CHARACTERIZATION OF PROPRANOLOL-INDUCED RELAXATION OF CORONARY ARTERY

Matao SAKANASHI and Satoshi TAKEO
Department of Pharmacology, School of Medicine, Faculty of Medicine,
University of the Ryukyus, Okinawa 903-01, Japan
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Abstract—Effects of propranolol on coronary artery were investigated in isolated dog coronary arteries and in isolated hearts of rats and guinea-pigs. Cumulative administration of dl-propranolol produced concentration-dependent relaxation of coronary arterial strips under potassium-contracture. There was no difference in relaxant potency between the optical isomers of propranolol. Lidocaine produced less relaxation than dl-propranolol, while atenolol and practolol scarcely affected the contraction. dl-Propranolol-induced relaxation was significantly restored by additional calcium or prevented by prior administration of calcium. In isolated hearts of rats and guinea-pigs which were perfused at a constant coronary inflow and paced at a constant rate, bolus administration of dl-propranolol produced dose-dependent falls in coronary perfusion pressure without significant changes in myocardial contractile force. Results indicate that propranolol may have relaxant activities on coronary arteries through inhibition of calcium influx in the cell membrane of coronary arterial smooth muscle.

It is well known that beta-adrenoceptor blocking agents including propranolol exert a local anesthetic activity on the cardiac cell (1–3) and the giant axon (4, 5) through inhibition of the dv/dt and amplitude of the action potential initiated by the sodium-current. Recently, it has been suggested that the calcium-current of the cardiac cell (6) and the Helix neurone (7, 8) is also inhibited by propranolol. Thus, propranolol has a possible inhibitory action on the calcium-channel in addition to the sodium-channel.

Coronary arteries have both alpha- and beta-adrenoceptors in their smooth muscles (9–11). Therefore, beta-adrenoceptor blockade by propranolol may induce contractions of coronary arteries through masked alpha-adrenoceptor activity which could be a cause of coronary arterial spasm. Clinically, however, propranolol has been utilized as a therapeutic drug against angina pectoris, and the drug has not always provoked vasospastic anginal attack. One reason why propranolol may not provoke coronary spasm could be an inhibitory action by this drug on the calcium-influx in the coronary arterial smooth muscle cell. The present study was designed to test this hypothesis, and the effects of propranolol on coronary arteries were compared with those of local anesthetics and some other beta-adrenoceptor blocking agents.

Materials and Methods
Experiments on isolated coronary arteries: Mongrel dogs of either sex weighing 10–16 kg were anesthetized with sodium pentobarbital 30 mg/kg i.v. The chest was widely opened by a fourth intercostal thoracotomy under artificial ventilation, and the heart was exposed. After cutting off all vessels connected to the heart, the heart was removed and placed in cold Krebs-Ringer bicarbonate
solution of the following composition (mM): NaCl, 117.7; KCl, 4.7; CaCl$_2$, 2.5; MgSO$_4$, 1.2; KH$_2$PO$_4$, 1.2; NaHCO$_3$, 24.4 and glucose, 10.0. A spiral strip, 2.0 mm wide and 20 mm long, was cut from the left circumflex coronary artery and placed in a 20 ml muscle bath filled with the same Krebs-Ringer bicarbonate solution. The solution in the bath was maintained at 37°C and continuously aerated with a gas mixture of 95% O$_2$ and 5% CO$_2$. The oxygen tension of the solution was 600 mmHg and the pH was 7.40 when measured by means of a bloodgas analyzer (Instrumentation Laboratory Micro-13). The strip was connected to a force-displacement transducer (Nihon Kohden TB-611T), and tension developments were isometrically recorded on an ink-writing recticorder (Nihon Kohden RJG-4004). The initial or passive tension was adjusted to an optimal tension, about 1.0 g, and the strip was allowed to equilibrate for 2 hr before any experiments were begun.

The drugs used in this experiment were as follows: dl-propranolol hydrochloride (ICI), I-propranolol hydrochloride (ICI), d-propranolol hydrochloride (ICI), procaine hydrochloride (Sigma), lidocaine hydrochloride (Fujisawa), atenolol (ICI) and practolol (ICI). All drugs were dissolved in physiological saline solution. Doses of drugs corresponded to the final molar concentrations of the salts in dl-propranolol, I-propranolol, d-propranolol, procaine and lidocaine and of free forms in atenolol and practolol. The drug solutions were cumulatively added to the bath in a volume of 0.2 ml. None of the solvents had any effect in the present experiment.

All values in the text are expressed as percent tension (mean±S.E.M.) of the control (100%=potassium 3×10^{-2} M-induced contraction), and the statistical analysis of the data was done with the Student's t-test.

In this experiment, isolated dog coronary arterial strips were contracted with 3×10^{-2} M potassium since high potassium depolarization would directly activate the calcium-channel in the cell membrane of dog coronary arterial smooth muscle (12).

**Experiments on isolated and perfused hearts:** Male Wistar rats and male guinea-pigs weighing about 250 g were killed by a blow on the neck. The heart with aorta was quickly removed into cold modified Krebs-Henseleit solution of the following composition (mM): NaCl, 120.0; KCl, 4.8; CaCl$_2$, 1.25; MgSO$_4$, 1.2; KH$_2$PO$_4$, 1.2; NaHCO$_3$, 25.0; glucose, 5.5 and sodium pyruvate, 2.0. All adipose and connective tissues were removed from the heart and aorta in cold Krebs-Henseleit solution. Then, the heart was transferred to a Langendorff apparatus without recirculation and perfused via aortic cannula at a constant flow rate of 8 ml/min in rat heart and 6 ml/min in guinea-pig heart with the same Krebs-Henseleit solution, which was maintained at 37°C and saturated with a gas mixture of 95% O$_2$ and 5% CO$_2$. When measured by a bloodgas analyzer (Instrumentation Laboratory Micro-13), the oxygen tension of the perfusate was 650 mmHg, and the pH was 7.40. The heart was paced at a constant rate of 300 beats/min in rat heart and 260 beats/min in guinea-pig heart with rectangular pulses of 2 msec in duration and 0.4 V in strength derived from an electronic stimulator (Nihon Kohden MSE-3R) through a pair of platinum electrodes attached on the surface of the right atrium. Initial basal tension was 0.9 g in rat heart and 1.0 g in guinea-pig heart, respectively. Contractile force of the heart was isometrically measured with a force-displacement transducer (Nihon Kohden SB-11T). Coronary perfusion pressure was monitored through a tube connected to the aortic cannula by means of an electric manometer (Nihon Kohden MP-4T). All the parameters were recorded on an ink-writing polygraph (Nihon Kohden RM-45). In this experiment, the conductance of the coronary artery was calculated as ratios of the flow.
rate to the perfusion pressure.

dl-Propranolol hydrochloride (ICI) was used in this study. The drug was dissolved in physiological saline solution, and it was injected into a rubber tube connected to the perfusion apparatus near the aortic cannula in a volume of 0.05 ml.

The data were expressed as percent changes (mean±S.E.M.) of the control (100%) and analyzed with a paired t-test.

Results

Experiments on isolated coronary arteries: Potassium at $3 \times 10^{-5}$ M induced contractions of isolated dog coronary arterial strips ($1146 \pm 47$ mg, $n=58$, Fig. 1). Cumulative administrations of dl-propranolol at concentrations from $10^{-6}$ M to $10^{-4}$ M produced concentration-dependent relaxations of the strips ($n=11$) which reached a steady state after administration of potassium, and additional calcium at $4.5 \times 10^{-3}$ M significantly restored tension of the strips to $68 \pm 4\%$, from $41 \pm 5\%$ ($P<0.01, n=11$, Fig. 1). Both $l$-propranolol ($n=6$) and $d$-propranolol ($n=6$) produced the same degree of relaxant responses on coronary arterial strips as dl-propranolol (Table 1). Procaine showed a tendency to relax the arterial strips ($n=5$) under potassium-contraction, but relaxations were not statistically significant (Table 1). Lidocaine-induced relaxations of the strips ($n=4$) under potassium-contraction were more marked than those by procaine, but not significantly different from the control value (100%) except ones by lidocaine $10^{-4}$ M (Table 1). Thus, coronary arterial relaxations induced by procaine and lidocaine were less than dl-propranolol. Atenolol at $10^{-6}$–$10^{-4}$ M did not change potassium-contraction of the coronary arterial strips ($n=5$, Table 1). Practolol also hardly affected the strips ($n=5$).

![Fig. 1. Typical recording of effect of dl-propranolol on the isolated dog coronary arterial strip which was contracted with potassium (K'). The interval between the left and the right panel is 30 min.](image)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration</th>
<th>n</th>
<th>10^{-6} M</th>
<th>3x10^{-6} M</th>
<th>10^{-5} M</th>
<th>3x10^{-5} M</th>
<th>10^{-4} M</th>
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<tr>
<td>dl-Propranolol</td>
<td>11</td>
<td>100±0</td>
<td>100±0</td>
<td>100±0</td>
<td>89±2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>41±5&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>l-Propranolol</td>
<td>6</td>
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<td>99±1</td>
<td>90±2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>47±4&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
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<td>100±0</td>
<td>98±1</td>
<td>97±1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>88±1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>46±5&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
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<td>100±0</td>
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<td>95±3&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>Lidocaine</td>
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<td>100±0</td>
<td>99±1</td>
<td>94±3</td>
<td>78±12&lt;sup&gt;c&lt;/sup&gt;</td>
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</tr>
<tr>
<td>Atenolol</td>
<td>5</td>
<td>100±0</td>
<td>100±0</td>
<td>100±0</td>
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<td>100±0&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Practolol</td>
<td>5</td>
<td>100±0</td>
<td>100±0</td>
<td>100±0</td>
<td>99±1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>101±0&lt;sup&gt;c&lt;/sup&gt;</td>
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Each value is expressed as the percent tension (mean±S.E.M.) of the control (100%=1146±47 mg, $n=58$). n: the number of strips. <sup>1</sup>P<0.05, <sup>2</sup>P<0.01 and <sup>3</sup>P<0.001 vs. control (100%). <sup>a</sup>P<0.05, <sup>b</sup>P<0.01 and <sup>c</sup>P<0.001 vs. dl-propranolol.
although the drug showed a tendency to relax the strips at a concentration of $3 \times 10^{-5}$ M and to constrict them at $10^{-4}$ M (Table 1).

When $4.5 \times 10^{-3}$ M calcium was applied 15 min prior to administrations of dl-propranolol, potassium-contracture was augmented to $112 \pm 5\%$ of the control, and dl-propranolol-induced relaxations of the strips ($n=6$) were significantly limited to $105 \pm 3\%$ at a concentration of $3 \times 10^{-5}$ M and to $82 \pm 4\%$ at $10^{-4}$ M (Table 2).

Experiments on isolated and perfused hearts: Figure 2 shows a typical recording of the effects of bolus injection of dl-propranolol on isolated and perfused guinea-pig heart. Bolus injections of dl-propranolol at doses from $3 \times 10^{-6}$ M to $3 \times 10^{-4}$ M produced dose-dependent falls in coronary perfusion pressure in guinea-pig hearts ($n=7$, Fig. 3) and rat hearts ($n=4$). Duration of falls in perfusion pressure were also dose-dependent. Conductance of coronary artery was dose-dependently increased by dl-propranolol in both guinea-pig hearts and rat hearts (Table 3). In guinea-pig hearts, the contractile force was almost unchanged by dl-propranolol at $3 \times 10^{-6}$–$10^{-4}$ M, except one by dl-propranolol at $3 \times 10^{-4}$ M, but basal tension was significantly elevated in proportion to the doses of dl-propranolol (Table 3). In rat hearts, contractile force showed a tendency to be depressed dose-dependently by $3 \times 10^{-6}$–

<table>
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<tr>
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<th>Concentration</th>
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<tr>
<td></td>
<td>$10^{-8}$ M</td>
<td>$3 \times 10^{-9}$ M</td>
</tr>
<tr>
<td>Non-treated</td>
<td>100±0</td>
<td>100±0</td>
</tr>
<tr>
<td>Ca$^{2+}$-pretreated</td>
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</tr>
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</table>

Each value is expressed as the percent tension (mean±S.E.M.) of the control ($100\% = 1146\pm 47$ mg, $n=58$). $n$: the number of strips. $^1P<0.05$ and $^2P<0.001$ vs. control (100%). $^aP<0.001$ vs. the value obtained without Ca$^{2+}$-pretreatment.

Fig. 2. Typical recording of effect of dl-propranolol on the isolated and perfused guinea-pig heart. Perfusion Pressure: Coronary arterial perfusion pressure. Tension: Myocardial contractile force. The transient increase in perfusion pressure followed by administration of propranolol is an artifact of injection.
Fig. 3. Time-course changes in perfusion pressure of isolated and perfused guinea-pig hearts by bolus administrations of dl-propranolol. Concentrations of dl-propranolol were $3 \times 10^{-6}$ M (solid triangles), $10^{-5}$ M (open squares), $3 \times 10^{-5}$ M (solid squares), $10^{-4}$ M (open circles) and $3 \times 10^{-4}$ M (solid circles). The initial perfusion pressure was $55 \pm 3$ mmHg. Each value is expressed as percent changes (mean ± S.E.M., n=7) of the control (100% = 55±3 mmHg).

Table 3. Effects of dl-propranolol on conductance of coronary artery, contractile force and basal tension of isolated and perfused hearts of guinea-pig and rat.

<table>
<thead>
<tr>
<th></th>
<th>Guinea-pig hearts (n=7)</th>
<th>Rat hearts (n=4)</th>
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<tbody>
<tr>
<td></td>
<td>Conductance</td>
<td>Contractile force</td>
</tr>
<tr>
<td>Control value</td>
<td>0.110±0.009 ml/min•mmHg</td>
<td>3.5±0.3 g</td>
</tr>
<tr>
<td>Dose of dl-propranolol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$3 \times 10^{-6}$ M</td>
<td>107±2$^a$</td>
<td>99±1</td>
</tr>
<tr>
<td>$10^{-5}$ M</td>
<td>112±5$^a$</td>
<td>100±3</td>
</tr>
<tr>
<td>$3 \times 10^{-5}$ M</td>
<td>129±7$^b$</td>
<td>104±3</td>
</tr>
<tr>
<td>$10^{-4}$ M</td>
<td>140±9$^b$</td>
<td>100±3</td>
</tr>
<tr>
<td>$3 \times 10^{-4}$ M</td>
<td>142±9$^b$</td>
<td>60±6$^b$</td>
</tr>
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</table>

The hearts were perfused at a constant flow rate of 6 ml/min in guinea-pigs and 8 ml/min in rats. Each value is expressed as percent changes (mean±S.E.M.) of the control value (100%). $^aP<0.05$ and $^bP<0.001$ vs. control (100%).
10^{-4} M dl-propranolol, and it was significantly depressed when dl-propranolol at a dose of 3\times10^{-4} M was applied (Table 3). Basal tension was not influenced by dl-propranolol at 3\times10^{-6}-3\times10^{-4} M (Table 3). In both guinea-pig and rat hearts, bolus injections of 3\times10^{-6}-3\times10^{-4} M atenolol and physiological saline solution as the solvent of the drug produced a transient increase alone in coronary perfusion pressure.

Discussion

In this experiment, dl-propranolol produced concentration-dependent relaxations of isolated dog coronary arterial strips under potassium-contracture. The fact that both l-propranolol and d-propranolol, which are optical isomers of dl-propranolol, produced the same degree of relaxant responses on coronary arterial strips as dl-propranolol suggests a possibility that such relaxant actions may be related to the membrane stabilizing activities of the drugs, particularly to a local anesthetic action, since it is well known that there is no difference in local anesthetic activities between dl-propranolol and its optical isomers (3). This view would be supported by the present observation that atenolol and practolol, which have no local anesthetic action (13-16), had no influence on coronary arterial strips under potassium-contracture.

Propranolol-induced relaxations of isolated dog coronary arterial strips cannot be due to the intrinsic sympathomimetic activity since dl-propranolol and its optical isomers produced relaxant responses of the strips in spite of possessing no intrinsic sympathomimetic activity (15, 16), and moreover, practolol having the intrinsic sympathomimetic activity (15) did not induce such relaxant responses in this study. Propranolol-induced relaxations cannot be related to the beta-adrenoceptor blocking activity since d-propranolol produced the same degree of relaxations as l-propranolol in the present study, although the beta-adrenoceptor blocking activity of d-propranolol corresponds to one hundredth that of l-propranolol (3). The fact that atenolol, which has an identical beta-adrenoceptor blocking activity to dl-propranolol (15, 16), did not change potassium-contracture of coronary arterial strips will further support this view.

To confirm participation of a local anesthetic activity in propranolol-induced relaxations of isolated dog coronary arterial strips under potassium-contracture, the effects of two local anesthetics were examined in this study. Procaine could not induce significant relaxations of coronary arterial strips at concentrations used in this experiment. On the other hand, lidocaine showed more marked relaxant responses than procaine did. This difference would be explained by the different potency of local anesthetic activity between procaine and lidocaine (17). It is described that the local anesthetic activity of lidocaine is about equal to that of propranolol (16). The present results, however, indicate that the relaxations induced by dl-propranolol were more marked than those by lidocaine. This discrepancy suggests that dl-propranolol-induced relaxations of coronary arterial strips under potassium-contracture may be caused by other mechanisms in addition to local anesthetic activity. The observation that additional calcium restored the tension of the strips during dl-propranolol-induced relaxations may show the possibility that dl-propranolol would impair the calcium-influx in the cell membrane of coronary arterial smooth muscle. This possibility has been presumed in our preliminary report (18). In 1979, Hashimoto et al. (6) reported that the calcium-current of the cardiac cell was inhibited by propranolol. Furthermore, Akaike et al. (7, 8) observed the inhibitory action of propranolol on the calcium-current in Helix neurones. Thus, also in coronary
arterial smooth muscle, it is fully expected that propranolol may impair the calcium-influx. This hypothesis is supported by the fact that exogenous calcium could significantly inhibit the relaxations induced by dl-propranolol when applied prior to the drug.

In isolated and perfused hearts of guinea-pigs and rats, dl-propranolol produced dose-dependent falls in coronary perfusion pressure, corresponding to dose-dependent increases in coronary arterial conductance. Since participation of the autonomic nervous system in this model is negligible because of the use of isolated hearts, dl-propranolol-induced increases in coronary arterial conductance would be due to the direct inhibitory action of this drug on coronary arterial smooth muscle. In this study, under the condition that the hearts were paced at a constant rate, dl-propranolol did not significantly affect myocardial contractile force unless an extremely large dose (3×10^{-4} M) was applied, suggesting that the inhibitory action of dl-propranolol may be more evident in coronary arteries than in the myocardium. Furthermore, the fact that dl-propranolol increased coronary arterial conductance in both guinea-pig hearts and rat hearts means that the drug would act on coronary arterial smooth muscle without distinction of species in experimental animals.

Thus, the present results suggest that it is plausible that relatively large doses of dl-propranolol will produce the relaxation of coronary arteries in part through inhibition of calcium-influx.

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References


