A SIMPLE METHOD TO DETERMINE THE RATIO OF CARDIAC TO VASCULAR $\beta$-RECEPTOR BLOCKADE IN THE RAT IN VIVO*

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We have devised a simple method to determine the ratios of cardiac to vascular $\beta$-receptor blockade (C.V ratios) in normotensive rats anesthetized with pentobarbital, and applied it to six $\beta$-adrenergic blocking drugs ($\beta$-blockers). Mean blood pressure (BP) and heart rate (HR) were recorded. Isoproterenol (3 ng-100 $\mu$g/kg, i.v.) was cumulatively injected before and after the intravenous administration of $\beta$-blockers at two dose-levels. Thus shifts of the dose-response curves in BP and HR were observed. "In vivo $pA_2"$ values were calculated in molar doses per kg by Schild plots. The C.V ratio was calculated as antilogarithm of the difference of the mean in vivo $pA_2$ value in HR from that in BP. Those of atenolol, bometolol, bunutrolol, pindolol, propranolol, and sotalol were 9.77, 8.13, 2.95, 1.38, 1.20, and 0.71, respectively.

Keywords—$\beta$-adrenergic blocking drugs; atenolol; bometolol; bunutrolol; pindolol; propranolol; sotalol; cardioselectivity; vasodoselectivity; anesthetized rat

INTRODUCTION

Various methods have been used to determine cardioselectivity of $\beta$-adrenergic blocking drugs ($\beta$-blockers).1-6) To compare potencies and efficacies of $\beta_1$ to $\beta_2$-receptor blockade, varieties of cardiac preparations in vivo or in vitro, and of tracheal or vascular smooth muscle preparations in several species of experimental animals have been utilized.

When one considers the antihypertensive effect of $\beta$-blockers, the comparison must be made against vascular receptor blocking activity. Blockade of the cardiac $\beta_1$-receptors decreases cardiac output, at least acutely, while blockade of the vascular $\beta_2$-receptors increases total peripheral resistance. Thus cardiac and vascular $\beta$-receptors influence blood pressure in an opposite direction. Resistance vessels are the primary participant in regulation of total peripheral resistance. Clinical experiences indicated that both nonselective and cardioselective $\beta$-blockers have antihypertensive action.7) Therefore, blockade of the cardiac $\beta_1$-receptor is more important and may be a common mechanism of the antihypertensive action, although many other mechanisms may possibly participate in.

Considering the above factors, we have devised a simple method to determine the ratio of cardiac to vascular $\beta$-receptor blockade in anesthetized normotensive rats. The ratios were determined for six $\beta$-blockers. Rats were selected, because they have been used widely to determine the antihypertensive effect of drugs in preclinical studies. They are relatively small in size and various types of hypertension can be induced experimentally.

METHODS

Rat Preparation—Female HOS®: Donryu rats of 10—12 weeks of age, weighing 210—260 g, were anesthetized with pentobarbital sodium (55 mg/kg, i.p.). The trachea was intubated, and the animal was allowed to breath spontaneously. The left femoral vein and the right femoral artery were cannulated for intravenous injection and

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FIG. 1. *A Typical Example of Heart Rate (HR) and Blood Pressure (BP) Records Used to Obtain Dose-Response Curves to Isoproterenol before and after the Treatment with α-Adrenergic Blocking Drugs in an Anesthetized Rat*

![Graph showing HR and BP responses to isoproterenol and propranolol.](image)

control
---
isoproterenol (μg/kg, i.v.)

propranolol
---
(300 μg/kg, i.v.)

treated (after 15 min)
---
isoproterenol (μg/kg, i.v.)

1 min

FIG. 2. *Cumulative Log Dose-Response (DR) Curves of Isoproterenol on Heart Rate (HR) and Blood Pressure (BP) without the Treatment of β-Blockers*

Between the two procedures for obtaining a DR curve, 0.9% NaCl solution (1.0 ml/kg, i.v.) was given. Each point represents mean ± S.E. in seven rats.

○ — ○ : control, ● — ● : saline.

![Graph showing cumulative log dose-response curves.](image)

\[
\frac{\Delta \text{HR}}{\Delta \text{HR}_{\text{max}}} = \%
\]

(DR curve 1: n = 7)

\[
\frac{\Delta \text{BP}}{\Delta \text{BP}_{\text{max}}} = \%
\]

(DR curve 2: n = 7)
blood pressure (BP) recording, respectively. Heparin sodium (200 U/kg, i.v.) was used as an anticoagulant. Mean arterial BP was recorded by an electronic system (CP-01, Century Technology; RP-5, and RTG-4008, Nihon Kohden). Heart rate (HR) was counted from electrocardiogram (lead I) by a cardiotachometer system (RB-5, KJ-5, and KJT-4008, Nihon Kohden). BP and HR were controlled at 100–125 mmHg and 325–400 beats/min, respectively, by supplemental pentobarbital as needed throughout the experiment. Rectal temperature was maintained at 37±0.2°C by adjusting the distance of an electric lamp to the rat.

**Determination of Dose-Response Curves** — Dose-response (DR) curves were constructed from responses in HR and BP to isoproterenol. dl-

Isoproterenol was injected at about 45 sec intervals in a volume of 0.5 ml/kg (Fig. 1). The venous cannula was flushed with 0.05 ml of 0.9% NaCl solution after each injection. Doses of isoproterenol were cumulatively increased from 3 ng/kg until the maximal responses in HR and BP were obtained. The maximal dose was 3 or 10 μg/kg. Whole procedures were completed within 5 min. Changes of HR and BP expressed as percent of the maximum changes (ΔHR/ΔHR, ΔBP/ΔBP) were used as the responses.

When HR and BP had recovered to the basal values, a β-blocker was administered intravenously in one min in a volume of 1.0 ml/kg. DR curves were obtained again after 15 min. The procedures were completed within 7 min. Two doses were

![Graph showing changes in HR and BP after intravenous injection of β-blockers](image)

**FIG. 3. Changes in Heart Rate (HR) and Blood Pressure (BP) after Intravenous Injection of β-Blockers**

Mean values in seven rats each. Vertical bars indicate S.E. Abbreviations: ATL, atenolol; BML, bometolol; BNL, bunitrolol; PDL, pindolol; PPL, propranolol; and STL, sotalol.
used for each β-blocker, but only one dose was tested in a rat. The first and second DR curves agreed well with each other unless the rat was treated with β-blockers (Fig. 2).

**Calculation** — In vivo pA₂ values in HR and BP responses were obtained by Schild plot as reported previously⁵.⁶ following the *in vitro* method. In vivo pA₂ means negative log of the molar dose per kg of an antagonist which produces the agonist dose ratio of two. They were expressed as pA₂ (HR) and pA₂ (BP), respectively. Dose ratios were determined at 50% of the maximal responses on a pair of DR curves in each rat. A least square regression line was drawn on a graph of log (dose ratio of isoproterenol - 1) versus negative log of dose (mol/kg) of a β-blocker (Schild plot). If action of the antagonist was simply competitive, the slope of this regression line becomes an unity. Potency ratios of β-blockers to propranolol were obtained as the antilog of differences in *in vivo* pA₂ values. Ratio of cardiac to vascular β-receptor blockade (C.V. ratio) was calculated by the following equation as the index of cardioselectivity:

\[
\text{C.V. ratio} = \text{antilog} \left[ pA_2 (\text{HR}) - pA_2 (\text{BP}) \right]
\]

**Drugs** — The following drugs from the suppliers listed were used: *dl*-isoproterenol hydrochloride (Sigma), atenolol (free base) (ICI Pharma), bethanolol hydrochloride monohydrate (Oruka Pharmaceutical), bunitrolol hydrochloride (Boehringer Sohn), pindolol (free base) (Sandoz), *dl*-propranolol hydrochloride (ICI Pharma), and sotalol hydrochloride (Bristol). The doses referred to free bases.

**RESULTS**

Treatments with β-blockers changed base line in HR and BP (Fig. 3): Atenolol, bethanolol, and propranolol lowered HR and BP in a dose-dependent fashion. Lowering of HR by sotalol was also dose-dependent, but decrease in BP was equal at the two doses (1.0 and 3.0 mg/kg). Bunitrolol and

**FIG. 4. Effect of Atenolol (ATL) on the Dose-Response Curves of Isoproterenol on Heart Rate (HR) and Blood Pressure (BP)**

Each point represents mean ± S.E. in seven rats.

〇 — ●: control to ATL 100 μg/kg, ● — ●: ATL 100 μg/kg, △ — △: control to ATL 300 μg/kg, ▲ — ▲: ATL 300 μg/kg.
**C.V Ratio of β-Blockers**

**FIG. 5. Effect of Propranolol (PPL)**

Details are the same as in Fig. 4.

- ○ ○ : control to PPL 100 μg/kg
- ● ● : PPL 100 μg/kg
- △ △ : control to PPL 300 μg/kg
- ▲ ▲ : PPL 300 μg/kg

**FIG. 6. Effect of Sotalol (STL)**

Details are the same as in Fig. 4.

- ○ ○ : control to STL 100 μg/kg
- ● ● : STL 100 μg/kg
- △ △ : control to STL 300 μg/kg
- ▲ ▲ : STL 300 μg/kg
pindolol increased HR and decreased BP in a dose-dependent manner, suggesting that they have an intrinsic sympathomimetic action. These changes in HR and BP did not influence the construction of DR curves, when changes in percent of the maxima were used as the responses. The absolute values of the maximal responses in HR and BP were about the same before and after the treatment of β-blockers.

Cumulative log DR curves of isoproterenol in HR and BP before and after the treatment of β-blockers were constructed in each of seven rats. Those of atenolol, propranolol, and sotalol are shown in Fig. 4-6. Bunitrolol, pindolol, propranolol, and sotalol shifted the DR curves to right in both HR and BP, suggesting that they inhibit the related receptors competitively. Atenolol and bometolol produced marked shifts of DR curves in HR, suggesting that the drugs inhibit the cardiac receptors selectively and competitively.

Analyses of the results on seven β-blockers are summarized in Table I. From in vivo pA₂, the order of potency in inhibiting isoproterenol induced tachycardia was pindolol > bunitrolol > atenolol, bometolol, propranolol > sotalol. Pindolol and bunitrolol were more than 10 times as potent as propranolol. The order of potency in inhibiting isoproterenol induced vasodepression was pindolol > bunitrolol > propranolol > sotalol, bometolol and atenolol. Slope values for prananolol in HR and BP were near unity, which coincided with those obtained previously in vivo.²,8²

C.V ratios of atenolol and bometolol were 9.77 and 8.13, respectively, indicating that these drugs are cardioselective. Bunitrolol, pindolol, propranolol, and sotalol were relatively nonselective, because C.V ratios ranged 2.95 to 0.71. Their respective cardioselectivities were in the order listed. C.V ratio of sotalol was less than 1.0, which meant that it is rather vasculoselective.

**TABLE I. Effects of β-Adrenergic Blocking Drugs on Heart Rate, Blood Pressure and C:V Ratio**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Heart rate</th>
<th>Blood pressure</th>
<th>C:V Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pA₂</td>
<td>Slope</td>
<td>RP</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atenol</td>
<td>7.07 (6.71-7.43)</td>
<td>0.85 (0.77-0.93)</td>
<td>1.41</td>
</tr>
<tr>
<td>Bometolol</td>
<td>6.97 (6.73-7.21)</td>
<td>0.93 (0.76-1.10)</td>
<td>1.12</td>
</tr>
<tr>
<td>Bunitrolol</td>
<td>7.99 (7.49-8.49)</td>
<td>0.72 (0.57-0.87)</td>
<td>11.80</td>
</tr>
<tr>
<td>Pindolol</td>
<td>8.14 (7.82-8.46)</td>
<td>0.77 (0.57-0.97)</td>
<td>16.60</td>
</tr>
<tr>
<td>Propranolol</td>
<td>6.92 (6.69-7.15)</td>
<td>1.03 (0.79-1.27)</td>
<td>1.00</td>
</tr>
<tr>
<td>Sotalol</td>
<td>5.97 (5.75-6.19)</td>
<td>0.89 (0.71-1.07)</td>
<td>0.11</td>
</tr>
</tbody>
</table>

a) In vivo pA₂
b) Slope of the regression line.
c) Relative potency (propranolol = 1).
d) Ratios of cardiac to vascular β-receptor blockade.
The numbers in parentheses are 95% confidence limits (n = 7).
DISCUSSION

We have devised a simple method to determine the ratio of cardiac to vascular β-receptor blockade (C:V ratio) in anesthetized normotensive rats. Cardiodeselectivity of a β-blocker was determined against its ability to inhibit β2-receptors in peripheral vasculature. Full DR curves of isoproterenol in HR and BP were obtained simultaneously by injecting isoproterenol cumulatively at about 45 sec intervals until the responses reached a maximum. The entire procedures to obtain a single DR curve can be completed within 5 to 7 min. The effect of a β-blocker administered intravenously should be stable for at least 7 min during which a DR curve was obtained. Dose ratios can be determined at 50% of the maximal responses, yielding reproducible and accurate results.

Various methods have been used to determine β2-receptor blockade. Tracheal preparations are not entirely satisfactory when one considers an antihypertensive action. Preparation of the larger arteries is not the choice, because they are not resistance vessels and participate minimally in regulation of total peripheral resistance. Perfusion of a regional vascular tree is one of the choices, but simultaneous determination of cardiodeselectivity is usually difficult.

We have selected inhibition of the increase in HR and the decrease in BP induced with isoproterenol as the indices of cardiac and vascular receptor blockades, respectively. Modification of HR or BP neural reflexes should be minimal, because cervical vagosympathectomy had no effect on DR curves for either HR or BP in preliminary experiments in our laboratory. Deep anesthesia with pentobarbital probably inhibited these reflexes. Vaugahn et al. reported the similar results. Although effect of isoproterenol on BP might be attenuated due to the increase in cardiac output, we obtained dose-dependent BP decreases, indicating that changes in BP represented mainly the effects on vascular β2-receptors. Receptors initiating HR changes are not solely responsible for changes in cardiac output. The same is true for receptors related to changes in BP and total peripheral resistance. In spite of the above limitations, changes in HR and BP approximated well the effects of agonists and antagonists on cardiac and vascular β-receptors, respectively. Therefore, C:V ratio obtained in this study is a good index for cardiodeselectivity of β-blockers. The same approximation has been used by many investigators. The ratios of atenolol and bometolol clearly indicate their cardiodeselectivity. It was also seen that bunitolol is slightly cardiodeselective.

We have calculated C:V ratio as described in this paper form the reported results in in vivo studies. The ratios of propranolol in the dog were 0.49, 0.23, 0.36, and 0.17, indicating that propranolol is relatively vasculoselective. It was 1.30 in the cat. C:V ratios of atenolol was calculated as 17.0 in the dog, and 14.0 in the cat, respectively. The differences from the present studies may be due to differences in species, and experimental methods.

Both nonselective and cardiodeselective β-blockers showed antihypertensive effect clinically. Therefore, the decrease in cardiac output caused by blockade of the cardiac β1-receptors may be a common mechanism in initiating the antihypertensive action. However, we do not know the exact mechanisms involved leading to a decrease in total peripheral resistances as the final hemodynamic change after treatment with β-blockers. It is significant to compare blocking activities of cardiac β1-receptors against those of vascular β2-receptors, because blockade of the vascular receptors increase total peripheral resistance. However, further studies are necessary to correlate the C:V ratio with antihypertensive efficacy of β-blockers.

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REFERENCES


