Difference in Mode of Action of Alpha$_1$-Adrenoceptor Antagonists on Some Vascular Smooth Muscles and Efficacy

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Abstract—Effect of YM-12617, a selective and potent alpha$_1$-adrenoceptor antagonist on dose-response curves of alpha$_1$-adrenoceptor agonists, norepinephrine, phenylephrine and naphazoline, was tested in isolated rabbit vascular smooth muscles such as the femoral vein, portal vein and aorta. YM-12617 shifted the dose-response curves for norepinephrine and phenylephrine to the right and also declined the maximum response in the femoral vein, where norepinephrine and phenylephrine behaved as low efficacy agonists. Similar results were obtained on the curve of naphazoline in the portal vein, where the efficacy of naphazoline was low. However, the efficacies of norepinephrine, phenylephrine and naphazoline were high in the aorta. The dose-response curves for three alpha$_1$-agonists were shifted by YM-12617 in a parallel manner in the aorta. The curves of norepinephrine and phenylephrine were also shifted by YM-12617 in the portal vein, where the efficacies of both the alpha$_1$-agonists were high. The present results suggest that the mode of antagonism between the alpha$_1$-agonist and alpha$_1$-antagonist is dependent on the efficacy of the alpha$_1$-agonist which depends upon the receptor-density in the organ used.

A number of alpha$_1$-adrenoceptor agonists and -antagonists have been developed. They compete with each other for the alpha$_1$-adrenoceptors. If we plot a log dose-response curve for the alpha$_1$-agonist in the presence of the alpha$_1$-antagonist, it is shifted to higher doses in a parallel manner. These phenomena were reported in isolated organs such as vasa deferentia from the guinea pig and rat, aortae from the rabbit and rat, and rabbit pulmonary artery (1-5). There are, however, kinetic conditions under which competitive antagonists shift the dose-response curves of agonists to higher doses, but also depress the maximum responses. These phenomena are encountered with persistent (low rate of off set) antagonists and low efficacy agonists, and they were described by Paton and Rang (6), Paton and Waud (7) and Kenakin (8) as the hemi-equilibrium state between agonist, antagonist and their receptors. Recently, 5-[[2-(2-ethoxyphenoxy)ethyl]amino]-propyl]-2-methoxybenzenesulfonamide hydrochloride was found to be a potent alpha$_1$-adrenoceptor antagonist (9). In this study, we tested the difference in the mode of action of this alpha$_1$-antagonist on the rabbit isolated aorta, femoral vein and portal vein using prazosin as a reference alpha$_1$-antagonist, and we studied the relationship between efficacy (10) and the mode of antagonism.

Materials and Methods

Male rabbits (2-3 kg) were killed by bleeding from the neck. The thoracic aorta, femoral vein and portal vein were dissected. The vascular smooth muscles were cut helically, stripped of their endothelial cells by mechanical rubbing and used as smooth muscle preparations. A strip (about $2 \times 10-20$ mm) of each preparation was suspended in a physiological solution (118 mM NaCl,
4.70 mM KCl, 2.54 mM CaCl₂, 1.20 mM MgCl₂, 1.19 mM KH₂PO₄, 25.0 mM NaHCO₃ and 11.0 mM glucose) kept at 32°C and gassed with a mixture of 95% O₂ and 5% CO₂. The solution also contained propranolol (10⁻⁶ M), desmethylimipramine (10⁻⁷ M) and normetanephrine (10⁻⁶ M) to inhibit beta-adrenoceptors, neuronal and extra-neuronal uptake, respectively (3). Responses to drugs were recorded isotonically under a tension of 1.5 g for the aorta and portal vein or 0.15 g for the femoral vein. To test the antagonism between an agonist and an antagonist, the preparations were pre-treated with the antagonist for 30 min, and the dose-response curve of the agonist was obtained in the presence of the antagonist. The competitive antagonistic activity of the drug was expressed as the pA₂-value, which is the negative logarithm of the dissociation constant, Kᵦ, of the antagonist. The pA₂-value was estimated by the method of Arunlakshana and Schild (11). In some experiments, the dose-response curves for the agonists declined in the presence of YM-12617. These phenomena were thought to be the hemi-equilibrium state between the agonist, antagonist and receptors as reported by Paton and Rang (6) and Paton and Waud (7). An estimate of the dissociation constant (Kᵦ) of the antagonist was made using the following equation (1):

\[
\frac{1}{[A]} = \frac{1}{Kₐ} \cdot \frac{(1-\rho)}{1-\rho} + \frac{1}{(1-\rho)} \cdot \frac{1}{[A']}
\]

where [A] and [A'] refer to equi-effective doses of the agonist in the absence and presence of the antagonist (B), and Kₐ is the dissociation constant of the agonist. Furthermore, ρ in the equation I is the fractional receptor occupancy, which is given from Gaddum's equation:

\[
\rho = \frac{1}{1 + \frac{Kₐ}{[A]}} \left(1 + \frac{[B]}{Kₐ}ight)
\]

where [A] and [B] are doses (M) of the agonist and antagonist, respectively, and Kₐ and Kᵦ refer to their respective equilibrium dissociation constants.

Therefore, a double reciprocal regression of 1/[A] upon 1/[A'] should yield a straight line with a positive intercept, according to equation I. The Kᵦ then could be calculated from equation III.

\[
Kᵦ = \frac{[B]}{\text{slope} - 1}
\]

In order to estimate the efficacy and dissociation constant of an agonist, the alpha₁-adrenoceptors were partially blocked by an irreversible antagonist, phenoxybenzamine, according to Furchgott (12). After the determination of the control dose-response curves for the agonists, the preparations were incubated with phenoxybenzamine (10⁻⁷ M) for 10 min. The preparations were then allowed to equilibrate for 60 min with repeated washing every 10 min, and second dose-response curves for the agonists were determined. The dissociation constant (Kₐ) was calculated from the following equation IV:

\[
\frac{1}{[A]} = \frac{1-q}{qKₐ} + \frac{1}{q[A']}
\]

where [A] and [A'] are the corresponding equi-effective doses (M) of the agonist before and after irreversible blockade of a fraction of alpha₁-adrenoceptors with phenoxybenzamine, respectively, and q is the remaining fraction of active receptors after phenoxybenzamine treatment. 1/[A] was plotted versus 1/[A'], and the straight line was fitted to the data by linear regression analysis. The dissociation constant (Kₐ) was obtained by the following equation V:

\[
Kₐ = \frac{\text{slope} - 1}{\text{intercept}}
\]

The efficacy (e) was calculated from equation VI:

\[
e = \frac{Kₐ}{ED₅₀} + 1
\]

where ED₅₀ is the dose (M) necessary to induce a 50% response.

Drugs used: Naphazoline hydrochloride (Sigma), norepinephrine bitartrate (Wako), phenylephrine hydrochloride (Sigma), prazosin hydrochloride (Sigma) and phenoxybenzamine hydrochloride (Tokyo Kasei). YM-12617 was kindly supplied by Yamano-uchi Pharmaceutical Co., Ltd. Other chemicals
used were of analytical grade.

**Results**

In the aorta, all the dose-response curves for norepinephrine, phenylephrine and naphazoline were parallelly shifted to higher doses by prazosin and YM-12617, indicating a competitive antagonism. In the portal vein, the dose-response curves for norepinephrine and phenylephrine were also shifted by prazosin and YM-12617 in a parallel manner (Fig. 1). The curve for naphazoline obtained in the portal vein was, however, shifted by prazosin and declined in the presence of YM-12617. YM-12617 declined the dose-response curves for norepinephrine and phenylephrine in the femoral vein, which were parallelly shifted by prazosin (Fig. 2).

As the response of the femoral vein to naphazoline was too small to estimate precisely, the effects of the alpha₁-blockers were not tested. The pA₂-values for prazosin and YM-12617 were calculated from the parallel shifts of the curves for the alpha₁-agonists, since the slopes of the Schild plot were not different from unity (P<0.05), and these are summarized in Table 1. The negative logarithm of the dissociation constant, pKₐ, of YM-12617 calculated by the equation 1 of Paton and Rang (6) was also summarized in Table 1. A difference between the pA₂-values for YM-12617 and prazosin was 1.0 to 1.7, suggesting that YM-12617 was 10 to 50 times as potent as prazosin. There is, however, no difference between the respective pA₂-value for YM-12617 and prazosin.
Each value is presented as the mean with S.E. in the parentheses. The number of experiments is 8 to 12.

*: the negative logarithm of the dissociation constant, Kα, calculated by the equation I.

<table>
<thead>
<tr>
<th></th>
<th>Norepinephrine</th>
<th>Phenylephrine</th>
<th>Naphazoline</th>
<th>Norepinephrine</th>
<th>Phenylephrine</th>
<th>Naphazoline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aorta</td>
<td>9.43 (0.04)</td>
<td>9.60 (0.09)</td>
<td>9.82 (0.03)</td>
<td>8.39 (0.08)</td>
<td>8.45 (0.06)</td>
<td>8.22 (0.06)</td>
</tr>
<tr>
<td>Portal vein</td>
<td>10.02 (0.07)</td>
<td>9.84 (0.06)</td>
<td>10.07 (0.05)*</td>
<td>8.29 (0.06)</td>
<td>7.99 (0.07)</td>
<td>8.41 (0.08)</td>
</tr>
<tr>
<td>Femoral vein</td>
<td>10.00 (0.08)*</td>
<td>9.94 (0.05)*</td>
<td>8.12 (0.06)</td>
<td>7.98 (0.06)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Each value is presented as the mean with S.E. in the parentheses. The number of experiments is 5 to 10.

Table 2. The efficacy and pKα-value for norepinephrine, phenylephrine and naphazoline

<table>
<thead>
<tr>
<th></th>
<th>Norepinephrine</th>
<th>Phenylephrine</th>
<th>Naphazoline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Efficacy</td>
<td>pKα</td>
<td>Efficacy</td>
<td>pKα</td>
</tr>
<tr>
<td>Aorta</td>
<td>7.89 (1.80)</td>
<td>6.11 (0.08)</td>
<td>17.26 (2.10)</td>
</tr>
<tr>
<td>Portal vein</td>
<td>7.55 (0.90)</td>
<td>5.40 (0.08)</td>
<td>8.96 (2.00)</td>
</tr>
<tr>
<td>Femoral vein</td>
<td>1.95 (0.30)</td>
<td>6.09 (0.20)</td>
<td>1.35 (0.24)</td>
</tr>
</tbody>
</table>

The efficacy and pKα-value (a negative log of Kα) calculated by the method of Furchgott (12) are summarized in Table 2. The efficacy of phenylephrine and norepinephrine in the femoral vein and that of naphazoline in the portal vein were less than 2, suggesting that the alpha1-stimulants used behaved as partial agonists in these organs.

Discussion

Chemical formulae of prazosin and YM-12617, alpha1-antagonists, are quite different from each other: prazosin is a quinazoline derivative and YM-12617 is a phenylethylamine derivative. Norepinephrine and phenylephrine, phenethylamine derivatives, and naphazoline, a imidazoline derivative, were used as alpha1-agonists. There is no difference between the respective pA2-values for prazosin and YM-12617 against all the agonists estimated in different organs as shown in Table 1. These results suggest that prazosin and YM-12617, though their chemical structures were different, interact with the same alpha1-receptors. Furthermore a comparison of the pA2-values (Table 1) indicates that YM-12617 is a potent alpha1-adrenoceptor antagonist, as reported previously (9, 13).

In the organs such as the femoral vein and portal vein, where alpha1-receptor stimulants behaved as low efficacy agonists, the potent alpha1-antagonist YM-12617 shifted their dose-response curves to the right and also depressed the maximum response. These phenomena are considered to be the hemi-equilibrium state (6-8). It is known that under the hemi-equilibrium state the antagonist behaves as an essentially irreversible blocker and produces unsurmountable antagonism. The depression of the maximal response for any given dose ratio is dependent upon the efficacy of the agonist (6-8). According to Paton's rate theory (14), the rate constant for dissociation of a potent competitive antagonist is smaller than that of a less potent antagonist, that is, the potent antagonist forms a complex with the receptor which is slowly broken up. YM-12617 is thought to dissociate more slowly from the alpha1-adrenoceptor than prazosin. Therefore the hemi-equilibrium state was observed in the experiments with YM-12617, but not in those with prazosin. The depression of the concentration response curves of guinea pig ileal longitudinal muscles to noctyltrimethylammonium by hyoscine was already reported by Rang (15). Using the equation I, Rang (15) calculated the dissociation constant Kα for hyoscine with noctyltrimethylammonium and found that it agreed with independent estimates by hyoscine antagonism of methyl fumethide. These results coincide with the
present observations that the $pA_2$ value (the negative logarithm of $K_a$) for YM-12617 estimated from the Schild plot was equal to the negative logarithm of $K_B$ calculated by the equation I of Paton and Rang (6) in this study.

Furchgott (12) defined the efficacy ($e$) as

$$e = z[R]$$

where $z$ and $[R]$ refer to an intrinsic efficacy, which should be constant for given drug receptor complexes across species and tissues, and the total concentrations of receptors or receptor-density. According to Furchgott (12), the efficacy is dependent on the receptor-density in the organ used. The present results indicate that the mode of antagonism between the agonist and antagonist is dependent upon the efficacy of the agonist which depends upon the receptor-density in the organ used, although it is necessary to estimate the receptor-density.

References


