A Difference in Mode of Antagonism between Optical Isomers of a Potent Selective Alpha₁-Adrenoceptor Blocker (YM-12617) and Norepinephrine in Isolated Rabbit Iris Dilator and Aorta

Issei TAKAYANAGI, Fukio KONNO, Hiroyuki KAMEDA, Haruichi KUBO, Akira FURUKAWA and Toshie TOYODA

Department of Chemical Pharmacology, Toho University School of Pharmaceutical Sciences, Funabashi, Chiba 274, Japan

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Abstract—Optical isomers of YM-12617, a potent and selective alpha₁-adrenoceptor blocker, were tested on the rabbit iris dilator and aorta. The order of potency was R(-)-isomer > racemate > S(+)-isomer. The R(-)-isomer and racemate behaved as an essentially irreversible antagonist to norepinephrine in the iris dilator where the efficacy of norepinephrine was small, although the S(+)-isomer was a competitive antagonist. These drugs behaved as a competitive antagonist of norepinephrine in the aorta where the efficacy of norepinephrine was large.

Many alpha₁-adrenoceptor blockers have been developed and clinically used as anti-hypertensive drugs. YM-12617, 5-[2-[[2-(O-ethoxyphenoxy)ethyl]amino]propyl]-2-methoxybenzenesulfonamide hydrochloride, was reported to be a potent and selective alpha₁-adrenoceptor blocker (1-3). In our previous paper (3), YM-12617 (a racemate) behaved as an essentially irreversible antagonist on alpha₁-adrenoceptors in the rabbit femoral vein where the efficacy of the alpha₁-agonist was small. In this paper, we studied the difference in the mode of antagonism between optical isomers of YM-12617 and norepinephrine in the isolated rabbit iris dilator and aorta and further estimated the efficacies (4) of norepinephrine to explain the difference in mode of antagonism.

Male albino rabbits weighing 2.0 to 3.0 kg were killed by bleeding from the neck. The eyeball and thoracic aorta were dissected. Pieces (about 3x4 mm) of iris dilators were carefully dissected from the iris of the enucleated eyeball (5). The iris dilators were rapidly mounted in a physiological saline solution of the following composition (in mM): NaCl, 118; KCl, 4.70; CaCl₂, 2.54; MgCl₂, 1.20; KH₂PO₄, 1.19; NaHCO₃, 25.0; and glucose, 11.0. The thoracic aorta was cut helically (about 2x10⁻²⁰ mm), stripped of its endothelial cells by mechanical rubbing, and used as a smooth muscle preparation. Each preparation was suspended in the physiological solution 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 and neural and extraneural uptakes, respectively (6). Responses to drugs were recorded isometrically under a tension of 20 to 30 mg for the iris dilator and 1.5 g for the aorta. The preparations were allowed to equilibrate for at least 60 min before the experiments started. To test an antagonism between norepinephrine and one of an agonists, the preparations were pretreated with the antagonist for 30 min after a control dose response curve of norepinephrine was obtained. Each preparation was suspended in the physiological solution 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 and neural and extraneural uptakes, respectively (6). Responses to drugs were recorded isometrically under a tension of 20 to 30 mg for the iris dilator and 1.5 g for the aorta.

The dose-response curve of norepinephrine was again 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 of the antagonist, and it was estimated by the method of Arunlakshana and Schild (7). In the iris dilators, the dose-response curves for norepinephrine declined isometrically (about 2x10⁻²⁰ mm), stripped of its endothelial cells by mechanical rubbing, and used as a smooth muscle preparation. Each preparation was suspended in the physiological solution 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 and neural and extraneural uptakes, respectively (6). Responses to drugs were recorded isometrically under a tension of 20 to 30 mg for the iris dilator and 1.5 g for the aorta. The preparations were allowed to equilibrate for at least 60 min before the experiments started. To test an antagonism between norepinephrine and one of antagonists, the preparations were pretreated with the antagonist for 30 min after a control dose response curve of norepinephrine was obtained cumulatively. The dose-response curve of norepinephrine was again 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 of the antagonist, and it was estimated by the method of Arunlakshana and Schild (7). In the iris dilators, the dose-response curves for norepinephrine declined in the
presence of the R(-)-isomer and racemate of YM-12617, an alpha1-adrenoceptor blocker. These phenomena were thought to be a hemi-equilibrium state between the agonist, antagonist and receptors as reported by Paton and Waud (8). The dissociation constant (Kd) of the R(-)-isomer and racemate in the iris dilator was estimated by the method of Paton and Waud (8). The precise procedures are described in previous papers (3, 8). The negative logarithm of the dissociation constant (Kd) obtained was listed as the pKd-value in Table 1.

The efficacy (e) of norepinephrine was calculated from equation I:

\[ e = \frac{K_d}{ED_{50} + 1} \]

where the ED50 is the dose (M) necessary to induce a 50% response and Kd is the dissociation constant (9). To estimate the dissociation constant (Kd) of norepinephrine in the rabbit iris dilator and aorta, alpha1-adrenoceptors were partially blocked by an irreversible antagonist, phenoxybenzamine (9). After the determination of the control dose-response curve of norepinephrine, 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 a second dose-response curve for norepinephrine was determined. The corresponding equi-effective doses (M) of norepinephrine before and after irreversible blockade of a fraction of the alpha1-adrenoceptors with phenoxybenzamine were obtained graphically. The dissociation constant (Kd) was calculated according to Furchgott (9).

The optical isomers of YM-12617, 5-[2-[[2-(O-ethoxyphenoxy)ethyl]amino]propyl]-2-methoxybenzenesulfonamide hydrochloride, were kindly supplied by the Yamanouchi Pharmaceutical Co., Ltd. Norepinephrine bitartrate (Wako) and phenoxybenzamine hydrochloride (Tokyo Kasei) were obtained commercially. Other chemicals used were of analytical grade.

Norepinephrine contracted the rabbit iris dilator dose-dependently, and the maximum tension developed by norepinephrine was 174.4±9.1 mg (a mean±S.E. of 20 experiments). The dose-response curve of norepinephrine was shifted to higher doses, but also its maximum response was depressed by the R(-)-isomer and racemate of YM-12617 (10^-9, 3x10^-9 and 10^-8 M), while the S(+) -isomer shifted the dose-response curve of norepinephrine to the right without a decrease in the maximal response at high doses of 10^-7, 3x10^-7 and 10^-6 M (Fig. 1).

The pA2-value for the S(+) -isomer was calculated from the parallel shifts of the curve (Table 1), since the slope of the Schild plot was different from unity (P<0.05). The values of the negative logarithm of the dissociation constant, pKd, for the R(-)-isomer and racemate calculated by the method of Paton and Waud (8) are summarized in Table 1. The relative order of the pA2 or pKd was R(-)-isomer > racemate >> S(+) -isomer.

The rabbit aorta was also contracted by norepinephrine. The R(-)-isomer (10^-9, 3x10^-9 and 10^-8 M), racemate (10^-9, 3x10^-9 and 10^-8 M) and S(+) -isomer (10^-8, 3x10^-8 and 10^-7 M) parallelly shifted the dose-response curve of norepinephrine to the right without decreasing the maximal response. The pA2-values were calculated from the parallel shifts of the curve of norepinephrine. A Schild plot of these results gave a straight line with a slope of one. The pA2-values obtained by the Schild plot analysis of the data are summarized in Table 1.

|                | Iris dilator | Aorta |
|----------------|-------------|-------|
| R(-)-Isomer    | 9.73±0.05*  | 9.77±0.14 |
| Racemate       | 9.64±0.09*  | 9.67±0.16 |
| S(+) -Isomer   | 7.76±0.01   | 7.46±0.15 |

Each value is presented as a mean±S.E. of 4 to 5 experiments. *: calculated by the method of Paton and Waud (8).
Fig. 1. Effects of the R(-)-isomer (upper) and S(+)-isomer (lower) of YM-12617 on the dose-response curve of norepinephrine in the rabbit iris dilator. Ordinate: contraction (%). Abscissa: negative logarithm of norepinephrine (M). Each value is presented as a mean±S.E. of 5 experiments. Upper: •, norepinephrine alone; ▲, with R(-)-isomer, 10^-9 M; △, with R(-)-isomer, 3x10^-9 M; and ▼, with R(-)-isomer, 10^-8 M. Lower: •, norepinephrine alone; ▲, with S(+)-isomer, 10^-8 M; △, with S(+)-isomer, 3x10^-8 M; and ▼, with S(+)-isomer, 10^-7 M.

The pKₐ-values for norepinephrine calculated by the method of Furchgott (9) were 5.43±0.07 (a mean±S.E. of 5 experiments) in the rabbit iris dilator and 5.68±0.10 (a mean±S.E. of 8 experiments) in the rabbit aorta. Both the pKₐ-values were identical suggesting that alpha₁-receptors in the rabbit iris dilator were qualitatively the same as those in the rabbit aorta. The efficacy (6.48±0.92) for norepinephrine in the iris dilator was significantly (P<0.05) smaller than that (18.22±3.40) in the aorta. The values were presented as the means±S.E. of 8 experiments in the latter and 5 experiments in the former, respectively.

The pA₂-values for the racemate of YM-12617 estimated herein are in agreement with the values reported previously (1, 3) and support the previous conclusion that YM-12617 is a potent and selective alpha₁-adrenoceptor blocker. In the rabbit iris dilator and aorta, the difference between the pA₂- or pKₐ-values for the R(-)-isomer and S(+)-isomer was 2 to 2.3, suggesting that the R(-)-isomer was 100 to 200 times as potent as the S(+)-isomer. These results are similar to the findings of Honda and Nakagawa (10) who reported that the R(-)-isomer was a 50 to 600 times more potent antagonist than the S(+)-isomer for alpha₁-adrenoceptors in the rabbit lower urinary tract, prostate and aorta. The stereochemical requirements of phenylethylamines including catecholamines to stimulate and block alpha- and beta-adrenoceptors have been reported (11). The fact that the relative order of pA₂- or pKₐ-values was R(-)-isomer>racemate>S(+)-isomer is in agreement with the previous findings (11).

In the rabbit femoral vein, the racemate of YM-12617 shifted the dose-response curves of alpha₁-adrenoceptor stimulants to the right and also depressed the maximum response (3). These phenomena were considered to be the hemi-equilibrium state (3, 8). It is known that under the hemi-equilibrium state the potent antagonist behaves as an essentially irreversible blocker and produces unsurmountable antagonism and that the depression of the maximal response for any given dose ratio is dependent upon the efficacy of the agonist. According to Paton's rate theory (12), 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. In the present study, the efficacy of norepinephrine in the iris dilator was significantly smaller than that in the aorta (P<0.05). Therefore, the R(-)-isomer and racemate of YM-12617, which were potent alpha₁-adrenoceptor blockers, behaved as essentially irreversible blockers in the iris dilator and depressed the maximum response to norepinephrine as discussed previously (3). However, both the potent alpha₁-blockers shifted the dose-response curve of norepinephrine to the right without decreasing the maximal response in the aorta where the efficacy of norepinephrine...
was large.

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