DJ-7141, a New Alpha-2 Agonist with Only a Mild Hypotensive Action

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Abstract—The pharmacological profile of a newly synthesized imidazole derivative, DJ-7141, was examined with special reference to alpha-2 adrenoceptors. In the rat vas deferens and dog mesenteric artery, DJ-7141 at concentrations over $10^{-9}$ M selectively acted on the presynaptic alpha-2 adrenoceptors on the sympathetic nerve terminals and inhibited the contractions induced by electrical transmural stimulation. The potency of DJ-7141 was almost the same as those of clonidine and guanabenz. DJ-7141 also acted on the postsynaptic alpha-2 adrenoceptors to contract the dog saphenous vein. However, no alpha-1 agonist and antagonist actions were found at concentrations showing presynaptic alpha-2 agonist activity. In contrast to DJ-7141, clonidine produced an apparent contraction in the dog mesenteric artery, and the response was inhibited by prazosin. In urethane-anesthetized rats, clonidine at doses ranging from 0.003 mg/kg to 0.03 mg/kg produced a marked and prolonged hypotension, while DJ-7141 at such doses failed to produce a reduction of blood pressure. From these results, it is suggested that, in contrast to clonidine and other alpha-2 agonists, DJ-7141 is a unique alpha-2 agonist which shows high affinity to peripheral alpha-2 adrenoceptors but only a mild hypotensive activity.

Since alpha adrenoceptors were classified into alpha-1 and alpha-2 subtypes (1, 2), many drugs selective to each subtype have been developed (3). Clonidine and guanabenz are representative alpha-2 agonists which are used clinically as hypotensive drugs (4, 5). Other alpha-2 agonists developed so far also show hypotensive activity and a common mechanism through central alpha-2 adrenoceptors has proposed (6, 7). However, alpha-2 adrenoceptors are widely distributed in not only central organs but also in peripheral organs (8). Because of this, we tried to develop a new alpha-2 agonist which has no hypotensive activity; that is, a peripheral type of alpha-2 agonist. In the present paper, we report that a newly synthesized imidazole compound, DJ-7141 (Fig. 1) (9), has high affinity to peripheral alpha-2 adrenoceptors but shows only a mild hypotensive action.

Materials and Methods

Rat vas deferens: Vasa deferentia were removed from Wistar rats (250–300 g) which had been stunned and exsanguinated. Each vas deferens was divided into three portions of equal length, and the prostatic and epididymal ends were mounted vertically in an organ bath containing 20 ml of Krebs
Henseleit solution of the following composition (mM): NaCl, 112; KCl, 5.9; MgCl₂, 1.2; CaCl₂, 2; NaHCO₃, 25; NaH₂PO₄, 1.2; glucose, 11.5. 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 0.5 g was applied, and the response was recorded isometrically through a force-displacement transducer. Before starting the experiment, preparations were equilibrated for 90 min. Electrical transmural stimulation was applied through a pair of platinum-wire electrodes. Stimulus parameters were a duration of 0.3 msec, supramaximal voltage of 10 V, and single pulse. Drugs were applied 30 min before stimulation.

**Dog mesenteric artery and saphenous vein:** Dogs of either sex, weighing 8 to 15 kg, were anesthetized with thiopental sodium (30 mg/kg, i.v.) and exanguinated from the common carotid arteries. The mesenteric artery and saphenous vein were isolated. The helical strip (approximately 2–3 mm in width and 15 mm in length) was mounted vertically under the resting tension of 1.0 g, and the mechanical response was recorded. Other experimental conditions were same as described above except electrical transmural stimulation was applied at 3 Hz for 5 sec.

**Rat blood pressure:** Male Wistar rats weighing from 250 to 300 g were anesthetized with urethane (1 g/kg, i.p.). Arterial blood pressure was measured from the left carotid artery with a pressure transducer. Simultaneously, heart rate was measured with a tachograph. Drugs were injected from the femoral vein.

**Statistical analysis:** Average values were given as the mean±S.E. pA₂ values for antagonists were obtained according to the method of Arunlakshana and Schild (10). Differences were tested for significance using Student's t-test for unpaired data.

**Drugs:** 2-(2-Chloro-6-fluorophenyl)-2,3,5,6-tetrahydro-1H-imidazo[1,2-a]imidazole hydrochloride (DJ-7141) was synthesized in and donated by Daiichi Seiyaku Co., Ltd. (Tokyo, Japan). The following drugs were used: yohimbine hydrochloride, dl-propranolol hydrochloride, phenoxybenzamine hydrochloride (Nakarai, Kyoto, Japan), guanabenz (WY-8678; Nippon-Shoji, Osaka, Japan), clonidine hydrochloride (Boehringer, Ingelheim, FRG), 1-phenylephrine hydrochloride (Sigma, St. Louis, MO), prazosin (Taito-Pfizer, Tokyo, Japan) and DG-5128 (Daiichi Seiyaku).

**Results**

**Rat vas deferens:** In the prostatic portion of the rat vas deferens, electrical stimulation of a single pulse produced a twitch contraction (Fig. 2Ba). This contraction was not affected by 10⁻⁷ M prazosin, but it was abolished by 3×10⁻⁶ M guanethidine. DJ-7141 at concentrations over 10⁻⁸ M attenuated the twitch contraction, and at 10⁻⁷ M, the response was abolished. The inhibition was concentration-dependent, the EC₅₀ value being (5.2±0.5)×10⁻⁹ M (n=17). Clonidine and guanabenz also attenuated the twitch contraction induced by electrical stimulation, and the EC₅₀ values were (3.0±0.2)×10⁻⁹ M (n=13) and (2.5±0.5)×10⁻⁸ M (n=11), respectively. These inhibitory effects of DJ-7141, clonidine and guanabenz were competitively antagonized by yohimbine (Fig. 2) or DG-5128. The pA₂ values and slope factors of DG-5128 and yohimbine were summarized in Table 1, showing no significant difference between the pA₂ values of DG-5128 or yohimbine against DJ-7141 and clonidine.

![Fig. 2. Effects of DJ-7141 on the twitch contractions induced by electrical stimulation in the prostatic portion of rat vas deferens. ▲: electrical stimulation of a single pulse. The responses marked as a, b and c in A were recorded at high speed and were shown in B.](image-url)
Table 1. pA2 values of yohimbine and DG-5128 against the inhibitory effects of DJ-7141 and clonidine on the responses to electrical transmural stimulation in the prostatic portion of rat vas deferens

|            | pA2 against DJ-7141 | pA2 against Clonidine |
|------------|----------------------|-----------------------|
| Yohimbine  | n=6, 7.97±0.10 (1.11±0.10) | n=5, 7.68±0.07 (1.10±0.13) |
| DG-5128    | n=6, 6.43±0.08 (1.14±0.08) | n=6, 6.74±0.22 (1.06±0.11) |

n: number of experiments. ( ) : slope factor obtained from an Arunlakshana and Schild plot.

In the epididymal portion, electrical stimulation of a single pulse produced a biphasic contraction (Fig. 3). The latter component was abolished by 10⁻⁷ M prazosin, while the former one was not affected by the alpha-antagonist. DJ-7141 attenuated both the components induced by electrical stimulation (Fig. 3B), and the inhibition was completely reversed by further addition of 10⁻⁵ M DG-5128 (Fig. 3) or 10⁻⁷ M yohimbine (n=5, for each of the drugs).

The resting tension of the prostatic or epididymal portions of vas deferens was not changed after the addition of DJ-7141 at concentrations less than 10⁻⁵ M (Figs. 2 and 3). However, at higher concentrations, DJ-7141 elicited spontaneous contraction. Such effects were also observed after addition of high concentrations of clonidine and guanabenz. Figure 4 shows representative results observed in the epididymal portion. Prazosin (3×10⁻⁷ M) abolished such contractile responses induced by these three drugs.

Phenylephrine produced tonic contraction in the epididymal portion. DJ-7141 (10⁻⁸–10⁻⁶ M) had no effect on the phenylephrine-response, but prazosin (10⁻⁷ M) abolished it (Fig. 5).

Dog mesenteric artery and saphenous vein: Electrical transmural stimulations at 3 Hz produced a phasic contraction in the dog mesenteric artery. This contraction was abolished by 3×10⁻⁶ M guanethidine and attenuated by 10⁻⁷ M prazosin, as reported previously (11). DJ-7141 at concentrations over 10⁻⁹ M attenuated the contractile response without changing the resting tension (Fig. 6A). This inhibitory effect of
DJ-7141 was reversed after addition of $10^{-5}$ M DG-5128 (Fig. 6B). Clonidine also attenuated the contractile response to electrical transmural stimulation at concentrations over $10^{-9}$ M, and the effect was antagonized by the treatment with DG-5128 (data not shown). DJ-7141 had no contractile action in the mesenteric artery. However, clonidine apparently contracted the artery (Fig. 7A), and the effect was inhibited by $10^{-7}$ M prazosin. In the dog saphenous vein, DJ-7141 produced a contraction, but the potency was lower than those of clonidine and noradrenaline (Fig. 7B). Under the conditions where alpha-1 adrenoceptors were blocked by $10^{-7}$ M phenoxybenzamine (12), the contractile responses to DJ-7141 and clonidine were competitively antagonized by yohimbine, the pA2 being $8.98\pm0.06$ (n=7) for clonidine.

Rat blood pressure: Figure 8 shows the representative recordings of rat blood pressure and heart rate after intravenous injection of DJ-7141 and clonidine. At a dose of 0.3 mg/kg, DJ-7141 slightly reduced the...
blood pressure and heart rate. In contrast, clonidine at 10 times lower dose produced a marked reduction in blood pressure and a prolonged bradycardia. In some cases, DJ-7141 and clonidine caused a transient increase in blood pressure after drug injection. Figure 9 shows the time course of the change of blood pressure and the dose-response curves measured 30 min after drug injection. The hypotensive activity of DJ-7141 was more than 30 times less potent than that of clonidine.
Discussion

Rat vas deferens is demonstrated to be suitable as a pharmacological preparation for the assessment of alpha-2 agonist activity (13), so we first used this preparation for the assay of alpha-2 activity. DJ-7141 inhibited the twitch contraction induced by electrical stimulation of a single pulse in the prostatic portion of vasa. The effective concentration was low ($10^{-9}$–$10^{-7}$ M), and the inhibition was antagonized by alpha-2 antagonists, yohimbine and DG-5128 (14). Comparison with clonidine and guanabenz shows that DJ-7141 is equipotent as such alpha-2 agonists and furthermore that this compound acts on the same receptors as clonidine, because $pA_2$ values of yohimbine or DG-5128 were almost the same between both agonists, DJ-7141 and clonidine. These results indicate that DJ-7141 has potent alpha-2 agonist activity.

In the epididymal portion, electrical stimulation produced biphasic contraction. Just as the monophasic contraction in the prostatic portion, the early phase is nonadrenergic in nature, while the latter phase is adrenergic, since prazosin selectively inhibited the latter component (13). DJ-7141 inhibited the both contractions, and the inhibition was antagonized by yohimbine and DG-5128. Since the contractile response to phenylephrine was not affected by DJ-7141, the inhibitory effect on the twitch contraction induced by electrical stimulation seems not to be related to alpha-1 antagonist action at the postsynaptic receptor sites. Thus, all results obtained in the rat vas deferens suggest that DJ-7141 acts on the presynaptic alpha-2 adrenoceptors and inhibits the twitch contraction, as well as in the cases of clonidine and guanabenz. Such potent alpha-2 agonist activity was also observed in the sympathetic response of the dog mesenteric artery.

At concentrations showing alpha-2 agonist activity, DJ-7141 itself had no contractile action on the vas deferens and dog mesenteric artery, suggesting that alpha-1 agonist activity is negligible at such concentrations. In contrast, DJ-7141 and clonidine produced apparent contractile responses in the dog saphenous vein. Since alpha-2 adrenoceptors are distributed postsynaptically in this vein (15, 16), the contractile response may be caused through such receptors. This possibility was confirmed by the evidence that the contractile responses to DJ-7141 and clonidine
Clonidine were competitively antagonized by yohimbine under the conditions where alpha-1 adrenoceptors were blocked by phenoxybenzamine (12).

The pharmacological profiles of DJ-7141 examined in the peripheral tissues are essentially the same as those of clonidine and guanabenz. However, the potency of DJ-7141 to produce hypotension in anesthetized rats was quite different from that of clonidine, the potency being at least 30 times less than that of clonidine. The hypotensive effect of clonidine is mainly caused through central alpha-2 adrenoceptors (3, 4, 17). Since presynaptic alpha-2 adrenoceptors of vasa are qualitatively similar to central ones (18), the low potency of DJ-7141 to produce hypotension would reflect difficult access of this drug to the central nervous system or other unknown differences in vivo. These points must be investigated in further studies.

Wide distribution of alpha-2 adrenoceptor in peripheral organs has been demonstrated (3, 8, 17, 19). If DJ-7141 has no other central action, this compound would become a useful probe for studying the functions of peripheral alpha-2 adrenoceptors and, furthermore, useful as a drug for the treatments of some disorders associated with alpha-2 adrenoceptors.

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References
1 Langer, S.Z.: Presynaptic regulation of the release of catecholamines. Pharmacol. Rev. 337–362 (1981)
2 Starke, K.: Alpha-adrenoceptor subclassification. Rev. Physiol. Biochem. Pharmacol. 88, 199–236 (1981)
3 Timmermans, P.B.M.W.M. and van Zwieten, P.A.: α2-Adrenoceptors: classification, localization, mechanisms, and targets for drugs. J. Med. Chem. 25, 1389–1401 (1982)
4 Pettinger, W.A.: The pharmacology of clonidine. J. Cardiovasc. Pharmacol. 2, S21–S28 (1980)
5 Holmes, B., Brogden, R.N., Heel, R.C., Speight, T.M. and Avery, G.S.: Guanabenz. A review of its pharmacodynamic properties and therapeutic efficacy in hypertension. Drugs 26, 212–219 (1983)
6 Scholtysek, G.: Pharmacology of guanfacine. Br. J. Clin. Pharmacol. 10, 215–245 (1980)
7 Ruffolo, R.R., Yaden, E.L., Timmermans, P.B.M.W.M., van Zwieten, P.A. and Hynes, M.D.: Characterization of the alpha adrenoceptor-mediated effects and antihypertensive activity of ICI 106270: comparison with clonidine. J. Pharmacol. Exp. Ther. 229, 58–66 (1984)
8 Timmermans, P.B.M.W.M. and van Zwieten, P.A.: The postsynaptic α2-adrenoceptor. J. Auton. Pharmacol. 1, 171–183 (1981)
9 Ishikawa, F., Kitagawa, M., Satoh, Y., Saegusa, J., Tanaka, S., Shibamura, S. and Chiba, T.: Cyclic guanidines. XV. Synthesis and biological activities of (substituted phenyl)-imidazo[1,2-a]imidazole derivatives. Chem. Pharm. Bull. (Tokyo) 33, 2838–2848 (1985)
10 Arunlakshana, O. and Schild, H.O.: Some quantitative uses of drug antagonists. Br. J. Pharmacol. 14, 48–58 (1959)
11 Muramatsu, I., Kigoshi, S. and Oshita, M.: Nonadrenergic nature of prazosin-resistant, sympathetic contraction in the dog mesenteric artery. J. Pharmacol. Exp. Ther. 229, 532–538 (1984)
12 Constantine, J.W., Lebel, W. and Archer, R.: Functional postsynaptic α2 but not α1 adrenoceptors in dog saphenous vein exposed to phenoxybenzamine. Eur. J. Pharmacol. 85, 325–329 (1982)
13 Brown, C., McGrath, J.C. and Summers, R.J.: The effects of α1-adrenoceptor agonists and antagonists on responses of transmurally stimulated prostatic and epididymal portions of the isolated vas deferens of the rat. Br. J. Pharmacol. 66, 553–564 (1979)
14 Muramatsu, I., Oshita, M. and Yamanaka, K.: Selective alpha-2 blocking action of DG-5128 in the dog mesenteric artery and rat vas deferens. J. Pharmacol. Exp. Ther. 227, 194–198 (1983)
15 DeMey, J.G. and Vanhoutte, P.M.: Uneven distribution of post-junctional alpha-1 and alpha-2 like adrenoceptors in canine arterial and venous smooth muscle. Circ. Res. 48, 875–884 (1981)
16 Fowler, P.J., Grous, M., Price, W. and Matthews, W.D.: Pharmacological differentiation of postsynaptic alpha adrenoceptors in the dog saphenous vein. J. Pharmacol. Exp. Ther. 229, 712–718 (1984)
17 Kobinger, W.: Central α-adrenergic systems as targets for hypotensive drugs. Rev. Physiol. Biochem. Pharmacol. 81, 39–100 (1978)
18 Doxey, J.C., Gadie, B., Lane, A.C. and Tulloch,
I.F.: Evidence for pharmacological similarity between $\alpha_2$-adrenoceptors in the vas deferens and central nervous system of the rat. Br. J. Pharmacol. 80, 155–161 (1983)

19 Sakakibara, Y., Fujiwara, M. and Muramatsu, I.: Pharmacological characterization of the alpha adrenoceptors of the dog basilar artery. Naunyn Schmiedebergs Arch. Pharmacol. 319, 1–7 (1982)