A Trial for Kinetic Evaluation of the Antagonistic Potency of Several \( \beta \)-Antagonists on Presynaptic \( \beta \)-Adrenoceptors

Misako KUWAHARA, Hiro AMANO, Takao KUBO and Yoshimi MISU
Department of Pharmacology, Yokohama City University School of Medicine, Yokohama 232, Japan
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Abstract—The antagonistic potency, \( pA_2 \), of several non-selective \( \beta \)-antagonists on presynaptic \( \beta \)-adrenoceptors was evaluated using a parallel line assay and MacKay's equation against isoproterenol-induced increases in \( ^3H \) release in isolated guinea-pig pulmonary arteries preloaded with \( ^3H \)-norepinephrine. Cumulatively applied isoproterenol at \( 10^{-9} \) M, \( 10^{-8} \) M and \( 10^{-7} \) M dose-dependently increased \( ^3H \) release evoked by transmural field stimulation at 1 Hz. \( \beta \)-Antagonists tested dose-dependently antagonized the isoproterenol-induced increases. The order of \( pA_2 \) was carteolol (11.23±0.09) > nadolol (9.78±0.05) > pindolol (9.59±0.03) > propranolol (9.26±0.17). Carteolol has the highest \( pA_2 \) and is a useful tool for clarifying whether or not presynaptic \( \beta \)-adrenoceptors tonically function.

It has been proposed that there is a positive feedback mechanism of the release of the transmitter norepinephrine through presynaptic \( \beta \)-adrenoceptors (1–3). For example, isoproterenol augments contractile responses of human saphenous vein to electrical field stimulation with small relaxation during contraction elicited by exogenously applied norepinephrine (4), whereas propranolol alone stereoselectively inhibits contractile responses of guinea-pig pulmonary arteries to sympathetic nerve stimulation without modifications of contraction induced by exogenously applied norepinephrine (5). Classical pharmacological techniques to determine the \( pA_2 \) values have been widely used for the evaluation of the antagonistic activities of various antagonists on different types of postsynaptic receptors (6). However, the kinetic analysis of the properties of presynaptic \( \beta \)-adrenoceptors has not been done (2, 3), although the subtype of these adrenoceptors was qualitatively determined as the \( \beta_2 \) type in the peripheral sympathetic nerves (2, 3, 5, 7). There is a possibility that presynaptic \( \beta \)-adrenoceptors might differ from postsynaptic \( \beta \)-adrenoceptors, probably in a similar manner as the dominant presynaptic and postsynaptic \( \alpha \)-adrenoceptors differ in their affinity for agonists and for antagonists (1). In general, there is extreme controversy or inconsistency concerning the blocking actions of \( \beta \)-antagonists alone on presynaptic \( \beta \)-adrenoceptors (2, 3). A potent \( \beta \)-antagonist on presynaptic \( \beta \)-adrenoceptors should be found to clarify whether or not these receptors tonically function in the positive feedback mechanism. Thus, a trial to estimate a \( pA_2 \) value has been done for a quantitative analysis of antagonistic activities of several non-selective \( \beta \)-antagonists against isoproterenol-induced increases in impulse-evoked release of \( ^3H \) from guinea-pig pulmonary arteries preloaded with \( ^3H \)-norepinephrine.

Spirally cut preparations of the pulmonary arteries from male guinea-pigs, weighing 200 to 250 g, were prepared and incubated at 37°C for 60 min with oxygenated Krebs-bicarbonate solution containing \( 10^{-7} \) M \( ^3H \)-norepinephrine (Amersham/Searle) and ascorbic acid 100 mg/l as described previously (5, 7). After rinsing for 10 min with norepinephrine-free medium, the preparations were mounted vertically between a pair of platinum stimulating electrodes and superfused with Krebs' solution at a constant rate of 1 ml/min. The solution had the following
composition (mM): NaCl, 120.7; KCl, 5.9; CaCl₂, 2.5; MgCl₂, 1.2; NaHCO₃, 15.5; NaH₂PO₄, 1.2; and glucose, 11.5. The solution, maintained at 37°C, pH 7.2 to 7.4, was bubbled with 5% CO₂ in O₂. A 90 min equilibration period was allowed and then transmural field stimulations (1 Hz, 2 msec, 10 V, 100 pulses) were repeated 4 times (S₁ to S₄ periods) at 15 min intervals, using an electrical stimulator with an isolator (SEN-3201 and SS-120J, Nihon Kohden). Superfusate for 2 min was collected before, during and after the nerve stimulation, respectively, and 12 ml of ACS-II solution was added, and total ³H activities expressed as disintegration per min were determined using a liquid scintillation spectrometer (Packard 2660). Impulse-evoked release of ³H was calculated as the difference between resting efflux before stimulation and total efflux detected in 3 successive samples during and after stimulation.

⁻⁻Isopropenol at 10⁻⁸ M, 10⁻⁸ M and 10⁻⁷ M was cumulatively applied 6 min after the S₁, S₂ and S₃ period of stimulation, respectively, in the absence or presence of each 3 to 4 dose of β-antagonists. β-Antagonists were applied 25 min before S₁ and were present throughout the experiments. The effect of each dose of isoproterenol was evaluated by the % release ratio of S₂/S₁, S₃/S₁ and S₄/S₁, respectively. According to the method of parallel line assay (8), the parallelism between the 2 dose-response curves of isoproterenol, one in the absence and the other in the presence of a given dose, [I], of a β-antagonist, was ascertained to be undeniable, the distance between 2 regression lines with a common regression coefficient was statistically calculated, and then a dose ratio (DR) was determined from the distance. A pA₂ value at the given dose of the β-antagonist was calculated from MacKay's equation (9): pA₂=log (DR-1)-log [I]. Log (DR-1) was plotted against a log molar dose (6), a regression line was drawn by the method of least squares, and the slope and its S.E. were calculated (10).

Drugs used were /-isoproterenol hydrochloride (Sigma), d/-carteolol hydrochloride (Otsuka), nadolol (Squibb-Dainippon), pindolol (Sandoz-Sanyo) and /-propranolol hydrochloride (ICI). Pindolol was dissolved in 0.1 N HCl, and further dilution was made with Krebs' solution. The other drugs were dissolved in distilled water.

The resting ³H efflux and the ³H efflux evoked by transmural field stimulation at 1 Hz from spirally cut pulmonary arteries preloaded with ³H-norepinephrine were 3286.9±670.1 dpm/tissue/2 min and 5380.3±302.6 dpm/tissue/100 pulses (n=7) 90 min after the start of superfusion. The evoked release ratios of S₂/S₁, S₃/S₁ and S₄/S₁.

Table 1. Effects of cumulatively applied isoproterenol at 10⁻⁹ M, 10⁻⁸ M and 10⁻⁷ M on impulse-evoked ³H release from spirally cut guinea-pig pulmonary arteries preloaded with ³H-norepinephrine and carteolol-induced antagonism

<table>
<thead>
<tr>
<th>Pretreatment (M)</th>
<th>N</th>
<th>S₂/S₁</th>
<th>S₃/S₁</th>
<th>S₄/S₁</th>
</tr>
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<tr>
<td>None</td>
<td>7</td>
<td>103.6±3.8</td>
<td>99.6±4.6</td>
<td>99.1±4.4</td>
</tr>
<tr>
<td>Control (Isopteronol alone)</td>
<td>16</td>
<td>106.3±5.2</td>
<td>117.2±7.1</td>
<td>131.4±6.4</td>
</tr>
<tr>
<td>Carteolol 10⁻¹¹</td>
<td>8</td>
<td>106.5±3.2</td>
<td>115.0±2.9</td>
<td>123.7±3.6</td>
</tr>
<tr>
<td>3x10⁻¹¹</td>
<td>6</td>
<td>99.4±4.9</td>
<td>107.2±5.4</td>
<td>119.8±6.1</td>
</tr>
<tr>
<td>10⁻¹⁰</td>
<td>6</td>
<td>94.2±4.7</td>
<td>103.2±4.8</td>
<td>110.7±3.6</td>
</tr>
</tbody>
</table>

Strips were incubated for 60 min with 10⁻⁷ M ³H-norepinephrine solution, rinsed, set up and then superfused with Krebs' medium. Transmural field stimulation (1 Hz, 2 msec, 10 V, 100 sec) was repeated 4 times, 90, 105, 120 and 135 min after the start of superfusion (S₁ to S₄). Isoproterenol at 10⁻⁹ M, 10⁻⁸ M and 10⁻⁷ M was cumulatively applied 6 min after the respective S₁, S₂ and S₃ stimulation in the control and in preparations pretreated with carteolol at 10⁻¹¹ M, 3x10⁻¹¹ M and 10⁻¹⁰ M, respectively. Carteolol was applied 25 min before S₁ and was present throughout the experiments. The respective ³H efflux evoked by the S₂, S₃ and S₄ stimulation is expressed as a % of that evoked by the S₁ stimulation, S₂/S₁, S₃/S₁ and S₄/S₁.
S₄/S₁ were almost the same (Table 1). Cumulatively applied isoproterenol at 10⁻⁸ M, 10⁻⁷ M increased the impulse-evoked ³H release in a dose-dependent manner without modifying the resting ³H efflux. The increases are via the activation of presynaptic β-adrenoceptors, since these were stereoselectively antagonized by propranolol (6). Carteolol at 10⁻¹¹ M, 3×10⁻¹¹ M and 10⁻¹⁰ M antagonized in a dose-dependent manner isoproterenol-induced increases in the evoked ³H efflux with a roughly parallel shift of the dose-response curve of isoproterenol to the right. No modifications of the resting ³H efflux occurred. Departure of the 2 dose-response curves of isoproterenol (Table 1) from parallelism was not significant in the absence and presence of each dose of carteolol (8). A pA₂ calculated at each dose of carteolol, the mean of the pA₂ (pA₂), and the slope and the S.E. of the Schild plot (6, 9, 10) are summarized in Table 2. The slope of the Schild plot of carteolol showed no significant difference from 1.0, the theoretical value of a competitive antagonism. The pA₂ values, the slopes and the S.E. of the Schild plots of nadolol, pindolol and propranolol were similarly calculated and are shown in Table 2. The parallelism between the 2 dose-response curves in the absence and presence of each dose of these β-antagonists was also undeniable. The slopes of the Schild plots were also not significantly different from the theoretical value 1.0. In the case of propranolol, however, the membrane stabilizing action appears to reflect on the high value of the slope of the Schild plot. These β-antagonists produced no modifications of the resting ³H efflux. The pA₂ value of carteolol was highest among the β-antagonists tested.

Although in the present experiments there were some methodological limitations in evaluating the pA₂ values of β-antagonists against isoproterenol-induced increases in the impulse evoked ³H release in guinea-pig pulmonary arteries preloaded with ³H norepinephrine such as narrow ranges of the dose-response curve of isoproterenol, the cumulative application of the agonist at a constant interval and different preparations in the absence and presence of β-antagonists, the β-antagonists tested produced a competitive type of antagonism on presynaptic β-adrenoceptors. This is the first trial for a quantitative analysis of β-antagonists-induced antagonism on presynaptic β-adrenoceptors. The order of the evaluated pA₂ values was

<table>
<thead>
<tr>
<th>β-Antagonist</th>
<th>Dose (M)</th>
<th>N</th>
<th>Dose ratio</th>
<th>pA₂</th>
<th>p₂A</th>
<th>Slope of regression line</th>
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<tr>
<td>Carteolol</td>
<td>10⁻¹¹</td>
<td>8</td>
<td>2.2</td>
<td>11.07</td>
<td>11.23±0.09</td>
<td>1.30±0.06</td>
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<td></td>
<td>3×10⁻¹¹</td>
<td>6</td>
<td>6.5</td>
<td>11.26</td>
<td>9.78±0.06</td>
<td>1.98±0.14</td>
</tr>
<tr>
<td></td>
<td>10⁻¹⁰</td>
<td>6</td>
<td>24.3</td>
<td>11.37</td>
<td>9.78±0.06</td>
<td>1.98±0.14</td>
</tr>
<tr>
<td>Nadolol</td>
<td>10⁻⁸</td>
<td>4</td>
<td>6.0</td>
<td>9.70</td>
<td>9.59±0.03</td>
<td>1.12±0.02</td>
</tr>
<tr>
<td></td>
<td>3×10⁻⁹</td>
<td>4</td>
<td>22.9</td>
<td>9.86</td>
<td>9.64</td>
<td>1.12±0.02</td>
</tr>
<tr>
<td></td>
<td>10⁻⁸</td>
<td>4</td>
<td>61.3</td>
<td>9.78</td>
<td>9.64</td>
<td>1.12±0.02</td>
</tr>
<tr>
<td>Pindolol</td>
<td>10⁻⁹</td>
<td>4</td>
<td>4.3</td>
<td>8.52</td>
<td>9.62</td>
<td>9.64</td>
</tr>
<tr>
<td></td>
<td>3×10⁻⁹</td>
<td>4</td>
<td>13.6</td>
<td>9.62</td>
<td>9.64</td>
<td>1.12±0.02</td>
</tr>
<tr>
<td></td>
<td>10⁻⁹</td>
<td>4</td>
<td>44.7</td>
<td>9.64</td>
<td>9.64</td>
<td>1.12±0.02</td>
</tr>
<tr>
<td>Propranolol</td>
<td>3×10⁻⁹</td>
<td>4</td>
<td>4.8</td>
<td>9.10</td>
<td>9.26±0.17</td>
<td>1.71±0.20</td>
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<tr>
<td></td>
<td>5×10⁻⁹</td>
<td>3</td>
<td>5.8</td>
<td>8.98</td>
<td>9.26±0.17</td>
<td>1.71±0.20</td>
</tr>
<tr>
<td></td>
<td>10⁻⁸</td>
<td>7</td>
<td>17.6</td>
<td>9.22</td>
<td>9.26±0.17</td>
<td>1.71±0.20</td>
</tr>
<tr>
<td></td>
<td>3×10⁻⁸</td>
<td>4</td>
<td>167.0</td>
<td>9.74</td>
<td>9.26±0.17</td>
<td>1.71±0.20</td>
</tr>
</tbody>
</table>

Each concentration of carteolol, nadolol, pindolol and propranolol was applied 25 min before the S₁ transmural field stimulation. Other details are as in Table 1.
carteolol > nadolol > pindolol > propranolol. Since the pA$_2$ value of carteolol was the highest, the sympathomimetic activity does not appear to reflect on the pA$_2$ value at the concentration ranges used. The already reported pA$_2$ values against isoproterenol on classical postsynaptic $\beta$-adrenoceptors in guinea-pig atria and tracheae are as follows: 9.26±0.61 in the tracheae for carteolol (11); 8.67±0.07 in atria and 8.83±0.09 (12) and 8.61±0.37 (11) in tracheae for pindolol; 8.51±0.08 (12) and 8.1 (13) in atria and 8.25±0.04 (12) and 7.69±0.04 (11) in tracheae for propranolol; and 7.7 in atria for nodolol (13). The order of the pA$_2$ values on postsynaptic $\beta$-adrenoceptors is carteolol > pindolol > propranolol > nadolol and is not consistent with our findings except for carteolol. Furthermore, the pA$_2$ values of $\beta$-antagonists on presynaptic $\beta$-adrenoceptors are higher by a factor of approximately 2 orders for carteolol and nadolol and of 1 order for pindolol and propranolol, compared to those on postsynaptic $\beta$-adrenoceptors. These results suggest that the properties of presynaptic $\beta$-adrenoceptors differ from those of classical postsynaptic $\beta$-adrenoceptors or that $\beta$-antagonists are readily accessible to presynaptic $\beta$-adrenoceptors, compared to postsynaptic $\beta$-adrenoceptors, and there is some difference of the accessibility of each $\beta$-antagonist to presynaptic $\beta$-adrenoceptors.

Carteolol is a useful tool for clarifying whether or not the presynaptic $\beta$-adrenoceptors involved tonically function in a positive feedback mechanism for the release of norepinephrine, as demonstrated in guinea-pig pulmonary arteries (14), and it is also suitable for clarifying what is an endogenous agonist for presynaptic $\beta$-adrenoceptors, as determined in rat hypothalamic slices (15).

References