Pharmacological Studies on the Selectivity of HV-723, a New Alpha-1 Adrenoceptor Antagonist

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Abstract—The pharmacological profile of a new alpha-1 adrenoceptor antagonist, \( \alpha \)-ethyl-3,4,5-trimethoxy-\( \alpha \)-(3-((2-(2-methoxyphenoxy)ethyl)amino)propyl)benzene-acetonitrile fumarate (HV-723), was studied in vitro. In dog mesenteric arteries, HV-723, prazosin and yohimbine competitively inhibited noradrenaline-induced contraction: the \( pA_2 \) value of HV-723 (9.37) was apparently larger than that of prazosin (8.22) and yohimbine (7.18). However, HV-723 showed no or only a slight inhibition on the contractile responses to 5-HT, KCl and prostaglandin \( \text{F}_2\alpha \). HV-723 also showed potent alpha-1 adrenoceptor antagonist activity in the dog mesenteric and saphenous veins. However, HV-723 showed little antagonist activity on the pre- and postsynaptic alpha-2 adrenoceptors, beta-1 and beta-2 adrenoceptors and muscarinic receptors. HV-723 also inhibited the sympathetic contraction induced by electrical transmural stimulation in the dog mesenteric arteries, and the inhibition of HV-723 was about 10 times more potent than that of prazosin. However, \( ^3\)H-noradrenaline release evoked by electrical stimulation was not influenced by HV-723. These results clearly show that HV-723 is a potent and selective alpha-1 adrenoceptor antagonist.

Alpha-adrenoceptors have been pharmacologically classified into alpha-1 and alpha-2 subtypes based on the development of selective agonists and antagonists (1–3). In general, alpha-1 adrenoceptors are most selectively activated by phenylephrine and antagonized by prazosin. In contrast, alpha-2 adrenoceptors are preferentially activated by clonidine, BHT-933 and UK-14304 and antagonized by yohimbine and rouwolsine.

Alpha-1 adrenoceptors in blood vessels are postsynaptic receptors mediating vasoconstriction, and the therapy with selective alpha-1 adrenoceptor antagonists is now established in the treatment of hypertension and cardiac failure (4).

We tried to develop a more selective alpha-1 adrenoceptor antagonist and found that a new compound, \( \alpha \)-ethyl-3,4,5-trimethoxy-\( \alpha \)-(3-((2-(2-methoxyphenoxy)ethyl)amino)propyl)benzeneacetonitrile fumarate (HV-723) (Fig. 1), has a higher affinity to alpha-1 adrenoceptors than prazosin. In this paper, we report the selectivity of HV-723 for alpha-1 adrenoceptors, as compared to prazosin and yohimbine.

Materials and Methods

Experiments with dog vascular vessels: Dogs of either sex, weighing 8–12 kg, were anesthetized with thiopental sodium (20 mg/kg, i.v.) and exsanguinated from the common carotid arteries. Mesenteric artery and mesenteric and saphenous veins were isolated.

For the measurement of mechanical re-
response, the blood vessels were helically cut approximately 2 mm in width and 15 mm in length, under a dissecting microscope. The strips were 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 CaCl₂, 1.2 mM MgSO₄, 1.2 mM KH₂PO₄, 25 mM NaHCO₃ 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% O₂ and 5% CO₂, during both preincubation and experimental periods. A resting tension of 1.5 g and 0.5 g was applied to the artery and veins, respectively, and the responses were recorded isometrically. All preparations were equilibrated for 90 min before starting the experiments. Cumulative concentration-response curves of agonists such as noradrenaline were determined by step-wise increase in the concentration of an agonist as soon as a steady response to the previous administration had been achieved. Antagonists to be tested were applied at least 30 min before recording the concentration-response curve. To block the beta adrenoceptors, 10⁻⁶ M propranolol was added to the bath solution throughout the experiments. When the response to clonidine in the dog saphenous vein was recorded, the preparations were treated with phenoxybenzamine (10⁻⁷ M) and indomethacin (3x10⁻⁶ M).

Electrical transmural stimulation was applied through a pair of platinum-wire electrodes. In this case, the preparation was placed in parallel between the electrodes. The distance between the electrodes was approximately 2 mm, this being sufficiently narrow to assure that the tension change would not be disturbed. Stimulus parameters were 0.3 msec in duration and supramaximal voltage for 10 sec and 10 Hz. Drugs were applied at least 15 min before stimulation.

The release of ³H-noradrenaline was determined according to the method reported by Muramatsu et al. (5). The strips were preincubated with ³H-noradrenaline (2x10⁻⁷ M) in Krebs-Henseleit solution containing 5.7x10⁻⁴ M ascorbic acid for 90 min at 37°C. These strips were suspended between a pair of parallel platinum-wire electrodes under 1.0 g of tension. The Krebs-Henseleit solution containing 5.7x10⁻⁴ M ascorbic acid, 10⁻⁶ M cocaine and 10⁻⁶ M cortico-sterone was bubbled with 95% O₂ and 5% CO₂ at 37°C and was superfused using a peristaltic pump at the flow rate of 1 ml/min. Before starting the experiments, each arterial strip was equilibrated for 90 min, after which electrical transmural stimulation was applied through a pair of electrodes. Stimulus parameters were a frequency of 3 Hz, duration of 0.1 msec and supramaximal voltage for 10 sec. Superfusate solution was collected every 1 min, and the radioactivity was determined by counting in Aloka LSC 653 liquid scintillation spectrometer. The spontaneous ³H-efflux decreased exponentially to reach a plateau during the equilibration period for 60–90 min. Thus, the increase in ³H-efflux induced by electrical transmural stimulation was calculated to be the net ³H-efflux by stimulation. The drugs to be tested were superfused 20 min before initiation of electrical transmural stimulation.

**Experiments with rat vas deferens:** The prostatic portion of vasa was vertically suspended in the organ bath containing Krebs-Henseleit solution at 37°C under 1.0 g resting tension. Alpha-2 adrenoceptor antagonist activity was assessed by the determination of the pA₂ value against the inhibitory effect of clonidine on the contractile response to the electrical transmural stimulation (0.3 msec, supramaximal voltage, 0.1 Hz). Propranolol at 10⁻⁶ M was added to the bath medium.

**Drugs:** HV-723 (α-ethyl-3,4,5-trimethoxy-a-((2-(2-methoxyphenoxy)ethyl)amino)propyl)benzenecetonitrile fumarate, Fig. 1) was synthesized in our laboratory. The following drugs were used: noradrenaline bitartrate, yohimbine hydrochloride (Wako, Osaka, Japan), clonidine hydrochloride (Boehringer, Ingelheim, F.R.G.), prazosin (Taito-Pfizer, Tokyo, Japan), 1-phenylephrine (Sigma, MO, U.S.A.), prostaglandin F₂α (PGF₂α) (Frostarmon F, Ono, Osaka, Japan), 5-hydroxytryptamine (5-HT) (Merck, Darmstadt, F.R.G.) and 1-[7,8-³H] noradrenaline (Amersham, Buckinghamshire, U.K.).

**Statistical analysis:** Experimental values
are given as the mean±S.E. The pA₂ values for antagonists were obtained according to the method of Arunlakshana and Schild (6).

**Results**

**Effects on the responses to noradrenaline and other drugs in the dog mesenteric arteries:** HV-723 at concentrations ranging from 10⁻¹⁰ M to 10⁻⁷ M had no significant effect on the resting tension of the dog mesenteric artery. However, the compound inhibited the contractile response to noradrenaline. Figure 2 shows the concentration-response curves before and after treatment with various concentrations of HV-723, together with the cases of two control drugs (prazosin and yohimbine). HV-723 and the control drugs competitively inhibited the responses to noradrenaline, as the slope factors were nearly equal to unity (Table 1). The pA₂ value of HV-723 was apparently higher than that of prazosin and yohimbine (Table 1).

On the other hand, HV-723 showed no or only a slight inhibition on the contractile responses to 5-HT, KCl and prostaglandin F₂α. Figure 3 shows the concentration-response curves of three agonists before and after treatment with 10⁻⁷ M HV-723. HV-723 slightly shifted the concentration-response curve of 5-HT to the right and attenuated the maximum contraction induced by KCl.

**Effects on alpha-1, alpha-2 and beta adrenoceptors and muscarinic receptors:** Selectivity of HV-723 for alpha-1 and alpha-2 adrenoceptors was examined in several tissues and was compared with that of prazosin and yohimbine. Alpha-1 adrenoceptor-mediated responses were recorded in the dog mesenteric artery and vein (against noradrenaline-response) and in the dog saphenous vein (against phenylephrine-response). Postsynaptic and presynaptic alpha-2 adrenoceptor-mediated responses were recorded in the dog saphenous vein (against clonidine-response in the presence of phenoxybenzamine) and in the rat vas

![Fig. 2. Effects of HV-723 (A), prazosin (B) and yohimbine (C) 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%.●, before; ○, ●, □, ▽, after treatment with 10⁻⁵, 10⁻⁶, 10⁻⁷, 10⁻⁸, 10⁻⁹ M, respectively, of each drug. Each value is the mean±S.E. of 5–7 experiments.](image)

| Table 1. pA₂ values and slopes of the Schild plots of HV-723, prazosin and yohimbine in dog mesenteric artery |
|---|---|---|
| Drugs | pA₂     | Slope   |
| HV-723 | 9.37±0.05 | 0.96±0.03 |
| Prazosin | 8.22±0.08 | 0.85±0.03 |
| Yohimbine | 7.18±0.08 | 0.82±0.02 |

Each value is the mean±S.E. of 5–7 experiments.
deferens (against clonidine-response), respectively. HV-723, prazosin and yohimbine at 10^{-7} M shifted the concentration-response curves to the right without attenuating the maximum responses. Table 2 shows the EC50 values of each agonist in the absence and presence of 10^{-7} M or 10^{-6} M antagonist and their ratios. HV-723 and prazosin selectively inhibited the alpha-1 adrenoceptor-mediated responses either with a slight or no effect on the alpha-2 adrenoceptor-mediated responses. Comparison of the ratios indicated that the antagonist activity of HV-723 for alpha-1 adrenoceptors was approximately 10 times more potent than that of prazosin. Yohimbine was more selective for alpha-2 rather than alpha-1 adrenoceptors.

HV-723 (10^{-7} and 10^{-6} M) had no effect on positive inotropic and chronotropic responses to isoproterenol in the guinea-pig atria, on the relaxing response to isoproterenol in the guinea-pig trachea and on the contractile response to acetylcholine in the guinea-pig ileum (data not shown).

Effects on the responses to electrical transmural stimulation in the dog mesenteric artery: Electrical transmural stimulation at 10 Hz produced a transient contraction in the dog mesenteric artery. This contraction was completely inhibited by 3 \times 10^{-7} M tetrodotoxin or 3 \times 10^{-6} M guanethidine (data not shown). HV-723 and prazosin inhibited but did not abolish this contraction (Fig. 4). The inhibition was concentration-dependent: the maximum inhibition was produced by 10^{-8} M HV-723 or 10^{-7} M prazosin. Approximately 40% of the amplitude of contraction was resistant to HV-723 and prazosin: no further reduction of the residual component was produced by combined treatments with HV-723 and prazosin. Yohimbine at concentrations ranging from 10^{-9} to 10^{-7} M augmented the contractile responses to electrical stimulation, but attenuated the response at 10^{-6} M.
Table 2. Effects of HV-723, prazosin and yohimbine on alpha-1 and alpha-2 adrenoceptors in several tissues

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Agonist</th>
<th>Control EC50 (M)</th>
<th>HV-723 (10^-7 M) EC50 (M)</th>
<th>Ratio</th>
<th>Prazosin (10^-7 M) EC50 (M)</th>
<th>Ratio</th>
<th>Yohimbine (10^-6 M) EC50 (M)</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog Mesenteric artery</td>
<td>NA</td>
<td>(1.24±0.17)×10^-6</td>
<td>(2.17±0.55)×10^-4</td>
<td>175.0</td>
<td>(1.52±0.29)×10^-5</td>
<td>12.3</td>
<td>(8.90±1.42)×10^-6</td>
<td>7.2</td>
</tr>
<tr>
<td>Dog Mesenteric vein</td>
<td>NA</td>
<td>(8.59±1.02)×10^-7</td>
<td>(2.11±0.56)×10^-4</td>
<td>245.6</td>
<td>(9.82±1.58)×10^-6</td>
<td>11.4</td>
<td>(1.19±0.27)×10^-6</td>
<td>13.9</td>
</tr>
<tr>
<td>Dog Saphenous vein</td>
<td>Phe</td>
<td>(2.58±0.13)×10^-6</td>
<td>(3.59±1.10)×10^-4</td>
<td>139.1</td>
<td>(3.93±1.00)×10^-5</td>
<td>15.2</td>
<td>(4.36±0.59)×10^-5</td>
<td>16.9</td>
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<tr>
<td>Dog Saphenous vein</td>
<td>Clo</td>
<td>(2.57±0.28)×10^-8</td>
<td>(5.17±0.57)×10^-8</td>
<td>2.0</td>
<td>(5.30±1.09)×10^-8</td>
<td>2.1</td>
<td>(4.00±1.10)×10^-6</td>
<td>155.6</td>
</tr>
<tr>
<td>Rat Vas deferens</td>
<td>Clo</td>
<td>(3.15±0.69)×10^-9</td>
<td>(3.38±1.07)×10^-9</td>
<td>1.1</td>
<td>(2.59±0.41)×10^-9</td>
<td>0.8</td>
<td>(3.42±0.44)×10^-7</td>
<td>108.6</td>
</tr>
</tbody>
</table>

NA: Noradrenaline, Phe: Phenylephrine, Clo: Clonidine. Ratio: ED50 value for agonist in the presence of antagonist was divided by the control ED50 value for agonist. Each value is the mean±S.E. of 5–7 experiments.
Effects on the 3H-efflux induced by electrical transmural stimulation in the dog mesenteric artery: In order to examine the presynaptic effect of HV-723, 3H-efflux was measured in the dog mesenteric artery which had been preincubated with 3H-noradrenaline. Electrical transmural stimulation at 3 Hz resulted in an increase in the 3H-efflux. This evoked efflux was abolished by 3×10^{-7} M tetrodotoxin or 3×10^{-6} M guanethidine (data not shown). HV-723 and prazosin (10^{-8}-10^{-7} M for each drug) had no significant effect on the basal and evoked 3H-efflux, but at 10^{-6} M, both the drugs slightly increased the efflux (Fig. 5). Yohimbine (10^{-8}-10^{-6} M) increased the evoked 3H-efflux twice.

Discussion

Postsynaptic alpha adrenoceptors of the dog mesenteric artery have been characterized as the alpha-1 subtype (7, 8). In the present study also, prazosin and yohimbine competitively inhibited the concentration-response curve of noradrenaline, and the pA2 value of prazosin was much higher than that of yohimbine (Table 1). Under the same conditions, HV-723, a new compound, shifted the concentration-response curve of noradrenaline to the right, and the slope factor of the Schild plot was nearly equal to unity. Furthermore, the estimated pA2 value of HV-723 was significantly larger than that of prazosin. These results suggest that HV-723 shows a potent and competitive alpha-1 adrenoceptor antagonist activity in the dog mesenteric artery.

Such alpha-1 antagonist action was also observed in the dog mesenteric and saphenous veins, and the potency was higher than that of prazosin. However, HV-723 had little or no antagonist action against pre- and postsynaptic alpha-2 adrenoceptors, beta adrenoceptors and muscarinic receptors. Furthermore, the contractile responses to 5-HT, KCl and PGF2α in the dog mesenteric artery were little affected by HV-723 at concentrations which caused a large reduction in the noradrenaline-response. These results suggest that the antagonist action of HV-723 is selective for alpha-1 adrenoceptors.

Since it has been recently demonstrated that sympathetic contraction of the dog mesenteric artery and other blood vessels consists of not only adrenergic but also purinergic components (9-12), we decided to examine the effects of HV-723 on the sympathetic response of the dog mesenteric artery. The results shown in Fig. 4 indicate that the sympathetic contraction was partially inhibited by HV-723 or prazosin and that the combined treatment with both drugs failed to produce further reduction of the sympathetic response. These results suggest that the drugs selectively attenuate the adrenergic component of sympathetic contraction. The results in Fig. 4 also show that the inhibitory action of HV-723 was produced at lower concentrations than that of prazosin. Since no presynaptic action of HV-723 and prazosin was observed, it is likely that the difference of the potencies reflects the difference in the affinities of both the drugs on the postsynaptic alpha-1 adrenoceptors.

In conclusion, the present in vitro study clearly shows that the newly synthesized compound HV-723 is a more potent and selective alpha-1 adrenoceptor antagonist as compared with prazosin, and suggests that it may be applicable for the treatment of...
diseases associated with alpha-1 adrenoceptors.

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References