Hypotensive Effect of Bunitrolol at Low Plasma Concentrations in Conscious, Unrestrained Spontaneously Hypertensive Rats

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Abstract—Effects of bunitrolol on mean arterial pressure (MAP) and heart rate (HR) were studied in conscious, unrestrained spontaneously hypertensive (SHR) rats at rest and during handling stress. Propranolol was employed as a reference drug. Plasma drug concentrations were determined to relate with the cardiovascular effects of the drugs. Bunitrolol produced a tachycardia for the first 2 hr and a significant reduction in resting MAP at 3 and 4 hr after the oral dose (5 mg/kg) when plasma bunitrolol concentration was less than 10 ng/ml, indicating the difference between cardiac and vascular beta adrenoceptors in sensitivity to intrinsic sympathomimetic action or direct vasodilator action. Propranolol (5 mg/kg) produced no discernible effects on resting MAP and HR. Stress-induced tachycardia was significantly inhibited by both drugs throughout the experiment, while significant inhibition of hypertensive response was observed only at 4 hr after the treatment. Both bunitrolol and propranolol were rapidly absorbed from the gastrointestinal tract. Plasma half-life of these drugs were almost the same values of around 2 hr. These results indicate that dose size, plasma concentrations, and procedures and the timing of blood pressure measurement are the important factors to be considered when the antihypertensive effect of beta-blockers is studied in SHR rats.

Bunitrolol is a potent non-selective beta-adrenoceptor blocking drug (beta-blocker) with an intrinsic sympathomimetic activity (ISA) (1-3). The beta-blocking potency of bunitrolol is higher than that of propranolol (2, 4). Bunitrolol also has vasodilator activity which is ascribed to ISA and a direct action on the peripheral vasculature (2). As a result of vasodilation, hypotension has been observed after the administration of bunitrolol in anesthetized dogs and rats (1, 2, 4). Although the hypotensive effect of bunitrolol is recognized in hypertensive patients during exercise (6), it is generally difficult to demonstrate the reduction of blood pressure in hypertensive animals (6). In three different models of experimentally hypertensive rats, Hasegawa et al. (7) failed to demonstrate any antihypertensive effect of bunitrolol even after chronic treatment, while Himori et al. (8) reported a hypotension in conscious, renal hypertensive dogs after a single oral dose of bunitrolol.

The purposes of the present study were to relate plasma drug concentrations with cardiovascular responses to bunitrolol and to elucidate the mechanism of its hypotensive action in conscious, freely moving spontaneously hypertensive (SHR) rats. The results of our study indicate that the hypotensive effect of bunitrolol in SHR rats can be observed at low plasma concentrations.

Materials and Methods

Procedures on animals: Twelve-week-old male SHR rats (F44) from the colony of the Department of Pharmacology, Jichi Medical School, were used in this study. Two days before the experiment, rats were anesthetized with pentobarbital sodium, 50 mg/kg, i.p., and an indwelling catheter (PE 10) was inserted into the lower abdominal aorta through the left femoral artery (9). Two electrodes were subcutaneously implanted in
the left chest and the right shoulder and exteriorized at the nape of the neck (10). After surgery, rats were placed in an individual plastic cage.

Before the experiment, the animals were fasted overnight and allowed free access to drinking water. A 0.3-ml reference blood sample was drawn from the arterial catheter into a syringe moistened with 15% EDTA-2NH₄. A pressure transducer (CP-01, Century Technology) was connected to the arterial catheter for the recording of mean arterial pressure (MAP) in conscious, freely moving rats using an electronic system (Type 1238, Sanei). Heart rate (HR) was monitored by attaching the electrodes to a cardiotachometer system (Type 1205D and 5149, Sanei). Resting MAP and HR were recorded in the home cage after the accommodation of the rat to the experimental conditions. Rats were considered to be in a resting condition when they were immobile for at least 2 min. Handling stress was loaded by lifting the rat by the tail so that the front paws could barely touch the floor of the cage and holding in that position for 30 sec (11). A marked rise in MAP and tachycardia were induced by this procedure. Handling stress was repeated intermittently until stable cardiovascular responses were observed. Then bunitrolol (5 mg/kg), propranolol (5 mg/kg), or the vehicle (1 ml/kg) was administered by gavage. At various times after the administration of drugs, MAP and HR were determined at rest and during handling stress. Blood samples for the determination of plasma drug concentrations were drawn from the arterial catheter as described above (0.2 ml at 5, 15, 30, 60 and 120 min, and 0.5 ml at 240 min after the dose of the drugs or the vehicle). Plasma was separated by centrifugation and stored at −20°C until assay was performed.

Radioimmunoassay for bunitrolol: The antiserum against d/-bunitrolol was obtained from a rabbit immunized with the conjugate of bunitrolol-hemiglutarate with bovine serum albumin (12). The specificity of the antiserum is shown in Table 1. The antiserum bound with both d- and l-bunitrolol to the same degree. The metabolites of bunitrolol, 4-hydroxybunitrolol and o-nitrolo-phenoxylactic acid did not show any appreciable cross-reactivities with the antiserum. Radioimmunoassay for d/-bunitrolol with a limit of sensitivity of 100 pg/tube was developed, using the antiserum and [³H]bunitrolol (28 Ci/mmol). Plasma samples were diluted at 1:10 with 0.1 M tris-HCl buffer, pH 7.4. To the tubes (10x75 mm) containing the antiserum, [³H]bunitrolol (42.1 pg, about 9,000 dpm) and a 20–50 µl portion of the diluted plasma sample was added, and then tris-HCl buffer was added to bring the total volume up to 0.5 ml. The tubes were incubated at 4°C overnight. Antibody-bound [³H]bunitrolol was separated from the free drug by the ammonium sulfate method (13). The radioactivity in the precipitate was determined by the liquid scintillation method. The values for serum samples were read directly from the standard curve.

Radioimmunoassay for propranolol: Plasma concentration of d/-propranolol was determined by radioimmunoassay utilizing the antiserum specific for d/-propranolol and [³H]propranolol as reported by Kawashima et al. (14).

Calculations and statistical analyses: Results were expressed as means±S.E.M. in the table and figures. Significance of difference among the means was ascertained.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>ID50 (ng)</th>
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<tbody>
<tr>
<td>d/-Bunitrolol</td>
<td>1.21</td>
</tr>
<tr>
<td>l-Bunitrolol</td>
<td>1.38</td>
</tr>
<tr>
<td>d-Bunitrolol</td>
<td>1.04</td>
</tr>
<tr>
<td>4-Hydroxybunitrolol</td>
<td>1762</td>
</tr>
<tr>
<td>o-Nitrolo-phenoxylactic acid</td>
<td>1242</td>
</tr>
</tbody>
</table>

ID50 refers to the amount of the compound required to produce 50% inhibition of [³H]bunitrolol-antibody complex formation.
using Fisher's least significance difference method.

Plasma drug concentration-time data were fitted to a two-compartment open kinetic model using the computer program TOPFIT (15). Kinetic parameters were calculated by standard procedures.

Drugs: dl-Bunitrolol hydrochloride, 4-hydroxybunitrolol, o-nitrilophenoxyactic acid and the optical isomers of bunitrolol were supplied by Nippon Boehringer Ingelheim Co., Ltd. dl-Propranolol hydrochloride was purchased from Sigma. Drugs were dissolved in distilled water. The doses were expressed as the base.

Results

Effects of bunitrolol and propranolol on resting MAP and HR: Resting MAP and HR were monitored for 4 hr after the administration of drugs (Fig. 1). Bunitrolol produced a gradual fall in MAP which was significant at 3 and 4 hr after the dose compared to the vehicle treated control (P<0.05). Resting HR was significantly increased by bunitrolol for the first 2 hr (P<0.05 or 0.01), and it returned to the control level by 3 hr after the administration. In propranolol treated rats, MAP increased slightly for the first 30 min and then decreased gradually. However, there was no significant difference between propranolol and vehicle treated animals in MAP. Propranolol (5 mg/kg, p.o.) did not attenuate resting HR in conscious, freely moving SHR rats.

Effects of bunitrolol and propranolol on stress-induced cardiovascular responses: Handling stress induced a rise in MAP by 41±2 mmHg and an increase in HR by 117±5 beats/min from the resting state (n=24). Percent changes in pressor and tachycardiac responses to handling stress after the administration of drugs or vehicle are shown in Fig. 2. Only at 4 hr after the

![Graph showing HR and MAP changes](image-url)
Fig. 2. Changes in tachycardiac and hypertensive responses to handling stress after the oral administration of bunitrolol (n=8) and propranolol (n=8) at the dose of 5 mg/kg or the vehicle in spontaneously hypertensive rats. The data are expressed as a percent of the values before the treatments. Each point represents the mean±S.E.M. *P<0.05, **P<0.01, compared to the vehicle.

Fig. 3. Semilogarithmic plot of plasma concentrations-time curves after oral administration of bunitrolol (5 mg/kg, n=8) and propranolol (5 mg/kg, n=8) in conscious, unrestrained spontaneously hypertensive rats. Each point represents the mean±S.E.M.
administration, both bunitrolol and propranolol produced a significant suppression of hypertensive response to stress compared to the vehicle (P<0.01 and 0.05, respectively). A significant suppression of tachycardiac response to stress was observed within 15 min after the administration of bunitrolol and propranolol (P<0.01 or 0.05), and this persisted throughout the experimental period of 4 hr. Bunitrolol produced a slightly greater and longer suppression in the tachycardiac response than propranolol.

### Disposition of bunitrolol and propranolol:
Plasma concentration-time curves for bunitrolol and propranolol after the oral administration (5 mg/kg) in SHR rats are shown in Fig. 3. Both bunitrolol and propranolol were rapidly absorbed from the gastrointestinal tract. Further kinetic analysis (Table 2) revealed a faster absorption of bunitrolol than propranolol. Propranolol had a higher plasma concentration and a larger AUC than bunitrolol. There was no difference between bunitrolol and propranolol in the value for plasma half-life at the β-phase.

### Table 2. Pharmacokinetic parameters in conscious, unrestrained spontaneously hypertensive rats after oral administration

<table>
<thead>
<tr>
<th>Drugs</th>
<th>(T_{\text{max}}) (hr)</th>
<th>(C_{\text{max}}) (ng/ml)</th>
<th>AUC (ng hr/ml)</th>
<th>(T_{1/2}\beta) (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bunitrolol (8)</td>
<td>0.14±0.03</td>
<td>190±35</td>
<td>142±22</td>
<td>1.80±0.19</td>
</tr>
<tr>
<td>6 mg/kg, p.o.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Propranolol (8)</td>
<td>0.21±0.02</td>
<td>366±80</td>
<td>373±62</td>
<td>1.77±0.22</td>
</tr>
</tbody>
</table>

Values given are means±S.E.M. Number of animals is shown in the parenthesis. Abbreviations used are: \(T_{\text{max}}\), time to reach maximum plasma concentration; \(C_{\text{max}}\), maximum plasma concentration; AUC, area under the concentration in the plasma versus time curve; \(T_{1/2}\beta\), half-life of the β-phase.

**Discussion**
ISA and direct action on the vascular smooth muscle are considered to be responsible for the vasodilator and acute hypotensive effect of bunitrolol in anesthetized animals (2). In conscious, freely moving SHR rats, bunitrolol produced a gradual fall in resting MAP accompanied by an initial increase in HR. After the subsidence of tachycardia by 3 hr after the administration of bunitrolol, a significant decrease in resting MAP was observed compared to the vehicle treated control. The time courses of hypotensive response to bunitrolol and plasma drug concentrations indicate the difference between cardiac and vascular beta-adrenoceptors in sensitivity to ISA or direct vasodilator action. At higher plasma concentrations, bunitrolol appears to stimulate both cardiac and vascular beta-adrenoceptors through ISA leading to the increase in cardiac output, and thus obtund its hypotensive effect. At lower plasma concentrations, bunitrolol may only stimulate vascular beta-adrenoceptors or exert vasodilator action, and produce an apparent hypotension. An alternative possibility is that accumulation of bunitrolol to the active sites which may exist in deep compartments is required to develop a hypotensive effect, and it takes some time to produce a significant fall in MAP. Similar results have been observed in SHR rats after the administration of oxprenolol, a beta-adrenoceptor antagonist with ISA (16).

Propranolol did not attenuate resting MAP after a single dose. This is consistent with the previous report (10). Thus, it appears to be difficult to demonstrate a hypotensive effect of propranolol in acute study using SHR rats. Furthermore, propranolol produced no effect on resting HR. These results indicate that the sympathetic tone to the heart is minimal in SHR rats under resting conditions.

During handling stress, both bunitrolol and propranolol significantly inhibited the tachycardiac response throughout the experiment. The degree of suppression roughly correlated with plasma drug concentrations. On the other hand, a significant decrease in hypertensive response to stress was observed only at 4 hr after the administration of the drugs. The data in the present study and
others (10, 16) show that suppression of stress-induced hypertensive response by beta-blockers can be observed at certain times after the administration in SHR rats. The mechanism of this effect is under investigation. It is well recognized that suppression of cardiac function by beta-blockers is not likely related to the attenuation of hypertensive response to stress (10, 16).

The failure to observe antihypertensive effects of bunitrolol in hypertensive rats in the previous study (7) could be due to the employment of the higher dose (50 mg/kg per day, p.o.) or the use of an indirect method for the measurement of blood pressure. Stressful stimuli such as heating and restraining associated with indirect blood pressure determination have been reported to exaggerate the degree of blood pressure elevation (17) and mask the hypertensive action of beta-blockers, especially at higher plasma concentrations (16).

Plasma half-life ($T_{1/2a}$) of bunitrolol and propranolol were almost the same value of around 2 hr in SHR rats. Bunitrolol seems to be more susceptible to the first-pass hepatic extraction than propranolol. After a single oral dose, propranolol showed much higher plasma concentrations and more than 2.6 times larger AUC value than bunitrolol. However, the degree of the suppression of tachycardiac response to handling stress was comparable between the two drugs or somewhat greater in rats treated with bunitrolol. This could be due to the higher potency of bunitrolol in beta-blocking activity than propranolol (1, 2, 4).

In summary, bunitrolol produced an initial increase in resting HR and a significant decrease in resting MAP at 3 and 4 hr after a single oral dose of 5 mg/kg in SHR rats. ISA or the vasodilator action of bunitrolol appears to be responsible for these acute hypertensive effects. The same dose of propranolol did not produce any discernible effect on resting MAP and HR. Both beta-blockers suppressed hypertensive response to handling stress only at 4 hr after the administration, while tachycardiac response was suppressed throughout the experiment. Dose size, plasma drug concentrations, and procedures and the timing of blood pressure measurement are the important factors to be considered when antihypertensive effect of beta-blockers is studied in experimentally hypertensive rats.

References


13 Farr, R.S.: A quantitative immunochemical measure of the primary interaction between I*-BSA and antibody. J. Infect. Dis. 103, 239–262 (1958)


