-1 and (S)-M-1, and stereoselectivity of the enantiomers (R)-M-1 and (S)-M-1 in functional in-vitro assays

Affinity estimates for the interaction of sarpogrelate,

Table 1. Inhibitory effects (pK_B values ± s.e.; n, numbers are in parentheses) of ketanserin, sarpogrelate, (*R,S*)-M-1, (*R*)-M-1 and (*S*)-M-1, and enantiomeric potency ratios (*R*)-M-1: (*S*)-M-1 from functional in-vitro assays.

Receptor	Ketanserin	Sarpogrelate	(*R,S*)-M-1	(*R*)-M-1	(*S*)-M-1	Stereoselectivity
5-HT_{2A}[a]	9·55 ± 0·02 (36)	8·46 ± 0·04 (16)	8·96 ± 0·06 (16)	9·04 ± 0·04 (16)	8·84 ± 0·05 (16)	1·6 : 1*
5-HT_1-like[b]	< 6·0[g]	5·38 ± 0·05 (8)	6·00 ± 0·07 (7)	5·82 ± 0·10 (5)	6·30 ± 0·12 (5)	1 : 3*
5-HT_3[c]	—	4·62 ± 0·05[f] (6)	—	5·54 ± 0·05[f] (6)	5·32 ± 0·05[f] (6)	1·7 : 1*
α_1[d]	7·99 ± 0·05 (23)	6·30 ± 0·08 (8)	6·82 ± 0·05 (8)	6·58 ± 0·11 (8)	6·80 ± 0·05 (8)	1 : 1·7
β[e]	5·35 ± 0·14 (3)	5·53 ± 0·02 (3)	—	5·84 ± 0·12 (4)	6·54 ± 0·15 (5)	1 : 5*
H_1[c]	8·53 ± 0·04 (23)	5·47 ± 0·06 (14)	6·46 ± 0·04 (12)	6·49 ± 0·04 (8)	6·12 ± 0·05 (8)	2·3 : 1*
H_2[e]	5·21 ± 0·12 (3)	5·01 ± 0·01 (3)	—	5·37 ± 0·04 (3)	5·36 ± 0·05 (3)	1 : 1
M_3[c]	4·18 ± 0·08[f] (4)	4·02 ± 0·11[f] (8)	4·84 ± 0·08[f] (12)	5·14 ± 0·09[f] (8)	4·74 ± 0·04[f] (8)	2·5 : 1*

[a] Rat-tail artery, [b] guinea-pig iliac artery, [c] guinea-pig ileum, [d] rat aorta, [e] guinea-pig atrium, [f] pD'_2 ± s.e.m., [g] from Pertz (1993). * $P < 0·05$.

(*R,S*)-M-1 and M-1 enantiomers with 5-HT_{2A} receptors, 5-HT_1-like receptors, 5-HT_3 receptors, α_1 adrenoceptors, β adrenoceptors, histamine H_1 receptors, histamine H_2 receptors and muscarinic M_3 receptors, based on functional studies, are summarized in Table 1 in comparison with the classical 5-HT_{2A} antagonist ketanserin. In addition, Table 1 shows the enantiomeric potency ratios of (*R*)- and (*S*)-M-1 for the different receptors studied. Sarpogrelate was found to be a relatively potent antagonist of 5-HT at 5-HT_{2A} receptors with weak (α_1) or minimal (5-HT_1-like, 5-HT_3, β, H_1, H_2 and M_3) affinity for other receptor mechanisms. Antagonism at 5-HT_{2A} receptors was one-twelfth that found for ketanserin. (*R,S*)-M-1 and M-1 enantiomers showed higher affinities for 5-HT_{2A} receptors than sarpogrelate with only one-fifth to one-third the antagonist activity of ketanserin. On the other hand, the metabolites also showed higher affinities than sarpogrelate for other receptors examined in the present study. The specificity of the metabolites toward 5-HT_{2A} receptors was still high but slightly lower than that found for sarpogrelate, for example (*S*)-M-1 showed weak affinity for 5-HT_1-like receptors ($pK_B = 6·30$), α_1- ($pK_B = 6·80$) and β- ($pK_B = 6·54$) adrenoceptors, and (*R*)-M-1 was weakly active as an antagonist at histamine H_1 receptors ($pK_B = 6·49$). The weak affinity of sarpogrelate and its metabolites for α_1-adrenoceptors and histamine H_1 receptors was in marked contrast to ketanserin. On the whole, M-1 enantiomers showed weak stereoselectivity for various receptors studied. Moderate discrimination was found at β-adrenoceptors and 5-HT_1-like receptors, where enantiomeric activity ratios in favour of (*S*)-M-1 were 5 and 3, respectively.

Competitive antagonism of 5-HT at 5-HT_{2A} receptors in rat tail artery by sarpogrelate, (R,S)-M-1 and M-1 enantiomers

Sarpogrelate, (*R, S*)-M-1 and M-1 enantiomers were investigated as antagonists of 5-HT in rat tail artery (Fig. 1). All compounds produced concentration-dependent antagonism of contractile responses to 5-HT, causing parallel dextral shifts of 5-HT concentration-effect curves with no or little effect on maximum response. Schild plots were linear with slopes of 0·95 ± 0·05 (sarpogrelate), 0·95 ± 0·05 ((*R,S*)-M-1), 1·03 ± 0·02 ((*R*)-M-1) and 1·02 ± 0·06 ((*S*)-M-1). Thus, sarpogrelate and its metabolites proved to be competitive antagonists of 5-HT at 5-HT_{2A} receptors of rat tail artery

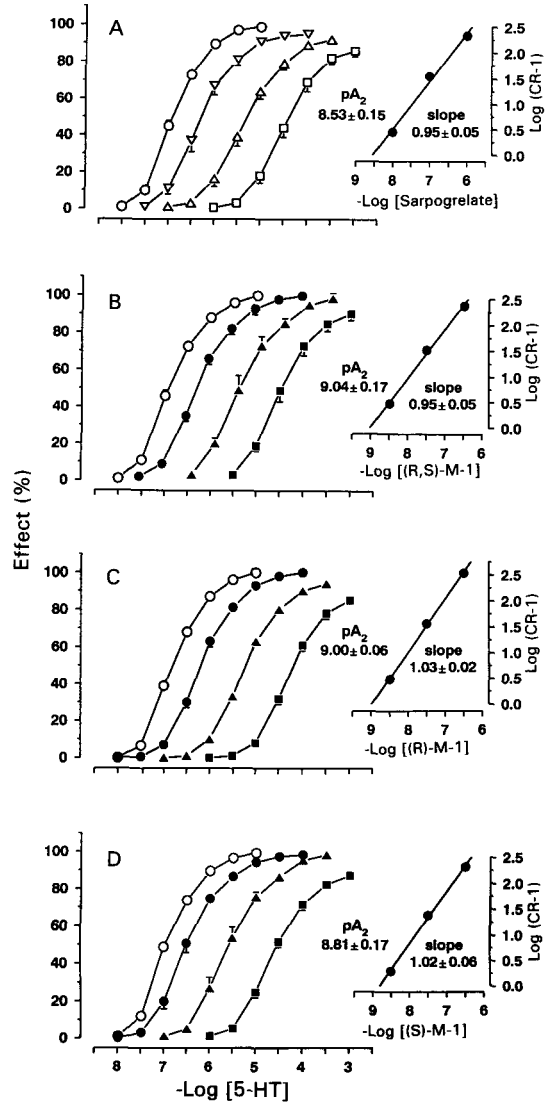

FIG. 1. Competitive antagonism of 5-HT-induced contractions in rat tail artery. A. Sarpogrelate at 10 nM (▽, n = 6), 100 nM (△, n = 6) and 1000 nM (□, n = 6), B. (*R,S*)-M-1, C. (*R*)-M-1, D. (*S*)-M-1 at 3 nM (●, n = 6), 30 nM (▲, n = 4–6) and 300 nM (■, n = 4–6). Left: 5-HT concentration-effect curves in the absence (○, n = 16) and presence of antagonists. Right: Schild analysis of antagonist-induced curve displacements. All values are means ± s.e. Slopes are given as means ± s.d.

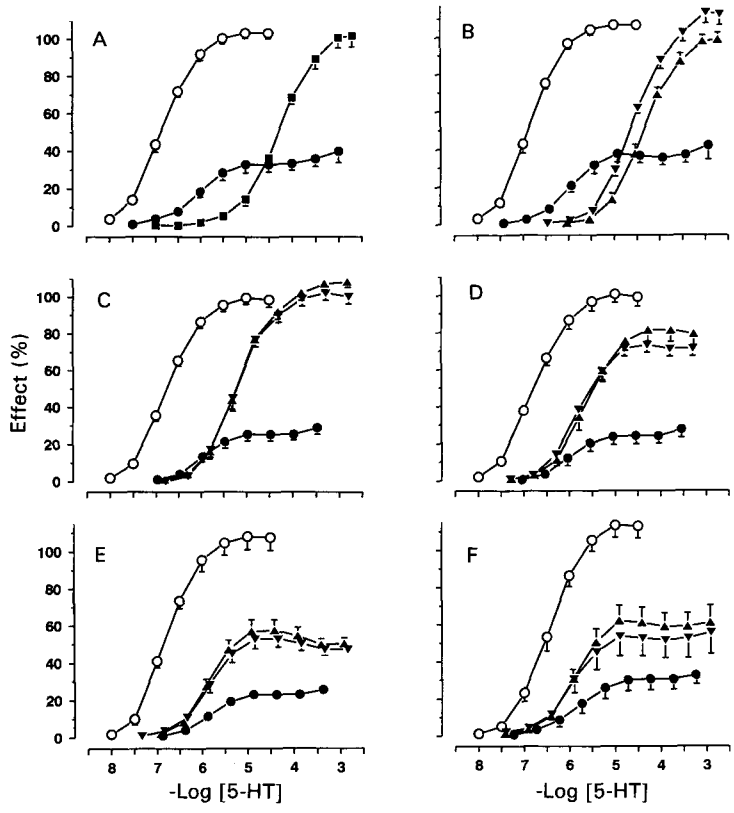

FIG. 2. Prevention by sarpogrelate and (*R*)- and (*S*)-M-1 enantiomers of the depression of 5-HT maximum responses caused by ritanserin in rat tail artery. ○ Absence of antagonists (n = 6–13), ● ritanserin (1 nM, n = 6–9), ■ ritanserin (1 μM) + sarpogrelate (1 μM, n = 7), ▲ ritanserin (1 nM) + (*R*)-M-1 (0·3–300 nM, n = 6–8), ▼ ritanserin (1 nM) + (*S*)-M-1 (0·3–300 nM, n = 5–8). Metabolite concentrations were A. 0, B. 300, C. 30, D. 3, E. 1, F. 0·3 nM. All values are means ± s.e.

with calculated pA_2 values of 8·53 ± 0·15 (sarpogrelate), 9·04 ± 0·17 ((*R,S*)-M-1), 9·00 ± 0·06 ((*R*)-M-1), and 8·81 ± 0·17 ((*S*)-M-1).

Allosteric modulation of 5-HT$_{2A}$ receptors in rat tail artery by sarpogrelate, and (R)- and (S)-M-1 enantiomers

These experiments were performed to determine whether sarpogrelate and M-1 enantiomers can mimic the ability of ketanserin to prevent the depression of the 5-HT maximum response caused by the insurmountable 5-HT$_{2A}$ antagonist ritanserin (Frenken & Kaumann 1987). Fig. 2 and Table 2 show that sarpogrelate and (*R*)- and (*S*)-M-1 enantiomers, respectively, can protect 5-HT$_{2A}$ receptors from blockade by ritanserin. Maximum effects of 5-HT-induced contractions of rat tail artery were strongly depressed in the presence of 1 nM ritanserin. When sarpogrelate, (*R*)-M-1 and (*S*)-M-1, respectively, were incubated 30 min before the administration of 1 nM ritanserin, the depression of the 5-HT maximum response was totally or partially prevented depending on the concentration of antagonist used. Total prevention of ritanserin-induced depression of the 5-HT maximum response was found at 1 μM sarpogrelate and 30 and 300 nM (*R*)-M-1 or (*S*)-M-1, whereas 0·3–3 nM (*R*)-M-1 or (*S*)-M-1, partially prevented the depression of the 5-HT maximum contractile effect caused by 1 nM ritanserin (Fig. 2, Table 2). The enantiomers failed to differ in restoring the ritanserin-induced depression of the maximum response to 5-HT. (*R*)- or (*S*)-M-1, partially prevented the depression of the 5-HT maximum response caused by ritanserin even at concentrations (0·3 and 1 nM) that did not block 5-HT$_{2A}$ receptors (see Table 2). It should be mentioned that in experiments where sarpogrelate and M-1 enantiomers totally prevented ritanserin-induced depression of the 5-HT maximum response (Fig. 2A–C), the dextral shift of the second 5-HT curves (Table 2) yielded pK_B values for the antagonists comparable with those found in 30-min incubation experiments (Fig. 1).

Discussion

Sarpogrelate belongs to a novel class of antiplatelet agents, [2-[ω-aminoalkoxyphenyl]ethyl]benzenes, whose inhibitory effects on platelet aggregation were found to be well correlated with their antagonist activities at vascular 5-HT$_{2A}$ receptors (Kikumoto et al 1990). The aim of the present study was to extend the affinity profile of sarpogrelate and its major metabolite (*R,S*)-M-1 in various functional in-vitro assays with special regard to stereoselectivity of M-1 enantiomers. Since the present study confirms previously published findings (Hara et al 1991) that sarpogrelate and (*R,S*)-M-1 are competitive antagonists of 5-HT at 5-HT$_{2A}$ receptors of rat tail artery, further experiments were performed to clarify the question whether sarpogrelate and M-1 enantiomers can mimic the ability of the classical

Table 2. △pEC50 (pEC50 1st curves—pEC50 2nd curves) and E_{max} values (2nd curves) of concentration-effect curves to 5-HT in rat tail artery in experiments with (*R*)-M-1 and (*S*)-M-1 preventing ritanserin-induced depressions of 5-HT maximum responses.

Antagonists (nM)			△pEC50	E_{max}(%)	
(*R*)-M-1	(*S*)-M-1	Ritanserin			
—	—	—	−0·20±0·03	107 ± 3	
300	—	1	2·33±0·05	99 ± 4	
—	300	1	2·12±0·05	114 ± 6	*
—	—	1	n.d.	42 ± 5*	
—	—	—	−0·12±0·04	100 ± 4	
30	—	1	1·48±0·08	108 ± 4	
—	30	1	1·41±0·07	102 ± 4	
—	—	1	n.d.	29 ± 4*	
—	—	—	−0·12±0·05	101 ± 5	
3	—	1	0·91±0·04	81 ± 6	*
—	3	1	0·87±0·04	73 ± 4	*
—	—	1	n.d.	28 ± 5	
—	—	—	−0·13±0·06	108 ± 7	
1	—	1	n.d.	55 ± 6	*
—	1	1	n.d.	52 ± 5	*
—	—	1	n.d.	25 ± 3	
1	—	—	0·17±0·04	99 ± 4	
—	1	—	0·13±0·10	104 ± 3	
—	—	—	−0·09±0·04	113 ± 7	
0·3	—	1	n.d.	62 ± 8	*
—	0·3	1	n.d.	51 ± 10	*
—	—	1	n.d.	32 ± 5	
0·3	—	—	−0·04±0·01	103 ± 1	
—	0·3	—	0·01±0·01	106 ± 8	

n.d. not determined. *$P < 0·05$.

5-HT_{2A} antagonist ketanserin to prevent the depression of 5-HT-induced contractions caused by the insurmountable 5-HT_{2A} antagonist ritanserin (Frenken & Kaumann 1987).

In the present study sarpogrelate has been shown to be a relatively potent antagonist of 5-HT at 5-HT_{2A} receptors with weak or minimal affinity for other receptor mechanisms. These findings are consistent with previously published observations based on radioligand binding data (Maruyama et al 1991) and support the specificity of sarpogrelate. The specificity of sarpogrelate is in marked contrast to ketanserin which was found to be a potent histamine H_1 antagonist ($pK_B = 8·53$) and a relatively potent α_1-adrenoceptor antagonist ($pK_B = 7·99$) in the present study. Sarpogrelate showed lower affinity than ketanserin for histamine H_1 receptors (11/150th) and α_1-adrenoceptors (1/50th). Further support of the findings of Maruyama et al (1991) consists in the fact that the metabolite of sarpogrelate in contrast to the parent drug showed higher affinity for 5-HT_{2A} receptors and also for various other receptor mechanisms investigated in the present study. The specificity of (*R*,*S*)-M-1 and M-1 enantiomers is somewhat lower than that found for sarpogrelate. The weak β-adrenoceptor blocking properties ($pK_B = 6·54$) of (*S*)-M-1 can be explained by its apparent structural resemblance to the classical β-adrenoceptor antagonist (*S*)-propranolol.

Because the major metabolite of sarpogrelate, (*R*,*S*)-M-1, is a mixture of two enantiomers, we sought to investigate the relative contribution of each of the enantiomers to the antagonist activity of (*R*,*S*)-M-1. Small but significant differences ($P < 0·05$) between the enantiomers were found at 5-HT_{2A}, 5-HT_3, histamine H_1 and muscarinic M_3 receptors, where (*R*)-M-1 showed 1·6-, 1·7-, 2·3- and 2·5-fold antagonist activity compared with (*S*)-M-1. On the other hand, affinity at β-adrenoceptors and 5-HT_1-like receptors was 5-fold and 3-fold, respectively, for (*S*)-M-1 compared with (*R*)-M-1. These data indicate that the stereochemical configuration of the metabolite (*R*,*S*)-M-1 is not crucial for the binding to the receptors examined in the present study. The observation is of special significance for the interactions with 5-HT_{2A} receptors, where (*R*,*S*)-M-1 was a potent and competitive antagonist of 5-HT-induced contractions ($pA_2 = 9·04$, slope 0·95 of Schild plot).

The ability of ketanserin to prevent insurmountable antagonism of 5-HT-induced contractions caused by ritanserin in rat tail artery (Frenken & Kaumann 1987) has been explained by a two-state model for the 5-HT_{2A} receptor (Kaumann 1989). The 5-HT_{2A} receptor interconverts between a highly active state R and a low active state R′. Ritanserin binds to an allosteric site which is distinct from the 5-HT_{2A} receptor and induces a conformational change of the receptor from R to R′, thereby causing insurmountable antagonism. Competitive antagonists (e.g. ketanserin) favour the R state and are able to reverse the effects of insurmountable antagonists (e.g. ritanserin) by competing not only with 5-HT for the 5-HT_{2A} receptor but also with the insurmountable antagonist for the allosteric site, thus shifting R′ back towards R.

The results from the present study show that sarpogrelate and M-1 enantiomers resemble ketanserin by preventing insurmoutable antagonism of 5-HT-induced contractions caused by ritanserin. Sarpogrelate and its enantiomeric metabolites totally or partially prevented ritanserin-induced depression of the maximum 5-HT response depending on the concentration of antagonist used. It is suggested that the threshold concentration of (*R*)- and (*S*)-M-1 enantiomers for interaction with the allosteric binding site is somewhat lower than for the 5-HT_{2A} receptor, since (*R*)- and (*S*)-M-1 partially prevented ritanserin-induced depression of the 5-HT maximum response even at concentrations (0·3 and 1 nM) that did not block 5-HT_{2A} receptors (Table 2). Unlike the enantiomers of α-methylketanserin (Elz 1991), which interact stereoselectively with the allosteric 5-HT_{2A} receptor site, ((*S*)-α-methylketanserin is a potent allosteric deactivator and (*R*)-α-methylketanserin is a weak allosteric activator), (*R*)- and (*S*)-M-1 are nearly equipotent allosteric activators of the 5-HT_{2A} receptor system in rat tail artery.

It is concluded that sarpogrelate and the enantiomers of its major metabolite M-1 are specific antagonists of 5-HT at 5-HT_{2A} receptors. Stereochemical aspects of the molecular structure seem to be of minor importance for binding to the 5-HT_{2A} receptor. Like the classical 5-HT_{2A} antagonist ketanserin, sarpogrelate and M-1 appear to be allosteric activators of the 5-HT_{2A} receptor system.

Acknowledgements

The studies were supported by a grant of Schwarz Pharma AG, Germany. The authors wish to thank W. Schunack for additional support of the studies. The generous gifts of compounds by the pharmaceutical companies mentioned in Materials and Methods are gratefully acknowledged.

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