The Affinity of Bopindolol and Its Two Metabolites for a \(\beta_2\)-Adrenoceptor in the Bovine Mesenteric Artery

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Bopindolol and its two metabolites (18–502 and 20–785) were examined for their affinity to a \(\beta_2\)-adrenoceptor in the bovine mesenteric artery using the radioligand binding assay method with \(^{3}H\)CGP12177 as a radioligand. The Scatchard analysis of the data demonstrated a uniphasic plot with \(K_d\) and \(R_{max}\) values of 0.86±0.16 nM, and 13.34±1.11 fmol/mg protein, respectively. The \(pK_i\) values of bopindolol and its two metabolites for \(\beta_2\)-adrenoceptors in the bovine mesenteric artery were 7.70±0.13, 8.07±0.13, 8.20±0.24, respectively, with 20–785 showing the highest values among these drugs. The present findings indicate that the bovine mesenteric artery membrane is predominantly \(\beta_2\)-adrenoceptor tissue, and that bopindolol and its two metabolites were potent for \(\beta_2\)-adrenoceptors in the bovine mesenteric artery.

Keywords bopindolol; bopindolol metabolite; bovine mesenteric artery; \(\beta_2\)-adrenoceptor; \(^{3}H\)CGP12177; radioligand binding assay

Bopindolol (4-(benzoyloxy-3-tert-butylaminopropyl)-2-methylindole hydrogenmalonate) is a potent and non-selective \(\beta_2\)-adrenoceptor antagonist with partial agonist activity.\(^1\)–\(^4\) In vivo, this drug is very rapidly metabolized to 18–502 (4-(3-tert-butylamino-2-hydroxypropoxy)-2-methyl indole), which is further metabolized to 20–785 (4-(3-tert-butylamino-propoxy)-2-carboxyl indole).\(^5\) These two metabolites also exert strong \(\beta\)-blocking action.\(^6\) Our previous report\(^6\) described that bopindolol and its two metabolites demonstrated potent and non-selective \(\beta_2\)-adrenoceptor antagonist activities using \(\beta_2\)-predominant heart tissue and \(\beta_2\)-predominant trachea tissue for the assessment of various \(\beta\)-blockers by radioligand binding assay and pharmacological observations. In addition, we also reported that both \(\beta_1\) and \(\beta_2\)-adrenoceptors coexisted in the bovine heart and trachea.\(^7\) Thus, in the present study we measured the binding characteristics of \(^{3}H\)CGP12177 to \(\beta_2\)-adrenoceptors in the bovine mesenteric artery, and we assessed the displacement potency of bopindolol and its two metabolites for \(\beta_2\)-adrenoceptors by means of the radioligand binding assay.

MATERIALS AND METHODS

Drugs (–)–\(^{3}H\)CGP12177, (–)–4-(3-tert-butylaminono-2-hydroxypropoxy)-[5,7,\(^3\)H]benzimidazol-2-one hydrochloride (50 Ci/mmol), was obtained from Amersham Japan Co., Ltd. Bopindolol, 18-502 and 20-785 were kindly donated by Sandoz Pharmaceuticals, Ltd. The sources of other drugs used in this work were as follows: betaxolol from Mitsubishi Kasei Co., Ltd., and atenolol, \(\pm\)-propranolol, (–)-propranolol and ICI 118 551 from ICI Pharma, Japan, butoxamine from Burroughs Wellcome Co., U.S.A.

Preparation of the Membrane-Enriched Fractions
Membrane-enriched fractions from bovine mesenteric artery were prepared by the following method. Bovine mesenteric artery was obtained from a local abattoir. In the laboratory, the mesenteric arteries were freed of excess fat, frozen in liquid nitrogen, and stored at –80°C until use. The mesenteric arteries (approximately 2 g) were minced with a small pair of scissors in 20 ml of 10 mm Tris–HCl, 250 mm sucrose buffer (pH 7.4), and then homogenized in a Polytron homogenizer, twice for 10 s at setting 8. The homogenate was filtered through 4 layers of gauze. The filtrate was centrifuged at 1000 \(\times\) g for 10 min, and the supernatant was again centrifuged at 4000 \(\times\) g for 30 min. The resultant pellets were rinsed once with the incubation buffer (120 mm Tris–HCl, 40 mm MgCl\(_2\) pH 7.4) and homogenized with a Polytron homogenizer, twice for 10 s at setting 8, in 20 mm of the same buffer. The membrane-enriched fraction was frozen in liquid nitrogen, stored at –80°C and diluted to appropriate concentrations immediately before use. Protein concentrations were determined by the method of Lowry et al.,\(^8\) using bovine serum albumin as the standard.

Binding Assay
Saturation binding assays were carried out in duplicate with \(^{3}H\)CGP12177 in the presence (non-specific) and absence (total) of 10 \(\mu\)M (–)-propranolol. In brief, 0.25 ml of membrane suspension (0.15 mg of protein) was incubated for 45 min at 23°C with various concentrations (0.05–10 nm) of \(^{3}H\)CGP12177 in a total volume of 0.5 ml containing 60 mm Tris–HCl and 20 mm MgCl\(_2\) (pH 7.4). Displacement experiments were done in the presence of various concentrations of \(\beta_2\)-adrenoceptor antagonists in duplicates of 1 nm \(^{3}H\)CGP12177. At the end of the incubation period, the incubation medium was immediately filtered through a GF/C glass fiber filter by the method previously described.\(^9\) The radioactivity was counted by scintillation spectrometry (Packard 2200 Tri-Carb Scintillation Analyzer). The difference in mean values between the total and non-specific binding was taken as the specific binding.

Kinetic Analysis
All kinetic analyses were carried out on an NEC PC-9801 computer system that performs iterative non-linear regression as described previously.\(^10\) Estimates of the dissociation constants (\(K_d\)) and maximum
binding capacity ($B_{max}$) of specific $[^3]$HCGP12177 binding were obtained by Scatchard analysis. In this report, $K_i$ values obtained from displacement experiments are expressed as $pK_i$ values ($-\log K_i$).

RESULTS AND DISCUSSION

Figure 1 depicts the Scatchard plot of the $[^3]$HCGP12177 binding to the bovine mesenteric artery. When Scatchard analysis was carried out in the absence (total) and presence (non-specific binding) of 10 $\mu$m (-)-propranolol, the best fit curve for specific binding was uniphasic ($K_i = 0.86 \pm 0.16$ $nM$, $B_{max} = 13.34 \pm 1.11$ fmol/mg protein). Under these conditions, specific binding was approximately 50–70% of the total binding.

$\beta_2$-Adrenoceptors in most vessels contribute to vasodilation, suggesting that antagonists to $\beta_2$-adrenoceptors in vessels antagonize vasodilation, and this vascular receptor was classified as a $\beta_2$-adrenoceptor. The present study indicates that the bovine mesenteric artery also contains $\beta_2$-adrenoceptors using the radioligand binding assay method and that only one subtype exists in this tissue from Scatchard analysis. However, the $K_i$ and $B_{max}$ values obtained from the bovine mesenteric artery in the present study were as follows: 10 times higher in $K_i$ values and one-half lower in $B_{max}$ values compared to those obtained using rat mesenteric arteries. The degree of affinity of $[^3]$HCGP12177 could vary somewhat depending on the species. The reasons for these differences of affinity of $[^3]$HCGP12177 binding to $\beta_2$-adrenoceptors in the mesenteric arteries between rats and cattle may be due to a difference in receptor structure, including amino acid sequences and/or the lipid composition in the vicinity of $\beta_2$-adrenoceptors.

Table I summarizes the $pK_i$ values of several $\beta$-adrenoceptor antagonists for the $\beta_2$-adrenoceptor subtype in the bovine mesenteric artery. The $pK_i$ values of bopindolol, 18-502 and 20-785 were 7.70 ± 0.13, 8.07 ± 0.13 and 8.20 ± 0.24, respectively. As compared to the $\beta_2$-antagonistic potencies of these drugs indicated in our previous report, no significant difference in the $pK_i$ values of bopindolol (7.82), 18-502 (8.58) in rat heart and the $pA_2$ value of 20-785 (8.03) in guinea pig trachea were shown, but the $pA_2$ values of bopindolol (8.82), 18-502 (9.65) and the $pK_i$ value of 20-785 (7.16) disagreed with the $pK_i$ values of these drugs obtained in the present study. These results may be due to differences in the experimental method (radioligand binding assay or pharmacological method), species or tissues (bovine mesenteric artery or rat heart, guinea pig trachea). However, the present study showed high potencies of the $\beta_2$-antagonistic effect of bopindolol and its two metabolites, even in the bovine mesenteric arteries.

ICI 118 551, a $\beta_2$-selective antagonist, was the most potent (8.31 ± 0.22) in the present study. As this $pK_i$ value of ICI 118 551 was compared to those of bovine trachea ($pK_i$ 9.19), bovine heart ($pK_i$ 9.05), rat cerebral cortex ($pK_i$ 8.75) or rat heart ($pK_i$ 8.40) tested in our previous reports, the $pK_i$ values of this agent in bovine mesenteric arteries obtained here were almost same as those of the rat heart, but were lower than those of the bovine trachea, the bovine heart and the rat cerebral cortex.

In contrast, low $pK_i$ values of propranolol (6.83) and butoxamine (5.28) were obtained in the present study. As it is well known that propranolol is a non-selective $\beta$-blocker and butoxamine is a selective $\beta_2$-blocker, high $pK_i$ values of these drugs to $\beta_2$-adrenoceptors in blood vessels should be obtained if this tissue contains $\beta_2$-adrenoceptors. Harms et al. reported, however, that the values of $-\log ED_{50}$ of propranolol blockade to isoprenaline-induced bronchorelaxation was 6.93 mol/kg and that propranolol was displaced with lower broncho-

<table>
<thead>
<tr>
<th>Drug</th>
<th>$pK_i$ value</th>
<th>Slope factor</th>
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<tbody>
<tr>
<td>Bopindolol</td>
<td>7.70 ± 0.13</td>
<td>0.97 ± 0.05 (5)</td>
</tr>
<tr>
<td>18-502</td>
<td>8.07 ± 0.13</td>
<td>1.05 ± 0.08 (6)</td>
</tr>
<tr>
<td>20-785</td>
<td>8.20 ± 0.24</td>
<td>1.17 ± 0.13 (6)</td>
</tr>
<tr>
<td>ICI 118 551</td>
<td>8.31 ± 0.22</td>
<td>1.11 ± 0.09 (9)</td>
</tr>
<tr>
<td>dl-Prropranolol</td>
<td>6.83 ± 0.20</td>
<td>1.00 ± 0.12 (7)</td>
</tr>
<tr>
<td>Betaxolol</td>
<td>6.38 ± 0.09</td>
<td>1.01 ± 0.08 (8)</td>
</tr>
<tr>
<td>Atenol</td>
<td>5.35 ± 0.11</td>
<td>1.00 ± 0.08 (9)</td>
</tr>
<tr>
<td>Butoxamine</td>
<td>5.28 ± 0.15</td>
<td>0.97 ± 0.09 (6)</td>
</tr>
</tbody>
</table>

Values are mean ± S.E. Numbers in parentheses represent the number of experiments.
lectivity than cardioselectivity. In bovine test muscles, the IC$_{50}$ value of butoxamine was 1295 nM when [3H]dihydroalpranolol was used as a radioligand. The results indicate that these drugs may have low affinities to $\beta_2$-adrenoceptors in these smooth muscles. In addition, it is of interest that Wasserman et al. already proposed that the $\beta$-adrenoceptors in blood vessels might be a third subtype which might be different from the $\beta_1$- and $\beta_2$-adrenoceptors subdivided by Land et al. These authors pointed out the existence of three different $\beta$-adrenoceptor subtypes in dogs. Therefore, the present results imply that a different $\beta_2$-adrenoceptor (atypical receptor) from bronchial muscles may exist in the mesenteric arteries, so that this $\beta$-adrenoceptor in the mesenteric arteries is justly sensitive to ICI 118 551, but with a low affinity for propranolol and butoxamine. Further studies, however, are required to establish this.

In conclusion, the present findings suggest that the bovine mesenteric artery contains predominantly ICI 118 551 sensitive $\beta_2$-adrenoceptors, and bopindolol and its two metabolites also have marked $\beta_2$-antagonistic effects in the bovine mesenteric artery.

REFERENCES