In Vitro Pharmacological Profile of the Novel α_1 -Adrenoceptor Antagonist HSR-175

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ABSTRACT—The pharmacological profile of HSR-175, a new α_1 -adrenoceptor antagonist, was studied in vitro and compared with those of other α_1 -antagonists. HSR-175, prazosin, bunazosin and yohimbine competitively antagonized the contractile responses induced by noradrenaline in the dog mesenteric arteries and the rabbit thoracic aorta. The pA_2 values for HSR-175 in the dog mesenteric arteries and the rabbit aorta were 10.38 and 9.63, respectively, which were significantly higher than those for prazosin (8.39 and 8.80), bunazosin (8.44 and 8.75) and yohimbine (7.34 and 6.10). HSR-175 also inhibited the sympathetic adrenergic contraction induced by electrical transmural stimulation in the dog mesenteric arteries, and the inhibitory effect of HSR-175 was more potent than those of prazosin and bunazosin. Although HSR-175 also possessed competitive antagonist properties at pre- and postsynaptic α_2 -adrenoceptors in the rat vas deferens and the dog saphenous veins, those affinities ($pA_2 = 6.41$ and 7.05) were much lower than those at postsynaptic α_1 -adrenoceptors. Furthermore, HSR-175 at concentration of 10^{-6} M showed no inhibition on the contractile responses to 5-HT, histamine, KCl and angiotensin II in the rabbit thoracic aorta. These results indicate that HSR-175 is a very potent and selective α_1 -adrenoceptor antagonist.

Keywords: α_1 -Adrenoceptor, α_1 -Adrenoceptor antagonist, Blood vessel, HSR-175

α -Adrenoceptors have been pharmacologically divided into two subtypes, i.e., α_1 and α_2 , on the basis of selective agonists and antagonists (1–3). In general, α_1 -adrenoceptors are most selectively activated by phenylephrine and cirazoline and are antagonized by prazosin and bunazosin. In contrast, α_2 -adrenoceptors are preferentially activated by clonidine and BHT-933 and are antagonized by yohimbine and idazoxan.

In various blood vessels α_1 -adrenoceptors are located postsynaptically, mediating vasoconstriction. Postsynaptic α_1 -adrenoceptors have also been demonstrated in the lower urinary tracts (4, 5). Therefore, it has now been confirmed clinically that α_1 -adrenoceptor antagonists are effective for treatments of hypertension, cardiac failure and lower urinary tract disorder (6, 7).

We tried to develop a more potent and selective α_1 -adrenoceptor antagonist and recently found that the newly synthesized compound *R*-(–)-5-[2-[[2-(5-fluoro-2-methoxyphenoxy)ethyl]amino]propyl]-2-methoxybenzenesulfonamide hydrochloride (HSR-175) (Fig. 1) had a

Fig. 1. Chemical structure of HSR-175.

higher affinity to α_1 -adrenoceptors than prazosin and bunazosin. In this paper, we report the α_1 -adrenoceptor selectivity of HSR-175, as compared with those of prazosin, bunazosin and yohimbine.

MATERIALS AND METHODS

Experiments with dog and rabbit vascular vessels

Mongrel dogs of either sex (7.5–13.0 kg) and male albino rabbits (2.1–2.8 kg) were anesthetized with sodium thiopental (20 mg/kg, i.v.) and sodium pento-

barbital (30 mg/kg, i.v.), respectively, and exsanguinated from the common carotid arteries. The dog mesenteric artery and saphenous vein and rabbit thoracic aorta were isolated and helically cut under a dissecting microscope. To avoid the possible involvement of endothelium-derived relaxing factor in the mechanical response (8), the endothelial cells of these vessels were removed by rubbing them with filter paper and the functional loss of endothelial cells was confirmed by the loss of relaxing response to acetylcholine (10^{-7} M) in agonist-precontracted vessels (9). Each strip was mounted vertically in an organ bath containing 10 ml of Krebs-Henseleit solution of following composition: 118 mM NaCl, 4.7 mM KCl, 2.6 mM CaCl_2 , 1.2 mM MgSO_4 , 1.2 mM KH_2PO_4 , 24.9 mM NaHCO_3 and 11.1 mM glucose.

The bath medium was maintained at 37°C, pH 7.4, and was equilibrated with a gas mixture consisting of 95% O_2 and 5% CO_2 . A resting tension of 2 g, 1 g and 0.5 g was applied to the rabbit thoracic aorta, dog mesenteric artery and saphenous vein, respectively, and the response was recorded isometrically through a force-displacement transducer (T7-30-240, Orientec, Tokyo, Japan) on a recorder (RJG-4028, Nihon Kohden, Tokyo, Japan). All preparations were equilibrated for 60 min before starting the experiments. Cumulative concentration-response curves of agonists such as noradrenaline were determined by a step-wise increase in the concentration of an agonist as soon as a steady response to the previous administration had been achieved. α -Adrenoceptor antagonists to be tested were applied 30 min before recording the concentration-response curve. Desmethylinipramine (10^{-7} M), deoxycorticosterone acetate (5×10^{-6} M) and propranolol (10^{-6} M) were added to the bath solution to block neuronal and extraneuronal uptake and β -adrenoceptors, respectively, when noradrenaline was applied as an agonist. When the responses to clonidine in the dog saphenous vein were recorded, the preparations were pretreated with phenoxybenzamine (10^{-7} M) for 30 min and then washed. Indomethacin (3×10^{-6} M) and propranolol (10^{-6} M) were added to the bath solution (10).

In the dog mesenteric arteries, electrical transmural stimulation was applied through a pair of platinum electrodes at 15-min intervals. The preparation was placed in parallel between the electrodes. The distance between the electrodes was approximately 2 mm. Stimulus parameters were 0.3 msec duration, frequency at 10 Hz and supramaximal voltage (10 V) for 10 sec. In this series of experiments, propranolol (10^{-6} M) and α, β -methylene ATP (10^{-5} M) were present throughout the experiments to block β -adrenoceptors and the sym-

pathetic purinergic component, respectively (11). Complete loss of the sympathetic purinergic component or response to 10^{-3} M ATP for 5 hours after application of α, β -methylene ATP was preliminary performed. α -Adrenoceptor antagonists to be tested were applied 60 min after the treatment of α, β -methylene ATP.

Experiments with rat vas deferens

Male Wistar rats (270–340 g) were killed by a blow on the head and exsanguinated from the common carotid arteries. The prostatic portion of the vas deferens was vertically suspended in an organ bath containing Krebs-Henseleit solution at 37°C under 1-g resting tension. The preparations were electrically stimulated through a pair of platinum-wire electrodes (0.1 Hz, 0.3 msec duration, 10 V), and the twitch responses to electrical stimulation were recorded. After an equilibration period of 90 min, cumulative concentration-response curves of clonidine in the absence of α -adrenoceptor antagonist were obtained in 12 preparations. In other preparations, the responses to clonidine were examined after a 30-min incubation with the antagonist. Propranolol (10^{-6} M) was added to the bath solution.

Drugs

HSR-175 (*R*-(–)-5-[2-[[2-(5-fluoro-2-methoxyphenoxy)ethyl]amino]propyl]-2-methoxybenzenesulfonamide hydrochloride) was synthesized at Central Research Laboratories, Hokuriku Seiyaku Co., Ltd.

The following drugs were used: *l*-noradrenaline bitartrate, and yohimbine hydrochloride (Wako, Osaka, Japan); angiotensin II, ATP, clonidine hydrochloride, desmethylinipramine hydrochloride, indomethacin, α, β -methylene ATP, and prazosin hydrochloride (Sigma, St. Louis, MO, USA); deoxycorticosterone acetate and histamine dihydrochloride (Nacalai, Kyoto, Japan); 5-hydroxytryptamine creatinine sulfate (5-HT) (Merck, Darmstadt, F.R.G.); propranolol hydrochloride (Inderal, Sumitomo, Osaka, Japan); guanethidine sulfate and phenoxybenzamine hydrochloride (Tokyo Kasei, Tokyo, Japan); acetylcholine hydrochloride (Ovisot, Daiichi, Tokyo, Japan); and tetrodotoxin (Sankyo, Tokyo, Japan). Bunazosin hydrochloride was prepared in our laboratory.

Deoxycorticosterone acetate, indomethacin and phenoxybenzamine were dissolved in ethanol and then diluted with distilled water. The other drugs were dissolved in distilled water.

Statistical analysis

Experimental values were expressed as means $\pm$ S.E. or means with 95% confidence limits. ED_{50} values for

the concentration-response curves were calculated graphically, and the pA_2 values for α -adrenoceptor antagonists were obtained according to the method of Arunlakshana and Schild (12).

RESULTS

Effects on postsynaptic α_1 -adrenoceptors in dog mesenteric arteries and rabbit thoracic aorta

Noradrenaline produced concentration-dependent contractions in the dog mesenteric arteries. HSR-175 (3×10^{-10} – 3×10^{-9} M), prazosin (10^{-8} – 10^{-7} M), bunazosin (10^{-8} – 10^{-7} M) and yohimbine (3×10^{-7} – 3×10^{-6} M) produced parallel shifts to the right of the concentration-response curves for noradrenaline

without a decrease in the maximal response (Fig. 2). The slope of the Schild plot for each antagonist was not different from unity (Table 1). The pA_2 value for HSR-175 (10.38 ± 0.16) was much higher than that for prazosin (8.39 ± 0.09), bunazosin (8.44 ± 0.10) and yohimbine (7.34 ± 0.19) (Table 1). None of the antagonists tested affected the resting tension of the mesenteric arteries at the concentrations used.

In the rabbit thoracic aorta, the effects of these antagonists on the contractile response to noradrenaline were similar to those in the dog mesenteric arteries. The pA_2 value for HSR-175 (9.63 ± 0.13) was higher than that for prazosin (8.80 ± 0.17), bunazosin (8.75 ± 0.13) and yohimbine (6.10 ± 0.07) (Table 1). The pA_2 values for HSR-175 and yohimbine in the dog mesenteric arteries

Fig. 2. Effects of HSR-175 (A), prazosin (B), bunazosin (C) and yohimbine (D) on the contractile response to noradrenaline in dog mesenteric artery. Contractile responses induced by 10^{-4} M noradrenaline before drug treatment were taken as 100%. Each value is the mean $\pm$ S.E. of 6 to 9 experiments.

Table 1. pA₂ values and slope of the Schild plot for HSR-175, prazosin, bunazosin and yohimbine at postsynaptic α_1 -adrenoceptors in dog mesenteric artery and rabbit thoracic aorta

Antagonist	Dog mesenteric artery			Rabbit thoracic aorta		
	n	pA ₂	slope	n	pA ₂	slope
HSR-175	22	10.38 $\pm$ 0.16	0.94 (0.72–1.16)	20	9.63 $\pm$ 0.13	0.96 (0.72–1.20)
Prazosin	21	8.39 $\pm$ 0.09	0.99 (0.81–1.17)	16	8.80 $\pm$ 0.17	0.83 (0.61–1.05)
Bunazosin	22	8.44 $\pm$ 0.10	0.97 (0.79–1.15)	17	8.75 $\pm$ 0.13	0.85 (0.68–1.03)
Yohimbine	21	7.34 $\pm$ 0.19	0.82 (0.59–1.05)	20	6.10 $\pm$ 0.07	0.87 (0.76–0.99)

pA₂ values were expressed as means $\pm$ S.E. The slope of the Schild plot was expressed as means with 95% confidence limits.

Fig. 3. Effects of HSR-175 (A), prazosin (B), bunazosin (C) and yohimbine (D) on the clonidine-induced inhibitory response to twitch contraction induced by electrical transmural stimulation (0.1 Hz) in rat vas deferens. Twitch contractile responses to electrical transmural stimulation before clonidine treatment were taken as 100%. Each value is the mean $\pm$ S.E. of 4 to 12 experiments.

Table 2. pA_2 values and slope of the Schild plot for HSR-175, prazosin, bunazosin and yohimbine at presynaptic α_2 -adrenoceptors in rat vas deferens and α_1 -adrenoceptor selectivities of these antagonists

Antagonist	n	Rat vas deferens		α_1/α_2 Ratio	
		pA_2	slope	α_1 (Me)/ α_2	α_1 (Ao)/ α_2
HSR-175	13	6.41 ± 0.11	0.96 (0.73–1.19)	9330	1660
Prazosin	8	5.38 ± 0.10	—	1020	2630
Bunazosin	13	5.95 ± 0.15	1.15 (0.79–1.51)	310	630
Yohimbine	14	7.68 ± 0.09	1.09 (0.84–1.35)	0.46	0.026

pA_2 values were expressed as means $\pm$ S.E. The slope of the Schild plot was expressed as means with 95% confidence limits. α_1 (Me)/ α_2 : Antilogarithm of the difference between pA_2 values in dog mesenteric artery and rat vas deferens. α_1 (Ao)/ α_2 : Antilogarithm of the difference between pA_2 values in rabbit thoracic aorta and rat vas deferens. The pA_2 value for prazosin was calculated as the negative logarithm of K_B ; therefore, the slope of the Schild plot was not determined.

were higher than those in the rabbit thoracic aorta. On the other hand, the pA_2 values for prazosin and bunazosin in the mesenteric arteries were lower than those in the rabbit thoracic aorta (Table 1).

Effects on presynaptic α_2 -adrenoceptor in rat vas deferens

In the rat vas deferens, clonidine inhibited the twitch contraction induced by electrical stimulation in a concentration-dependent manner. The concentration-inhibition curves for clonidine were shifted to the right in a parallel manner in the presense of HSR-175 (10^{-6} – 10^{-5} M), bunazosin (3×10^{-6} – 3×10^{-5} M) and yohimbine (3×10^{-8} – 3×10^{-7} M) (Fig. 3). The slopes of the Schild plot for HSR-175, bunazosin and yohimbine were not different from unity (Table 2). In the case of prazosin, only two concentration (3×10^{-6} and 10^{-5} M) were tested due to the low solubility at higher concentrations (Fig. 3). Therefore, the pA_2 value for prazosin was calculated as the negative logarithm of K_B , where K_B is a dissociation constant (13). None of the antagonists tested affected the resting tension of the rat vas deferens.

From the pA_2 values obtained in the dog mesenteric arteries, rabbit thoracic aorta and rat vas deferens, the α_1 - and α_2 -adrenoceptor selectivity of each antagonist was estimated. For this purpose, the antilogarithm of the difference between the pA_2 values obtained in the dog mesenteric arteries or the rabbit aorta (postsynaptic α_1 -adrenoceptors) and the rat vas deferens (presynaptic α_2 -adrenoceptors) was calculated (Table 2). HSR-175, prazosin and bunazosin were more selective at postsynaptic α_1 -adrenoceptors than at presynaptic α_2 -adrenoceptors. The α_1 (Me)/ α_2 ratio for HSR-175 was relatively greater than the α_1 (Ao)/ α_2 ratio (9330 versus 1660). On the other hand, the α_1 (Me)/ α_2 ratio for prazosin

Table 3. pA_2 values and slope of the Schild plot for HSR-175 and yohimbine at postsynaptic α_2 -adrenoceptors in dog saphenous vein

Antagonist	n	pA_2	Slope
HSR-175	15	7.05 ± 0.16	0.84 (0.58–1.10)
Yohimbine	15	8.88 ± 0.25	0.89 (0.55–1.23)

pA_2 values were expressed as means $\pm$ S.E. The slope of the Schild plot was expressed as means with 95% confidence limits.

and bunazosin were relatively smaller than the α_1 (Ao)/ α_2 ratio (1020 versus 2630 and 310 versus 630, respectively). In contrast, yohimbine was more selective at presynaptic α_2 -adrenoceptors.

Effects on postsynaptic α_2 -adrenoceptor in dog saphenous veins

Clonidine produced concentration-dependent contraction in the dog saphenous veins where the postsynaptic α_1 -adrenoceptors were masked by phenoxybenzamine. HSR-175 (3×10^{-7} – 3×10^{-6} M) and yohimbine (10^{-8} – 10^{-7} M) produced parallel shifts to the right of the concentration-response curves for clonidine without a decrease in the maximal response. The slopes of the Schild plot for HSR-175 and yohimbine were not different from unity (Table 3). The pA_2 value for HSR-175 was 7.05 ± 0.06 , which was much lower than those at postsynaptic α_1 -adrenoceptors. On the other hand, the pA_2 value for yohimbine (8.88 ± 0.25) was much higher than those at postsynaptic α_1 -adrenoceptors (Table 3).

Effects on the responses to electrical transmural stimulation in the dog mesenteric artery

In the presence of α, β -methylene ATP (10^{-5} M) and

Fig. 4. Effects of HSR-175 (●), prazosin (▲) and bunazosin (■) on the contractile response to electrical transmural stimulation (10 Hz for 10 sec) in dog mesenteric artery. Contractile responses to electrical transmural stimulation before drug treatment were taken as 100%. Each value is the mean $\pm$ S.E. of 6 experiments.

propranolol (10^{-6} M), electrical transmural stimulation at 10 Hz for 10 sec produced a transient contraction in the dog mesenteric artery. This contraction was abolished by guanethidine (3×10^{-6} M) or tetrodotoxin (3×10^{-7} M) (data not shown). HSR-175 (3×10^{-12} – 10^{-9} M), prazosin (10^{-10} – 10^{-7} M) and bunazosin (10^{-10} – 10^{-7} M) inhibited the contraction in a concentration-dependent manner (Fig. 4). The ED_{50} value for HSR-175 ($6.5 \pm 1.2 \times 10^{-11}$ M) was much lower than those for prazosin ($1.7 \pm 0.3 \times 10^{-9}$ M) and bunazosin ($1.4 \pm 0.4 \times 10^{-9}$ M).

Effects on the contractile responses to 5-HT, histamine, KCl and angiotensin II in rabbit thoracic aorta

HSR-175 (10^{-6} M) did not affect of concentration-response curves for 5-HT, histamine, KCl and angiotensin II in the rabbit thoracic aorta (Fig. 5)

DISCUSSION

Postsynaptic α -adrenoceptors of the dog mesenteric artery and the rabbit thoracic aorta are characterized as the α_1 -adrenoceptor subtype (10, 14, 15). Prazosin, bunazosin and yohimbine competitively inhibited the concentration-response curves of noradrenaline in both the arteries. The pA_2 values for prazosin and bunazosin were higher than that for yohimbine (Table 1). These results confirm the previous conclusion that the contractile response to noradrenaline is mediated through postsynaptic α_1 -adrenoceptors in the dog mesenteric artery and the rabbit thoracic aorta (10, 14, 15).

Fig. 5. Effects of HSR-175 (10^{-6} M) on the contractile response to 5-HT (A), histamine (B), KCl (C) and angiotensin II (D) in rabbit thoracic aorta. Contractile responses induced by 10^{-5} M 5-HT, 10^{-4} M histamine, 60 mM KCl and 10^{-7} M angiotensin II before HSR-175 treatment were taken as 100%, respectively. ●, before; ○, after treatment with 10^{-6} M HSR-175. Each value is the mean $\pm$ S.E. of 4 to 5 experiments.

HSR-175 antagonized the contractile responses to noradrenaline in such preparations, and the slopes of the Schild plot were close to unity. The pA_2 values for HSR-175 were much higher than those for prazosin and bunazosin. Furthermore, HSR-175 inhibited the sympathetic adrenergic contraction in the dog mesenteric artery and the potency for the inhibitory effect of HSR-175 was much greater than those for the inhibitory effects of prazosin and bunazosin. These results strongly indicate that HSR-175 is a very potent and competitive antagonist on postsynaptic α_1 -adrenoceptors.

In addition to this, HSR-175 showed presynaptic and postsynaptic α_2 -adrenoceptor blocking activities, which

were evaluated in the rat vas deferens and the dog saphenous vein, respectively. However, the potencies were markedly weaker than the postsynaptic α_1 -adrenoceptor blocking activity. The ratio of the pA_2 value at postsynaptic α_1 -adrenoceptors to that at presynaptic α_2 -adrenoceptors was more than 1000, which was the same order as that of prazosin but significantly greater than that of bunazosin and yohimbine. Therefore, it is likely that HSR-175 is highly selective to α_1 -adrenoceptors as compared with α_2 -adrenoceptors.

HSR-175 at a high concentration of 10^{-6} M showed no inhibitory effects on the contractions induced by 5-HT, histamine, KCl and angiotensin II. Furthermore, HSR-175 was without effect on the responses to isoproterenol in the guinea pig atria and to acetylcholine in the guinea pig ileum (S. Hashimoto et al., unpublished observations). Therefore, it is likely that HSR-175 at the concentrations tested shows no effect on β -adrenoceptors, muscarinic receptors, 5-HT receptors, histamine receptors and angiotensin II receptors.

Recently, there has been increasing evidence for the existence of α_1 -adrenoceptor subtypes. In functional studies with vascular smooth muscles, α_1 -adrenoceptors have been divided into α_{1N} , α_{1H} and α_{1L} subtypes, which are based on their affinities to prazosin and HV-723 (16, 17). Noradrenaline-induced contraction of the dog mesenteric artery and the rabbit thoracic aorta are mediated through α_{1N} - and both α_{1L} - and α_{1H} -adrenoceptor subtypes, respectively (16, 17). It is very interesting to note that the affinities of HSR-175 for α_1 -adrenoceptors are different between these two preparations. This may suggest a possibility that HSR-175 can discriminate between different α_1 -adrenoceptor subtypes. Further studies are needed to verify this point.

In conclusion, the present in vitro study shows that the newly synthesized compound HSR-175 is a potent and selective postsynaptic α_1 -adrenoceptor antagonist. The extremely high affinity to postsynaptic α_1 -adrenoceptors may warrant its future use for the treatment of α_1 -adrenoceptor-associated diseases such as hypertension, cardiac failure and lower urinary tract disorders.

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