COMPARISON OF PRE- AND POSTSYNAPTIC α-ADRENOCEPTOR BLOCKING EFFECTS OF E-643 IN THE ISOLATED VAS DEFERENS OF THE RAT

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Abstract—Effectiveness of E-643, a newly developed α-blocker, and four α-antagonists in blocking pre- and postsynaptic α-adrenoceptors were compared in the isolated rat vas deferens. The inhibitory effect of clonidine on the field-stimulated twitch response was antagonized in the presence of the α-antagonists. The order of affinity (pA₂) for presynaptic α-receptors, as assessed from parallel shift of the dose-response curve to clonidine, was: phentolamine > yohimbine > tolazoline > E-643 > prazosin. At concentrations from 10⁻⁸ to 10⁻⁶ M, neither E-643 nor prazosin had any effect on the twitch which had been depressed by the treatment with clonidine, whereas phentolamine, yohimbine and tolazoline partially reversed it. Contractile effects of cumulative concentrations of noradrenaline were also antagonized by α-antagonists. The order of affinity (pA₂) for postsynaptic α-receptors was: E-643 > prazosin > phentolamine > yohimbine > tolazoline. Selectivity for pre- versus postsynaptic α-receptors was assessed by comparing Kᵦ values for pre- and postsynaptic α-receptors. The order of selectivity for the presynaptic α-receptors was: yohimbine > tolazoline > phentolamine > prazosin > E-643. It is concluded that E-643 is a potent and highly selective postsynaptic α-blocker.

E-643, 2-[4-(n-butyryl)-homopiperazine-1-yl]-4-amino-6,7-dimethoxyquinazoline, is a newly developed α-blocker with potent antihypertensive activity (1–4). In a previous study using the isolated rabbit aorta, we showed that E-643, like phentolamine, was a specific and competitive blocker of noradrenaline (5). On the other hand, a difference was noted concerning hypotensive effects of these two drugs; that is, in both anesthetized and unanesthetized rats, hypotensive effects of E-643 were more remarkable than those of phentolamine on the basis of equipotent α-receptor blockade (2, 5). E-643 had no direct relaxing action on isolated rabbit aortic strips (5). These findings suggested the possibility that E-643 might selectively block the postsynaptic α-adrenoceptors without significant interference with presynaptic α-receptors (5). The purpose of the present study was to compare the blocking activity of E-643 on the pre- and postsynaptic α-adrenoceptors.

Experiments were conducted using the isolated rat vas deferens, an organ in which motor transmission is thought to be modulated by an inhibitory presynaptic α-receptors (6–9). The results were compared with those of yohimbine, tolazoline and phentolamine, compounds which are known to block presynaptic α-adrenoceptors (10, 11) and with prazosin, a drug which is thought to
preferentially block postsynaptic \( \alpha \)-adrenoceptors (12–14).

**MATERIALS AND METHODS**

Preparation and recording: The vas deferens was removed from Sprague-Dawley rats weighing 280–350 g and dissected free from the adhering connective tissues. A section of 2 cm, including mainly the central portion of the vas deferens was excised and suspended in a 20-ml organ bath containing Krebs solution of the following composition (mM): NaCl, 118.4; KCl, 4.7; CaCl\(_2\), 2.52; MgSO\(_4\)-7H\(_2\)O, 1.2; KH\(_2\)PO\(_4\), 1.2; NaHCO\(_3\), 25.0 and glucose, 11.1. Magnesium sulfate was reduced to 0.6 mM when the twitch response to field stimulation was studied. The solution was gassed with a mixture of 95% O\(_2\) and 5% CO\(_2\). Temperature of the bathing solution was maintained at 37°C for study of the twitch response and it was reduced to 30°C in order to decrease the spontaneous contractions when noradrenaline-induced contractions were studied.

Contractions of the isolated preparations were measured isometrically under a tension of 0.5–1.0 g with a force-displacement transducer (Nihonkohden, SB-1T) connected to a carrier amplifier (Nihonkohden, RP-5). Recordings were made on a pen-writing oscillograph (Nihonkohden, RJG-4000).

Field stimulation: A pair of Ag-AgCl ring-electrodes were placed near the top and bottom of the vas deferens. Field stimulation was carried out at 0.2 Hz using square pulses of 3 msec duration at submaximum voltage (about 10 V). This stimulation condition was fixed throughout the experiment.

Effects on the presynaptic \( \alpha \)-adrenoceptors: Blocking activity of the \( \alpha \)-antagonists on the presynaptic \( \alpha \)-adrenoceptors was assessed by the antagonism with \( \alpha_2 \)-adrenoceptor agonist, clonidine. Clonidine inhibits twitch responses of the field-stimulated vas deferens by acting on the presynaptic \( \alpha \)-adrenoceptors (8, 10, 12, 15).

Two different methods were used to study interference by \( \alpha \)-antagonists of the twitch-inhibiting effect of clonidine. One of these was to assess blocking activity of an antagonist from the shift of the dose-response curve to clonidine. Control dose-response curves and those in the presence of antagonists were obtained in separate preparations as a full recovery from maximally effective concentrations of clonidine could not be obtained by washout. After the twitch tension to field stimulation became constant, the antagonists were added to the bath and allowed to contact with the vas deferens for 15 min prior to initiation of the cumulative addition of clonidine. Control preparations were treated with 20 μl of distilled water instead of the antagonists.

Another method was to assess the antagonistic potency of an antagonist from reversal of the twitch tension which had been potentially depressed by the treatment of clonidine. The twitch response was first depressed below 20% of the original peak tension by \( 10^{-8} \) M of clonidine. Increasing concentrations (10\(^{-9}\) to 10\(^{-3}\) M) of the antagonists were then added to the bath and reversal of the twitch was tested (see an example in Fig. 1). The tissue was exposed to each successive concentration of an antagonist until the response had reached an equilibrium.

Effects on the postsynaptic \( \alpha \)-adrenoceptors: Blocking activity of the \( \alpha \)-adrenoceptor antagonists on the postsynaptic \( \alpha \)-adrenoceptors was assessed by the antagonism with \( \beta \)-noradrenaline-induced contractions of the vas deferens in the presence of cocaine (5×10\(^{-8}\) M) and propranolol (5×10\(^{-6}\) M). Noradrenaline was added to the bath in increasing concentrations (3×10\(^{-8}\) to 3×10\(^{-4}\) M), so that a cumulative dose-response curve was pro-
duced. After two successive dose-response curves of equal size had been obtained, antagonists were added and thereby exposed to the tissue for 30 min. The addition of increasing concentrations of noradrenaline was repeated, and dose-response curves in the presence of antagonists were obtained.

**Effects on the twitch response:** When the twitch tension of the vas deferens to field stimulation became constant, \( \alpha \)-antagonists were added to the bath in a cumulative fashion \((10^{-8} \text{ to } 10^{-5} \text{ M})\) and an alteration in the twitch tension was observed. The tissue was allowed to contact with each successive concentration of the antagonists until the response had reached an equilibrium.

**Drugs:** Drugs used were clonidine hydrochloride (Boehringer-Ingelheim), \( \beta \)-noradrenaline hydrochloride (Fluka), yohimbine hydrochloride (Sigma), tolazoline hydrochloride (CIBA-Geigy), phentolamine hydrochloride (CIBA-Geigy), cocaine hydrochloride (Takeda) and \( \beta / \alpha \)-propranolol hydrochloride (Sigma). E-643 hydrochloride and prazosin hydrochloride were synthesized in the Chemical Synthetic Laboratories of Eisai Co. Ltd. for the present study.

The \( \alpha \)-adrenoceptor agonists and antagonists were dissolved in distilled water and added to the bath in a volume of 20 \( \mu \text{l} \). \( \beta \)-Noradrenaline was dissolved with an equimolar amount of \( \beta \)-ascorbic acid. When cocaine and propranolol were used, they were included in the bathing solution throughout the experiment.

**Statistics:** \( pA_2 \) values were calculated according to the method of van Rossum (16). Statistical evaluation of the data was made by Student’s t-test. Differences with \( P \) values less than 0.05 were considered to be significant.

**RESULTS**

**Effects on the dose-response curve to clonidine:** Field stimulation of the vas deferens at 0.2 Hz produced rapid twitch contractions. Peak tension of the twitch varied among preparations and ranged from 0.4 to 1.5 g. As shown in a typical trace in Fig. 1, clonidine produced a dose-dependent inhibition of the twitch response in a low concentration range.

All of the \( \alpha \)-antagonists caused a parallel shift of the dose-response curve to clonidine, without reduction of the maximum inhibitory response, thereby suggesting a competitive-

![Fig. 1. Typical traces of twitch inhibition caused by increasing concentrations of clonidine (upper record) and reversal by phentolamine of the twitch which had been depressed by \( 10^{-6} \text{ M} \) of clonidine (lower record). Clonidine and phentolamine were added to the bath at the point indicated by the arrows. Horizontal and vertical calibrations are 1 min and 1 g, respectively.](image-url)
type of antagonism (Fig. 2). pA2 values calculated from these dose-response curves are given in Table 1. The decreasing order of affinity was: phentolamine > yohimbine > tolazoline > E-643 > prazosin. The pA2 values for E-643 were significantly less (P<0.001) than those of phentolamine, yohimbine and tolazoline. There was no significant difference between pA2 values for E-643 and prazosin. The affinity of E-643 for the presynaptic α-receptors was calculated to be one sixty-eighth of phentolamine.

Reversal of the clonidine-induced inhibition of the twitch: The results with the five α-antagonists are shown in Fig. 3. Under conditions in which the twitch responses were inhibited below 20% of the original size by the treatment of clonidine (10-8 M), subsequent addition of phentolamine antagonized and completely reversed the depressed twitch in a concentration-dependent manner (10-7 to 10-5 M) (see a typical trace in Fig. 1). In contrast, E-643 and prazosin had no effect at concentrations ranging from 10-8 to 10-6 M. At a higher concentration of 10-5 M, both drugs caused partial antagonism. Almost full antagonism was obtained with tolazoline at 10-5 M, but only partial reversal of the twitch was obtained at a lower concentration of 10-6 M. Yohimbine produced a biphasic effect, a dose-dependent antagonism occurring at concentrations up to 10-6 M followed by an inhibition at 10-5 M.

Effects on the postsynaptic α-adrenoceptors: All of the α-antagonists used in this study caused a parallel displacement of the dose-response curve to noradrenaline to the right (Fig. 4). pA2 values calculated from these dose-response curves are given.

Table 1. Blocking activities of α-antagonists on the pre- and postsynaptic α-adrenoceptors.

<table>
<thead>
<tr>
<th>Antagonists</th>
<th>Presynaptic α-receptors</th>
<th>Postsynaptic α-receptors</th>
<th>Kd-post/Kd-pre</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Conc. (M)</td>
<td>pA2</td>
<td>n</td>
</tr>
<tr>
<td>E-643</td>
<td>10^-4</td>
<td>6.09±0.05</td>
<td>6</td>
</tr>
<tr>
<td>Prazosin</td>
<td>10^-6</td>
<td>6.03±0.07</td>
<td>5</td>
</tr>
<tr>
<td>Phentolamine</td>
<td>10^-7, 10^-6</td>
<td>7.86±0.05***</td>
<td>10</td>
</tr>
<tr>
<td>Tolazoline</td>
<td>10^-6</td>
<td>6.56±0.09***</td>
<td>5</td>
</tr>
<tr>
<td>Yohimbine</td>
<td>10^-7, 10^-6</td>
<td>7.53±0.07***</td>
<td>10</td>
</tr>
</tbody>
</table>

Kd-post/Kd-pre was used as an index of relative selectivity of the antagonists on the presynaptic α-adrenoceptors. Kd-post and Kd-pre are the apparent dissociation constants (10^-pA2) of the antagonist-postsynaptic and antagonist-presynaptic receptor complex, respectively. ***: Significantly different (P<0.001) when compared with the mean value of E-643.
The decreasing order of affinity for the postsynaptic α-receptors was: E-643 > prazosin > phentolamine > yohimbine > tolazoline. The pA₂ value for E-643 was significantly greater (P<0.001) than the values obtained for phentolamine, yohimbine and tolazoline. There was no statistically significant difference between the values for E-643 and prazosin. The affinity of E-643 for the postsynaptic α-receptors was calculated to be 13, 676 and 1290 times greater than those of phentolamine, yohimbine and tolazoline, respectively.

**Effects on the twitch response:** The results are shown in Fig. 3. E-643 slightly reduced the twitch peak tension at the concentrations of 10⁻⁷ and 10⁻⁶ M. At the concentration of 10⁻⁵ M, E-643 potentiated the twitch to a slight extent and the response tended to return towards the original level. Phentolamine produced a biphasic effect, inhibition of the twitch at lower concentrations (up to 10⁻⁸ M) followed by marked enhancement at a higher concentration (10⁻⁵ M). Prazosin and yohimbine produced a dose-dependent reduction of the twitch at concentrations higher than 10⁻⁷ M. Tolazoline did not significantly affect the twitch contractions at concentrations up to 10⁻⁶ M, but did markedly enhance the twitch at 10⁻⁵ M.

**DISCUSSION**

The vas deferens of the rat has been repeatedly used for the study of presynaptic α-adrenoceptors (6–9, 12, 15, 17–19). Field stimulation of the epididymal portion of the vas deferens with single pulses elicits a biphasic response consisting of an initial fast
component (twitch) followed by a slower component, whereas in the prostatic portion, the response consists principally of a single fast component (20-23). The second slower component is thought to be adrenergic and the initial fast component is considered to be of nonadrenergic origin (20-22). Both the fast and slow components are inhibited by low concentrations of clonidine (22).

Preparations used in the present study consisted mainly of a central portion of the vas deferens. Field stimulation (0.2 Hz, 3 msec, submaximal voltage) produced only a rapid twitch response. Thus, it seems that the contractions elicited by the stimulation consisted mainly of the fast component. This assumption may be partly supported by the modest inhibition of the twitch caused by the highest concentration of E-643 and prazosin.

Clonidine produced a dose-dependent inhibition of the twitch at low concentrations similar to those reported by other workers (8, 10, 12, 19). This inhibitory action was antagonized by the α-antagonists. pA₂ values showed a distinct variation in affinity for the presynaptic α-receptors. Thus, E-643, as well as prazosin, had a much lesser affinity than those of phentolamine and yohimbine.

Contractile effects of cumulative concentrations of noradrenaline were also antagonized by α-antagonists. pA₂ values revealed a difference in affinity for the postsynaptic α-receptors among the antagonists. E-643, as well as prazosin, had much greater affinity than either yohimbine or tolazoline. The pA₂ values for E-643 and phentolamine were much the same as those we found in preparations of the rabbit aorta (5).

Selectivity of each α-antagonist for pre-versus postsynaptic α-adrenoceptors was assessed by the ratio of Kᵦ values derived from pA₂ values against noradrenaline and clonidine, respectively (Kᵦ-pre/Kᵦ-post) (Table 1). The decreasing order of selectivity for the presynaptic α-receptors was: yohimbine > tolazoline > phentolamine > prazosin ≥ E-643. Thus, it is evident that E-643, like prazosin, can be classified into a category of α-antagonists which preferentially block postsynaptic α-receptors. Yohimbine and tolazoline, on the other hand, are drugs which preferentially block presynaptic α-adrenoceptors. Phentolamine is an α-antagonist which has fairly equal affinities for both pre- and postsynaptic α-receptors. This spectrum of selectivity concerning prazosin, phentolamine, tolazoline and yohimbine is in good agreement with data on the rat vas deferens (8, 10, 12, 15, 19) and other tissues (see ref. 11). It should be noted, however, that the difference between E-643 and tolazoline was mainly due to variation in postsynaptic potency; the presynaptic affinity of tolazoline was only 2.9 times greater than...
that of E-643 while postsynaptic affinity of the former drug was as little as 1/1290 of the latter.

That E-643, as well as prazosin, has less blocking activity on the presynaptic \( \alpha \)-receptors than that of three other \( \alpha \)-antagonists was confirmed in the experiment where reversal of clonidine-induced twitch inhibition was examined. At concentrations up to \( 10^{-6} \) M, neither E-643 nor prazosin showed any effect on the twitch which had been depressed by clonidine, whereas, the three other antagonists partially reversed the twitch response. It should be mentioned, however, that the dose-response curves for the \( \alpha \)-antagonists, particularly at high concentrations, clearly reflect a potentiating or inhibitory action of the antagonists.

E-643 produced an inhibitory effect on the field-stimulated twitch response with a slight potentiating effect at the highest concentration, while prazosin produced dose-dependent reduction of the twitch. This difference cannot be attributed to variation in the potency for the postsynaptic \( \alpha \)-blocking activity, since the blocking activity of prazosin on the noradrenaline-induced contraction did not exceed that seen with E-643.

An effect which had not been anticipated was the marked enhancement of the twitch by high concentrations of tolazoline. This is not consistent with the finding reported by Drew (8). It seems unlikely that this potentiation results from removal of presynaptic \( \alpha \)-adrenoceptor-mediated negative feedback, since there was no correlation between the potency to antagonize presynaptic \( \alpha \)-adrenoceptors and the potentiating effect on the twitch response for the \( \alpha \)-antagonists tested.

It is concluded that E-643 is a potent and highly selective postsynaptic \( \alpha \)-adrenoceptor blocking agent.

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