Bopindolol Is a Slowly Dissociating β₁-Adrenoceptor Antagonist When Compared to Other β-Blockers

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This study used radioligand binding assay methods and pharmacological experiments to examine whether bopindolol, possessing a long-lasting action in addition to potent β-adrenoceptor antagonistic effects, is a slowly dissociating antagonist. In addition, the slow dissociation of two of its metabolites, 18–502 (4-(3-tert-butylamino-2-hydroxypropoxy)-2-methyl indole) and 20–785 (4-(3-tert-butylaminoproxy)-2-carboxyl indole), which have potent β-blocking activities, was also assessed. The blockade of ³H-CGP12177 binding sites in rat heart and brain induced by pindolol was readily reversed by washing, whereas this inhibition by bopindolol and 18–502 was not easily reversed by washing. In addition, specific bindings of the hearts of the treatment animals with 20–785, atenolol, (±)propranolol, nadolol and celiprolol and of washout were 86.7, 78.8, 77.5, 82.3 and 79.9% of the control, respectively. These blockade by the treatment of drug and washout in the brain were, however, lower than those in the hearts. On the other hand, when the left and right atra were treated with propranolol, bopindolol and 18–502, the inotropic and chronotropic actions of isoprenaline were inhibited by these drugs even though they were not present in the extracellular medium. Pretreatment with 20–785, atenolol and nadolol was readily reversed for both inotropic action and chronotropic rate, and inhibition by celiprolol and pindolol remained at 25% of the control at 240 min after treatment with these drugs. A good correlation between inhibitory binding percentage in the hearts and inhibitory inotropic or chronotropic actions were observed, although it was not observed in the brain. Thus, these results suggest that bopindolol and 18–502 are slowly dissociating β-adrenoceptor antagonists and this property may explain its long-lasting anti-hypertensive effects when compared to other β-blockers.

Key words bopindolol; metabolite; slow dissociating antagonist; ³H-CGP12177 binding; β-blocking action; prolonged action

It is well known that bopindolol, a non-selective β-adrenoceptor with antagonistic effects with moderate partial agonist activities, has been found to possess long-lasting action in animal models, and patients with hypertension. It has also been reported that the long-lasting effects of bopindolol may be due to the slowly reversible nature of this drug when bound to a receptor, in the presence of active metabolites, at the shallow slope of the plasma concentration-effect relationship and as a small dissociation constant of the drug-receptor complex. Bopindolol is metabolized to benzoic acid and hydrolyzed bopindolol (18–502 (4-(3-tert-butylamino-2-hydroxypropoxy)-2-methyl indole)), and this hydrolyzed bopindolol is further metabolized to 4-(3-tert-butylaminoproxy)-2-carboxyl indole (20–785). These two metabolites also have potent β-blocking activities. However, the precise mechanisms of bopindolol's long-lasting effect of β-blocking action is not known.

Our previous reports have shown that ³H-CGP12177 bindings to β-adrenoceptors in rat hearts and brains were saturable, with high affinity to those receptors. Thus, in order to elucidate the possibilities of long-lasting action, this study was designed to examine the inhibitory effect of ³H-CGP12177 binding to rat heart and brain after the washout of membranes pre-treated with drugs and the residual inhibition of drugs in the medium to inotropic and chronotropic actions induced by isoprenaline after washout of these drugs in guinea pigs in comparison with those of other β-blockers.

MATERIALS AND METHODS

Preparation of Membrane-Enriched Fraction Membrane-enriched fractions of rat hearts and brains were prepared by the method described previously. In brief, hearts and brains were removed from male Wistar rats weighing about 200–300 g and then minced in 10 mm Tris–HCl containing 250 mm sucrose (pH 7.4). The heart was homogenized by a Polytron for two 10 s periods at a setting of 8. The homogenates were filtered through 4 layers of gaze, and the filtrates were centrifuged at 4000 x g for 30 min. The rat brain was homogenized using a glass-homogenizer and centrifuged at 4000 x g for 30 min. The resultant pellets obtained from rat hearts and brains were immediately rinsed with the incubation medium containing 120 mm Tris–HCl and 40 mm MgCl₂ (pH 7.4), and then homogenized in the same medium with a Polytron homogenizer (heart) and glass homogenizer (brain). Protein was determined by the method of Lowry et al.

Binding Assay The membranes were incubated with a concentration (0.4 nm) of ³H-CGP12177 in a total volume of 0.5 ml of 60 mm Tris–HCl buffer and 20 mm MgCl₂ (pH 7.4) at 23 °C. After the incubation, the membranes were rapidly vacuum-filtered through glass fiber filters (Whatman GF/C). The tissue-bound radioactivity was counted with a scintillation counter. The specific binding was defined as the difference in binding determined in the absence and presence of 0.1 mm (-)-propranolol. The specific binding of ³H-CGP12177 to rat heart membrane

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increased linearly with increasing protein concentrations in the range of 0.05—0.25 mg per assay and was at equilibrium at 30 min.12,13) Thus, the binding reaction was carried out for 30 min, with a protein concentration of 0.2 mg per assay.

The inhibitory effects of 3H-CGP12177 binding by β-blockers after washout of the membranes with pretreated with drugs were studied according to the method described previously.14) The membranes were preincubated with or without antagonists added for 30 min at 23°C. The concentration of drugs used for hearts and brains were boipindolol (10^-7 M), 18-502 (3.16 x 10^-9 M), 20-785 (3.16 x 10^-7 M), atenolol (10^-4 M), propranolol (3.16 x 10^-8 M), pindolol (10^-8 M), nadolol (10^-6 M), and celiprolol (10^-6 M), respectively. These concentrations of these β-blockers used here inhibited 30—40% of 3H-CGP12177 bindings to β-adrenoceptors in the membranes of rat hearts and brains. The preincubated membranes were washed 3 times in 60 mm Tris—HCl buffer and 20 mm MgCl2 (pH 7.4) by centrifugation and resuspension. The washed membranes were then assayed for the 3H-CGP12177 binding used condition mentioned above. The concentration of 3H-CGP12177 used was 0.4 nm. The membranes preincubated without antagonists were also assayed using the incubation conditions mentioned above.

Pharmacological Experiments Strips of right and left atria were obtained from male guinea pig, weighing 300—400 g, as described previously.1 The in vitro test positive chronotropic potencies, the rate of spontaneous contraction of the right atrium was recorded on an inking-writing oscillograph. To evaluate the inotropic effect, the left atrium was stimulated electrically by a square-wave stimulator (Nihon Koden SEN-3101) at a frequency of 1 Hz, with voltages 30% above the threshold. The contractile tension was recorded on an ink-writing oscillograph with a strain gauge transducer and a carrier amplifier. The bathing solution used was Krebs-Henseleit solution (containing, in mm: NaCl, 118; KCl, 4.7; CaCl2, 2.5; NaHCO3, 25.0; MgSO4.7H2O, 1.2; KH2PO4, 1.2; glucose, 12), which was aerated with a mixture of 95% O2 and 5% CO2. The temperature of the solution was maintained at 37±0.1°C. After the chronotropic effect of the right atria and the contractile response of the left atria to 1 μM isoprenaline used as an agonist were determined, the preparations were preincubated with β-blockers for 30 min at 37°C and were again exposed to isoprenaline at 30 min intervals until 240 min. The concentrations of β-blockers used here were boipindolol (10^-7 M), 18-502 (10^-8 M for inotropic action and 3.16 x 10^-9 M for chronotropic rate), 20-785 (10^-5 M), atenolol (10^-5 M), (+)propranolol (10^-6 M), pindolol (10^-7 M), nadolol (10^-6 M) and celiprolol (10^-6 M), and these concentrations of drugs induced both inotropic actions and heart rates of between approximately 60% and 70% inhibition. Identical concentrations of drugs for those experiments, except for 18-502, were used. The preparation was washed three times with drug-free Krebs-Henseleit solution. Exposure of the preparation to 1 μM isoprenaline was repeated at 30 min intervals until 240 min after removal of the drug.

The drugs used in the present study were dissolved in double distilled water.

**Drugs** Bopindolol, 19-502, 20-785 and pindolol were gifts from Sandoz Pharma AG, Basel, Switzerland. Atenolol (ICI pharma, Japan), (+)propranolol (ICI Pharma, Japan), nadolol (Dainippon Pharmaceuticals, Co., Ltd.) and celiprolol (Nippon Shinyaku Co., Ltd.) were kindly donated from each company, respectively. 3H-CGP12177 (50Ci/mmol) was purchased from New England Nuclear/Du Pont, Inc., Boston, MA, U.S.A.

**RESULTS**

Inhibition by boipindolol and other β-adrenoceptor antagonists on 3H-CGP12177 binding to rat heart and rat brain is shown in Tables 1 and 2. In addition, the reversibility of the inhibition of heart and brain 3H-CGP12177 binding by these drugs is also illustrated in Fig. 1. In the presence of each β-blocker tested here (without preincubation with drugs), the % of control in the specific 3H-CGP12177 bindings in the membrane preparations of rat hearts were between 25.7% and 39.1% (open columns in Fig. 1a). When membranes previously preincubated with boipindolol and other β-blockers tested here at the concentrations mentioned above for 30 min were washed extensively and subsequently assayed for 3H-CGP12177 binding, there was little recovery of specific 3H-CGP12177 binding in membranes treated with boipindolol and 18-502 (hatched columns in Fig. 1). In contrast, binding was significantly restored following pindolol treatment, and the % of specific bindings in the membranes of treatments with 20-785, atenolol, pro-

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Table 1. Inhibition by β-Adrenoceptor Antagonists on 3H-CGP12177 Binding to Rat Heart Membranes

<table>
<thead>
<tr>
<th>Drugs (n)</th>
<th>Drug conc. (m)</th>
<th>fmol/mg protein</th>
<th>% of control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Without drugs¹</td>
<td>With drugs²</td>
</tr>
<tr>
<td>Bopindolol (7)</td>
<td>10^-7</td>
<td>10.36 ± 1.79</td>
<td>15.13 ± 3.04</td>
</tr>
<tr>
<td>18-502 (7)</td>
<td>10^-8.5</td>
<td>4.95 ± 0.65</td>
<td>8.67 ± 1.74</td>
</tr>
<tr>
<td>20-785 (4)</td>
<td>10^-6.5</td>
<td>8.44 ± 0.58</td>
<td>10.84 ± 1.04</td>
</tr>
<tr>
<td>Atenolol (6)</td>
<td>10^-5</td>
<td>4.38 ± 0.84</td>
<td>9.80 ± 1.08***</td>
</tr>
<tr>
<td>(+)propranolol (6)</td>
<td>10^-7.5</td>
<td>5.74 ± 0.60</td>
<td>10.04 ± 0.81***</td>
</tr>
<tr>
<td>Pindolol (6)</td>
<td>10^-8</td>
<td>4.83 ± 0.62</td>
<td>12.11 ± 1.40***</td>
</tr>
<tr>
<td>Nadolol (6)</td>
<td>10^-7.5</td>
<td>4.85 ± 0.90</td>
<td>10.03 ± 2.14*</td>
</tr>
<tr>
<td>Celiprolol (6)</td>
<td>10^-6</td>
<td>6.22 ± 0.56</td>
<td>9.79 ± 0.74***</td>
</tr>
</tbody>
</table>

Data are the mean ± S.E. Values in parentheses represent number of experiments. * p < 0.05, ** p < 0.02, *** p < 0.01 vs. control (without drugs). a) Preincubation without drugs. b) Preincubation with drugs.
Table 2. Inhibition by β-Adrenoceptor Antagonists on $^3$H-CGP12177 Binding to Rat Brain Membranes

<table>
<thead>
<tr>
<th>Drugs (n)</th>
<th>Drug conc. (m)</th>
<th>fmol/mg protein</th>
<th>% of control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Without drugs&lt;sup&gt;a&lt;/sup&gt;</td>
<td>With drugs&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Without drugs&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Bopindolol (7)</td>
<td>$10^{-7}$</td>
<td>8.08 ± 0.69</td>
<td>17.11 ± 1.50&lt;sup&gt;***&lt;/sup&gt;</td>
</tr>
<tr>
<td>18-502 (7)</td>
<td>$10^{-4.5}$</td>
<td>8.64 ± 1.52</td>
<td>18.45 ± 2.47&lt;sup&gt;***&lt;/sup&gt;</td>
</tr>
<tr>
<td>20-785 (7)</td>
<td>$10^{-4.5}$</td>
<td>7.90 ± 0.80</td>
<td>24.04 ± 2.73&lt;sup&gt;***&lt;/sup&gt;</td>
</tr>
<tr>
<td>Atenolol (7)</td>
<td>$10^{-3}$</td>
<td>9.42 ± 1.25</td>
<td>26.74 ± 2.12&lt;sup&gt;***&lt;/sup&gt;</td>
</tr>
<tr>
<td>(±)Propranolol (6)</td>
<td>$10^{-3}$</td>
<td>10.16 ± 1.25</td>
<td>31.42 ± 2.85&lt;sup&gt;***&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pindolol (6)</td>
<td>$10^{-4}$</td>
<td>9.71 ± 1.43</td>
<td>26.27 ± 2.85&lt;sup&gt;***&lt;/sup&gt;</td>
</tr>
<tr>
<td>Nadolol (6)</td>
<td>$10^{-4}$</td>
<td>16.22 ± 0.85</td>
<td>31.91 ± 2.69&lt;sup&gt;***&lt;/sup&gt;</td>
</tr>
<tr>
<td>Celiprolol (7)</td>
<td>$10^{-6}$</td>
<td>10.89 ± 0.99</td>
<td>29.13 ± 1.45&lt;sup&gt;***&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data are the mean ± S.E. Values in parentheses represent number of experiments. * p < 0.05, ** p < 0.02, *** p < 0.01 vs. control (without drugs). <sup>a</sup> Incubation without drugs. <sup>b</sup> Preincubation with drugs.

Fig. 1. Assessment of the Slowly Dissociating Abilities of Each β-Antagonist by the Radioligand Binding Assay Method

Inhibitions by β-antagonists on $^3$H-CGP12177 binding to rat heart (a) and brain (b) membranes were determined. Open column: each β-blocker was added directly to the incubation medium mixture and the membrane fractions were incubated with $^3$H-CGP12177 in the presence of each β-blocker for 30 min at 23°C. Hatched column: the identical membrane fractions were preincubated with each β-blocker, and then they were washed as described in Materials and Methods. The washed membranes were then assayed for $^3$H-CGP12177 binding. The ordinate represents $^3$H-CGP12177 binding expressed as a percentage of the specific binding of 0.4 nM $^3$H-CGP12177 in the presence (open column) and absence (hatched column) of any antagonist. The concentrations of the β-blockers used in these experiments are described in Materials and Methods. Each column represents the mean ± S.E. of 4-7 experiments. * p < 0.05, ** p < 0.02, *** p < 0.01 vs. the corresponding values before washing (paired standard t test).

Fig. 2. Residual Inhibitory Effects of β-Blockers after Washout on Isoprenaline-Induced Contraction in the Left Atria

Thirty minutes after the addition of each β-blocker, the atria were washed three times with the normal buffer and then stimulated with isoprenaline. This cycle was repeated at 30 min intervals. Ordinate: % inhibition of isoprenaline-induced isotropic contractions. Symbols represent bopindolol (●), 18-502 (▲), 20-785 (■), atenolol (○), propranolol (△), pindolol (□), nadolol (●), celiprolol (◇). Each value is the mean ± S.E. of 4-7 experiments. * p < 0.05, ** p < 0.02, *** p < 0.01 vs. inhibition (%) of each drug at 0 min (paired standard t test).

Fig. 3. Residual Inhibitory Effects of β-Blockers after Washout on Isoprenaline-Induced Heart Rate in the Right Atria

Thirty minutes after the addition of each β-blocker, the atria were washed three times with the normal buffer and then stimulated with isoprenaline. This cycle was repeated at 30 min intervals. Ordinate: % inhibition of isoprenaline-induced chronotropic rates. Symbols represent bopindolol (●), 18-502 (▲), 20-785 (■), atenolol (○), propranolol (△), pindolol (□), nadolol (●), celiprolol (◇). Each value is the mean ± S.E. of 4-5 experiments. * p < 0.05, ** p < 0.02, *** p < 0.01 vs. inhibition (%) of each drug at 0 min (Paired standard t test).
pranolol, nadolol and celiprolol were 86.7, 78.8, 77.5, 82.3 and 79.9% of control, respectively.

On the other hand, the inhibitory bindings of $^3$H-CGP12177 by the pretreatment with pindolol, propranolol and 20-785 in the brain were recovered to the control levels, but 18-502 and bopindolol were not recovered to the control levels (Fig. 1b). In addition, those potencies of nadolol, atenolol and celiprolol were intermediate.

As depicted in Fig. 2, pretreatment with bopindolol, 18-502, 20-785, atenolol, (+)propranolol, pindolol, nadolol and celiprolol inhibited an isoproterenol-induced increase in the force of a contraction similarly, by almost 65—85%, in the left atria before washout. Although the time courses of isometric action recovery following the washout of these $\beta$-blockers are shown in Fig. 2, the isoproterenol-induced contraction did not recover during the 240 min period after treatment with bopindolol, 18-502 and (+)propranolol. 20-785, atenolol and nadolol were completely recovered to the control levels at 60 min, but pindolol and celiprolol were restored to approximately 75% of the control at 240 min after pretreatment with drugs. The inhibitory effects of these drugs on the chronotropic effects after repeated washout for 240 min are shown in Fig. 3. Some $\beta$-blockers (20-785, nadolol, celiprolol, atenolol and pindolol) were completely restored within 60—120 min after pretreatment with these drugs. As depicted in Fig. 4, there was a good correlation between the inhibition of $^3$H-CGP12177 binding to the heart membranes and the inhibition of the isoproterenol-induced isometric and chronotropic action after their removal, although such good correlation in the brain was not observed (Fig. 5).

**DISCUSSION**

Many investigators$^{3,5,9,10,16,17}$ have reported that bopindolol possesses a long duration of $\beta$-blocking action with a high affinity and a partial agonist activity to the $\beta$-adrenoceptors in volunteer subjects and animal models, suggesting that less frequent administration may be necessary than with most other $\beta$-blockers.$^{2,3,7}$ A variety of clinical studies show that this drug has long-lasting effects on blood pressure and heart rate and that bopindolol is about 10 times more potent than pindolol.$^5$ In
clinical trials, the administration of bopindolol sustained the reduction of the mean heart rate for 24 h after exercise, and several explanations for this phenomenon also indicated that the long duration of this drug may be a consequence of (1) the high potency of bopindolol, (2) the shallow slope of the plasma concentration-effect relationship, (3) the higher affinity of a metabolite drug (18-502) than that of the parent drug to the β-adrenoceptors, or (4) a "deep compartment" irreversible binding to the receptor, and (5) a small dissociation constant. Although 8 kinds of β-blockers in the present study were selected as standard non-selective β-blockers (±propranolol), a standard β₁-selective β-blocker (atenolol), long-lasting β-blockers (nadolol and celiprolol) and β-blocker possessing intrinsic sympathomimetic activity (ISA) (pindolol), the present study apparently showed that bopindolol maintained its inhibitory effects on HCGP12177 binding to rat heart and brain membranes, as well as its residual inhibitory inotropic and chronotropic effects, even though the membranes and tissues were washed out by the buffer solution. This inhibition was resisted to the removal of free drug by washing. Thus, the results obtained here imply that bopindolol could tightly bind to β-adrenoceptors in membranes, and suggest that the dissociation of bopindolol from bound β-adrenoceptors was definitely slow when compared to other β-blockers. This tightly bound bopindolol to β-adrenoceptors may affect the high affinity, flat plasma concentration, or the slowly reversible dissociating nature of binding to receptors proposed by some of the investigators mentioned above.

There is another explanation for the long action of bopindolol: a metabolic transformation of the parent compound into the active drug (18-502) can occur. Bopindolol is metabolized to hydrolyzed bopindolol (18-502), and this metabolite also has higher β-blocking action than that of bopindolol. This metabolite (18-502) also showed long-inhibitory inotropic and chronotropic effects on the aorta of guinea pig, although a stronger prolonged inotropic action than that of chronotropic action was observed. The residual inhibition of bopindolol in membrane fractions or in tissues pretreated with this drug may also depend on the potency of this metabolite. Conversely, another metabolite (20-785), which has a weaker β-blocking action than that of bopindolol and 18-502, showed rapid dissociation from β-adrenoceptors, although the persisting effects of bopindolol may be due to these two metabolites. This contribution of 20-785 to the long-duration of β-blocking action may, however, be minor. Thus, the only difference in chemical structure between 20-785 and bopindolol or 18-502 is the presence of a carboxylic acid ester (20-785) or a methyl substitute at the 2-position of an indole nucleus. Probably, these differences affect the potencies of dissociation of these chemicals from bound β-adrenoceptors, and only one metabolite (18-502) of bopindolol may contribute to the long-duration of β-blocking action. As 20-785, however, possesses mild β-blocking action, this metabolite may contribute to the extent of this β-blocking action of bopindolol.

The plasma elimination half-life of bopindolol is approximately 8 to 10 h, and β-blocking effects could persist for up to 96 h. Several investigators have reported that bromoacetylpropranolololentane and NHNPNE (N(2-hydroxy-3-(1-naphthoxy)-propyl)-N-bromoacetylnitroguenimine) were irreversible β-adrenoceptor antagonists. Terasaki et al. reported that the irreversible effects of aminobenzylpropranolol persisted during extensive washing of the atra until 17 h. Although the anti-hypertensive effects of bopindolol were sustained for 15—20 h, the blood pressure of spontaneously hypertensive rat (SHR) models eventually recovered to the control levels before drug treatments. Thus, it seems that bopindolol and 18-502 are slowly reversible β-adrenoceptor antagonists as suggested by Doggrel because these agents tend to recover their chronotropic actions inhibited after washout with the buffer solution presented here.

The potencies of the β-adrenoceptor antagonists tested here for dissociating from β-adrenoceptors could be divided into two groups. One group is readily reversible, such as 20-785, atenolol, nadolol, celiprolol, pindolol, and another group is bopindolol and 18-502, which are irreversible after the washout. In the present study, (±)propranolol and bopindolol also showed that the contractile responses of the electrically-driven guinea pig atra to isoproterenol were inhibited by these drugs at 120 min after washout, and these drugs were also able to depress the maximum response to isoproterenol (1 μM). It is generally considered that the important pharmacological effects of β-blockers include their membrane stabilizing activity in addition to β-blocking action. Recent evidence suggests that propranolol was able to depress the maximal responses of the electrically driven rat right ventricle to isoproterenol. This author also suggests that this depression by (±)propranolol may be due to its membrane stabilizing activity. In addition, bopindolol was also shown to have this membrane stabilizing action. Thus, depression of the maximum response to chronotropic rates by bopindolol, 18-502 and (±)propranolol may be due to this membrane stabilizing action, and these effects may also contribute to the persisting effect of these drugs. Although the lipid solubilities or bindings to proteins of these β-blockers can't be ruled out, further experiments will be needed for the clarification of these roles.

The present study proved that bopindolol is a long-acting drug using the radioligand binding assay method and a functional experiment. These results on hearts, obtained from these two types of experiments, showed a good correlation, but not the results in brains. The different results of the dissociation in hearts and brains may be due to different structures (different subtypes) and/or a different environment of β-adrenoceptors.

In conclusion, as (1) bopindolol was a slowly dissociating β-adrenoceptor antagonist compared to other β-blockers, and in addition, (2) its metabolite (18-502) possessed the same slowly reversible nature of bopindolol, these actions by bopindolol and 18-502 may contribute largely to its prolonged antihypertensive effects.
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9) Grevel J., Cardiovasc. Pharmacol., 8 (Suppl. 6), S16 (1986).