EFFECTS OF 1-(2-CHLORO-4-HYDROXYPHENYL)-t-BUTYLAMINOETHANOL (HOKU-81), A NEW BRONCHODILATOR, ON ISOLATED TRACHEA AND ATRIA OF GUINEA PIG

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Abstract—Effects of 1-(2-chloro-4-hydroxyphenyl)-2-t-butylaminoethanol hydrochloride (HOKU-81), one of the metabolites of tulobuterol, on isolated trachea and atria of guinea pigs were compared with those of various bronchodilators. All test drugs abolished the resting tone of tracheal muscle completely. The potencies of test drugs which induced relaxation were in the order of: trimetoquinol>HOKU-81>isoproterenol>salbutamol>terbutaline>tulobuterol>metaproterenol>clorprenaline. In acetylcholine-, histamine or potassium-stimulated preparations, the intrinsic activities of 2-chlorophenyl derivatives were less than those of 3,4- or 3,5-dihydroxyphenyl derivatives and that of HOKU-81, 2-chloro-4-hydroxyphenyl derivative, lay between two groups. HOKU-81 showed weak positive chronotropic and inotropic actions and the potency ratio of HOKU-81 to isoproterenol was about 3/100 and less than 3/1000, respectively. Both chronotropic and inotropic actions of 2-chlorophenyl derivatives, including HOKU-81, were weak and the inotropic actions of drugs with N-t-butylamino radical were weaker than chronotropic actions. Effects of test drugs on trachea and atria were antagonized by propranolol $1 \times 10^{-6}$ or $1 \times 10^{-7}$ M. HOKU-81 appears to be a potent and selective $\beta_2$-stimulant with a slight inotropic action.

Since Konzett (1) reported that isoproterenol was a potent bronchodilator, various $\beta$-stimulants have been used as antiasthmatic agents (2). These $\beta$-stimulants, however, induce cardistimulation as well as bronchodilation. According to the hypothesis of Lands et al. (3), adrenergic $\beta$-receptors are classified into two groups: $\beta_1$-receptors which mediate cardistimulation and $\beta_2$-receptors which mediate relaxation of tracheal smooth muscle. Therefore, drugs possessing a selective activity on $\beta_1$-receptors are effective as bronchodilators and various $\beta_2$-receptor selective drugs have been developed (4-13). Kubo et al. (14) reported that tulobuterol was a bronchodilator possessing a high selectivity for $\beta_2$-receptors.

In the present study we examined the pharmacological effects and selectivity of 1-(2-chloro-4-hydroxyphenyl)-2-t-butylaminoethanol (HOKU-81), one of the metabolites of tulobuterol obtained from rat urine (15), on isolated trachea and atria of guinea pigs.

MATERIALS AND METHODS

Animals: Male Hartley guinea pigs weighing 300-400 g were used.

Isolated tracheal muscle preparation: Guinea pigs were stunned by a blow on the head, the trachea was removed and placed in Tyrode's solution at 37°C. The connective tissue was removed. The trachea was cut into 12 strips of equal width and a pair of tracheal chain
preparations was made by connecting every 6 pieces of strips (16). The preparations were suspended in aerated Tyrode's solution at 37°C and changes in tone of tracheal muscle were recorded isotonically on a smoked drum. The preparations were loaded to give a resting tension of 0.7 g. Drugs were administered after the preparations were equilibrated for more than 1.5 hr.

**Isolated right atrial preparation:** Guinea pigs were stunned by a blow on the head and the right atrium was excised from the isolated heart and suspended in an organ bath containing 30 ml of Krebs-Henseleit's solution equilibrated with 95% O₂ + 5% CO₂ and kept at 30°C. Spontaneous contractions of the preparations were recorded isometrically on a pen-oscillograph by means of a force-displacement transducer (Nihon Kohden SB-1T) and a carrier amplifier (Nihon Kohden RP-3). The rate of spontaneous beating was recorded simultaneously by a cardiotachometer (Nihon Kohden RM-5). Resting tension was adjusted to 0.5 g and the experiments were begun after the rate and amplitude of spontaneous contraction reached a steady state.

**Drugs:** 1-(2-Chloro-4-hydroxyphenyl)-2-tert-butyraminoethanol hydrochloride (HOKU-81), tulobuterol hydrochloride, clorprenaline hydrochloride, metaproterenol hemisulfate, terbutaline hemisulfate, salbutamol hemisulfate and trimetoquinol hydrochloride were kindly donated by Hokuriku Seiyaku Co., Ltd. Other drugs used were: acetylcholine chloride (Daiichi Seiyaku Co.), histamine dihydrochloride (Wako Pure Chemical Ind.), l-isoproterenol hydrochloride (Nikken Kagaku Co.), propranolol hydrochloride (Sumitomo Chemical Co.) and potassium chloride (guaranteed reagent). All these drugs were dissolved with 0.9% NaCl immediately before experiment. The test drugs were added cumulatively to the organ bath using a 0.1 ml syringe.

Tyrode's solution included: NaCl 137, KCl 2.7, CaCl₂ 1.8, MgCl₂ 1.1, NaH₂PO₄ 0.5, NaHCO₃ 11.9 and D-glucose 5.6 mM. The composition of Krebs-Henseleit's solution was: NaCl 118, KCl 4.7, CaCl₂ 2.5, MgSO₄ 1.2, KH₂PO₄ 1.2, NaHCO₃ 25 and D-glucose 1.0 mM.

**Calculation of the dissociation constant (Kₐ) of propranolol-receptor complex:** The

![Fig. 1. Chemical structures of test drugs.](image-url)
dissociation constant of propranolol-receptor complex was calculated using the following equation (according to Furchgott (17)), in which \((B)\) is the concentration of propranolol and \(R\) is equipotent-dose ratio of agonist before and after application of propranolol.

\[
K_B = \frac{(B)}{1 - \frac{1}{R}}
\]

RESULTS

Effects on the resting tone of isolated tracheal muscle preparations: All test drugs decreased the resting tone of tracheal muscle and the levels of maximum relaxation induced by test drugs were comparable to those in preparations suspended in calcium-free medium. In a few preparations, the maximum responses to tulobuterol and clorprenaline were smaller than the maximum responses to isoproterenol. Dose-response curves to test drugs for relaxation were expressed by percentages of the maximum response induced by each drug in each preparation (Fig. 2), and ED50 was calculated (Table 1). HOKU-81 was the most potent among the test drugs, except trimetoquinol and was about 10 and 1.3 times as potent as tulobuterol and isoproterenol, respectively.

Relaxations induced by test drugs were inhibited by propranolol at the concentration of \(1 \times 10^{-7}\) or \(1 \times 10^{-6}\) M and the dose-response curves of test drugs shifted to the right, except in the case of tulobuterol and clorprenaline (Table 2). This suggests that the relaxations induced by test drugs, except tulobuterol and clorprenaline, are due to the activation of adrenergic \(\beta\)-receptors. The reason why propranolol showed lesser activity against isoproterenol than other test drugs could not be determined.

The maximum response to tulobuterol was decreased to about 40% of the control and the relaxation induced by clorprenaline was completely inhibited by propranolol \(1 \times 10^{-7}\) M. The cumulative administration of tulobuterol and clorprenaline up to a concentration of

![Fig. 2. Dose-response curves to test drugs for relaxation of isolated trachea of guinea pigs. •—• HOKU-81, ▲—▲ tulobuterol, △—△ clorprenaline, ○—○ isoproterenol, □—□ metaproterenol, ■—■ terbutaline, ●—● salbutamol, ‡—‡ trimetoquinol. Standard errors, ranging from 0.5 to 8.0 in extreme cases \((n=6)\), are not shown.](image_url)
### Table 1. Bronchodilator and positive chronotropic actions of test drugs

<table>
<thead>
<tr>
<th>Test drug</th>
<th>Bronchodilator action ED50 (M)(^a)</th>
<th>Positive chronotropic action ED25% (M)(^b)</th>
<th>Selectivity for trachea ED 25% ED50</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOKU-81</td>
<td>3.2 × 10(^{-9})</td>
<td>7.9 × 10(^{-7})</td>
<td>247</td>
</tr>
<tr>
<td>Tulobutanol</td>
<td>3.1 × 10(^{-8})</td>
<td>7.4 × 10(^{-6})</td>
<td>239</td>
</tr>
<tr>
<td>Clorproprenaline</td>
<td>2.5 × 10(^{-7})</td>
<td>5.3 × 10(^{-6})</td>
<td>21</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>4.1 × 10(^{-9})</td>
<td>3.7 × 10(^{-8})</td>
<td>9</td>
</tr>
<tr>
<td>Metaproterenol</td>
<td>9.2 × 10(^{-8})</td>
<td>3.7 × 10(^{-7})</td>
<td>4</td>
</tr>
<tr>
<td>Terbutaline</td>
<td>2.4 × 10(^{-8})</td>
<td>7.4 × 10(^{-7})</td>
<td>31</td>
</tr>
<tr>
<td>Salbutamol</td>
<td>6.9 × 10(^{-9})</td>
<td>1.9 × 10(^{-7})</td>
<td>28</td>
</tr>
<tr>
<td>Trimetoquinol</td>
<td>1.1 × 10(^{-9})</td>
<td>2.4 × 10(^{-8})</td>
<td>22</td>
</tr>
</tbody>
</table>

Bronchodilator action and positive chronotropic action of test drugs were estimated graphically from Figs. 2 and 6, respectively.

\(a\) Dose which produced half the maximal response in isolated trachea of guinea pig.

\(b\) Dose which increased heart rate by 25% of the basal value.

### Table 2. Antagonistic effects of propranolol against test drugs in isolated trachea and right atria of guinea pigs

<table>
<thead>
<tr>
<th>Test drug</th>
<th>Propranolol (M)</th>
<th>n(^d)</th>
<th>Equipotent-dose ratio of test drug</th>
<th>Observed</th>
<th>Corrected</th>
<th>K&lt;sub&gt;B&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting tone of trachea</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HOKU-81</td>
<td>1.0 × 10(^{-7})</td>
<td>6</td>
<td>0.010 (0.0085–0.012)(^{b})</td>
<td>0.013(^{c})</td>
<td>1.3 × 10(^{-9})</td>
<td></td>
</tr>
<tr>
<td>Tulobutanol</td>
<td>3.2 × 10(^{-7})</td>
<td>6</td>
<td>0.015 (0.014–0.017)</td>
<td>0.017</td>
<td>5.5 × 10(^{-9})</td>
<td></td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>3.2 × 10(^{-7})</td>
<td>6</td>
<td>0.077 (0.061–0.097)</td>
<td>0.086</td>
<td>3.0 × 10(^{-8})</td>
<td></td>
</tr>
<tr>
<td>Metaproterenol</td>
<td>3.2 × 10(^{-7})</td>
<td>6</td>
<td>0.013 (0.0099–0.016)</td>
<td>0.0093</td>
<td>3.0 × 10(^{-9})</td>
<td></td>
</tr>
<tr>
<td>Terbutaline</td>
<td>3.2 × 10(^{-7})</td>
<td>6</td>
<td>0.016 (0.014–0.018)</td>
<td>0.011</td>
<td>3.4 × 10(^{-9})</td>
<td></td>
</tr>
<tr>
<td>Salbutamol</td>
<td>3.2 × 10(^{-7})</td>
<td>6</td>
<td>0.011 (0.0095–0.012)</td>
<td>0.0086</td>
<td>2.8 × 10(^{-9})</td>
<td></td>
</tr>
<tr>
<td>Trimetoquinol</td>
<td>3.2 × 10(^{-7})</td>
<td>6</td>
<td>0.015 (0.014–0.016)</td>
<td>0.018</td>
<td>5.8 × 10(^{-9})</td>
<td></td>
</tr>
<tr>
<td>Heart rate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HOKU-81</td>
<td>1.0 × 10(^{-7})</td>
<td>9</td>
<td>0.039 (0.012–0.091)</td>
<td>0.050</td>
<td>5.2 × 10(^{-9})</td>
<td></td>
</tr>
<tr>
<td>Clorproprenaline</td>
<td>1.0 × 10(^{-6})</td>
<td>3</td>
<td>0.016 (0.0039–0.065)</td>
<td>0.021</td>
<td>2.1 × 10(^{-8})</td>
<td></td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>1.0 × 10(^{-6})</td>
<td>4</td>
<td>0.081 (0.021–0.18)</td>
<td>0.11</td>
<td>1.3 × 10(^{-8})</td>
<td></td>
</tr>
<tr>
<td>Metaproterenol</td>
<td>1.0 × 10(^{-6})</td>
<td>9</td>
<td>0.0066 (0.0023–0.017)</td>
<td>0.0050</td>
<td>5.0 × 10(^{-9})</td>
<td></td>
</tr>
<tr>
<td>Terbutaline</td>
<td>1.0 × 10(^{-7})</td>
<td>3</td>
<td>0.047 (0.028–0.078)</td>
<td>0.046</td>
<td>4.8 × 10(^{-8})</td>
<td></td>
</tr>
<tr>
<td>Salbutamol</td>
<td>1.0 × 10(^{-6})</td>
<td>3</td>
<td>0.035 (0.012–0.072)</td>
<td>0.027</td>
<td>2.8 × 10(^{-9})</td>
<td></td>
</tr>
<tr>
<td>Trimetoquinol</td>
<td>1.0 × 10(^{-7})</td>
<td>3</td>
<td>0.026 (0.007–0.061)</td>
<td>0.0001</td>
<td>2.6 × 10(^{-9})</td>
<td></td>
</tr>
<tr>
<td>Contractile force</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>1.0 × 10(^{-6})</td>
<td>9</td>
<td>0.070 (0.019–0.21)</td>
<td>0.066</td>
<td>7.1 × 10(^{-8})</td>
<td></td>
</tr>
<tr>
<td>Metaproterenol</td>
<td>1.0 × 10(^{-6})</td>
<td>3</td>
<td>0.21 (0.16–0.28)</td>
<td>0.20</td>
<td>2.5 × 10(^{-7})</td>
<td></td>
</tr>
<tr>
<td>Trimetoquinol</td>
<td>1.0 × 10(^{-7})</td>
<td>3</td>
<td>0.65 (0.20–1.72)</td>
<td>0.25</td>
<td>3.3 × 10(^{-8})</td>
<td></td>
</tr>
</tbody>
</table>

\(a\) Number of experiments. \(b\) Confidence limit (p=0.05). \(c\) Corrected by control value. \(d\) Decrease in maximum response was observed.
over $1 \times 10^{-4}$ and $1.8 \times 10^{-5}$ M, respectively, induced contraction in resting and isoproterenol- or propranolol-treated preparations and these contractions were not inhibited by phentolamine $1 \times 10^{-6}$ M, atropine $1 \times 10^{-5}$ M or diphenhydramine $1 \times 10^{-5}$ M.

Effects on acetylcholine-, histamine- or potassium-treated tracheal muscle preparations: The isolated tracheal muscle preparations pretreated with ED80 of acetylcholine ($1 \times 10^{-4}$ M), histamine ($3.2 \times 10^{-5}$ M) or potassium chloride ($3 \times 10^{-2}$ M) were relaxed dose-dependently by the test drugs. Dose-response curves to the test drugs for relaxation were expressed as percentages of the maximum relaxation level obtained with isoproterenol $1 \times 10^{-7}$ M before

Fig. 3. Dose-response curves to test drugs for relaxation of acetylcholine-treated isolated trachea of guinea pigs. • - • HOKU-81, ▲ - ▲ tulobuterol, △ - △ clorprenaline, ○ - ○ isoproterenol, □ - □ metaproterenol, ■ - ■ terbutaline, ● - ● salbutamol, + - + trimetoquinol. Responses are expressed by percentages of the maximum relaxation obtained with isoproterenol $1 \times 10^{-7}$ M before application of acetylcholine $1 \times 10^{-4}$ M. Standard errors, ranging between 0.4 and 7.1 (n=4-6), are not shown.

Fig. 4. Dose-response curves to test drug for relaxation of histamine-treated isolated trachea of guinea pigs. • - • HOKU-81, ▲ - ▲ tulobuterol, △ - △ clorprenaline, ○ - ○ isoproterenol, □ - □ metaproterenol, ■ - ■ terbutaline, ● - ● salbutamol, + - + trimetoquinol. Responses are expressed by percentages of the maximum relaxation obtained with isoproterenol $1 \times 10^{-7}$ M before application of histamine $3.2 \times 10^{-5}$ M. Standard errors, ranging between 0.3 and 7.9 (n=6) except metaproterenol (0.8-10.4, n=6), are not shown.
application of the stimulant in each preparation (Figs. 3, 4 and 5). The levels of maximum relaxation induced by the test drugs in histamine-treated preparations were comparable to those in potassium-treated preparations. Isoproterenol was the most potent in both preparations. Trimetoquinol, salbutamol, metaproterenol and terbutaline produced about 80-90% relaxation of the maximum relaxation obtained with isoproterenol. The maximum relaxation produced by HOKU-81 was smaller than the relaxation induced by the above mentioned drugs. Tulobuterol and clorprenaline induced little relaxation. The relaxations induced by the test drugs in acetylcholine-treated preparations were weaker than those in histamine- or potassium-treated preparations. The maximum relaxations by isoproterenol, metaproterenol and terbutaline which produced the stronger relaxation were only about 60, 50 and 45% of the maximum relaxation obtained with isoproterenol before application of acetylcholine.

Effects on right atria of guinea pigs: Dose-response curves for positive chronotropic and inotropic actions of test drugs on isolated right atria of guinea pigs are shown in Figs. 6 and 7, respectively. In these figures, responses are expressed by percentages of the basal values. Isoproterenol had the greatest effect regarding the inotropic action, while trimetoquinol was the most effective regarding the chronotropic action.

In the positive chronotropic action, the maximum responses induced by test drugs, except tulobuterol and clorprenaline, were over 80% of the maximum response to isoproterenol. The maximum responses to tulobuterol and clorprenaline were about 50% of the responses induced by isoproterenol. When compared at doses which increased heart rate by 25% (ED 25%), trimetoquinol and isoproterenol were the most potent, and were followed by salbutamol, metaproterenol, terbutaline, HOKU-81, clorprenaline and tulobuterol in decreasing order of potency. HOKU-81 was about 3/100 as potent as isoproterenol. In the positive inotropic action, the maximum responses induced by metapro-
terenol and trimetoquinol were comparable to those induced by isoproterenol. The maximum responses induced by other test drugs were only 50% of the responses induced by isoproterenol.

**FIG. 6.** Cumulative dose-response curves to test drugs for positive chronotropic action on isolated right atria of guinea pigs. • • HOKU-81, ▲ ▲ tulobuterol, △ △ clorprenaline, ○ ○ isoproterenol, □ □ metaproterenol, ■ ■ terbutaline, ● ● salbutamol, + + trimetoquinol. Standard errors, ranging between 0.7 and 8.7 (n=5-9), are not shown.

**FIG. 7.** Cumulative dose-response curves to test drugs for positive inotropic action on isolated right atria of guinea pigs. • • HOKU-81, ▲ ▲ tulobuterol, △ △ clorprenaline, ○ ○ isoproterenol, □ □ metaproterenol, ■ ■ terbutaline, ● ● salbutamol, + + trimetoquinol. Standard errors, ranging between 0.3 and 6.2 (n=5-9), are not shown.

terenol and trimetoquinol were comparable to those induced by isoproterenol. The maximum responses induced by other test drugs were only 50% of the responses induced by isoproterenol.

**Antagonistic effect of propranolol in right atrial preparation of guinea pigs:** The influences of propranolol on positive chronotropic and inotropic actions of test drugs on isolated right atria were examined. However, reproducibilities of effects of test drugs on the same atria were not so satisfactory that R values could be corrected with an equipotent-dose ratio of the test drug in the control experiment when this test drug was applied repeatedly on the same preparation without propranolol. The positive chronotropic actions of clorprenaline and tulobuterol were completely inhibited by propranolol $1 \times 10^{-6}$ M and the maximum response of tulobuterol was significantly decreased by propranolol $1 \times 10^{-7}$ and $1 \times 10^{-8}$ M. The dose-response curves of other test drugs were shifted to the right. In a few cases,
maximum responses of some test drugs were decreased by propranolol. However, since
the differences in maximum responses were not significant, corrected R values and dis-
sociation constant (K_b) of propranolol-receptor complex were calculated from these results
(Table 2).

The positive inotropic actions of salbutamol, clorprenaline and tulobuterol were
abolished and those of HOKU-81 and terbutaline were inhibited to less than 15% by pro-
pranolol 1x10^-7 M. Thus, R values for these test drugs could not be calculated.

**DISCUSSION**

HOKU-81 is a metabolite of tulobuterol obtained from rat urine of which the 4th
position of benzene ring is hydroxylated. This compound is presumed to be an adrenergic
β-receptor stimulant similar to its parent compound. Therefore, the effects of HOKU-81
on isolated trachea and atria of guinea pigs were compared with those of various broncho-
dilators. HOKU-81 dose-dependently reduced the resting tone of tracheal muscle, but
did not affect the tone of acetylcholine-, histamine- or potassium-treated preparations. In
isolated right atria, weak positive chronotropic and inotropic actions of HOKU-81 were
observed. These effects of HOKU-81 on resting trachea and right atria were inhibited by
propranolol. This indicates that effects of HOKU-81 are mediated via adrenergic β-
receptors in both preparations. The ratio of the positive chronotropic action to the in-
hibitory effect on resting tone of tracheal muscle was taken as an index of selectivity for
β_2-receptors (Table 1). Selectivity for β_2-receptors of HOKU-81 was equal to that of
tulobuterol and was higher than the selectivity seen with the other test drugs.

Some discussion should be made concerning why maximum responses induced by tulo-
buterol, clorprenaline or HOKU-81 were smaller than those induced by other test drugs.
Since the activities of drugs with the chloro-substituted ring were weak, higher concen-
trations of drugs were necessary to induce the relaxation of tracheal muscle. But, clorpren-
aline induced the contraction of tracheal muscle at a higher concentration (Kasuya et al.:
personal communication). We also found that higher concentrations of clorprenaline and
tulobuterol produced contractions of resting, isoproterenol-treated and propranolol-treated
preparations. If the contraction was elicited before the maximum relaxation was obtained
by cumulative administration of a test drug, the real maximum relaxation could not be
obtained. This may explain why maximum responses induced by tulobuterol or clorpren-
aline were smaller than the maximum responses induced by more potent drugs, especially
in the preparations stimulated by acetylcholine, histamine or potassium chloride.

The bronchodilators used in this study are structurally related to HOKU-81 with respect
to having the moieties: chloro-substituted benzene ring, hydroxy-substituted benzene ring
and/or t-butyl group as N-substituent. The intrinsic activities of test drugs to induce
maximum relaxation were in the order of (I) isoproterenol, trimetoquinol, salbutamol, ter-
buterine and metaproterenol>(II) HOKU-81>(III) tulobuterol and clorprenaline. This
classification is consistent with the classification based on chemical structure: group-I con-
tains drugs with the hydroxy-substituted ring, group-II contains a drug with the chloro- and
hydroxy-substituted ring and group-III contains drugs with the chloro-substituted ring.

Van den Brink (18) proposed a model to explain the various types of functional antagonism. According to his hypothesis the drugs with the hydroxy-substituted ring could induce a greater inhibitory stimulus than those with the chloro-substituted ring and stimulus-inducing activity of the drug with chlorohydroxy-substituted ring lay between the two groups. Moreover, it was also estimated that excitatory stimulus caused by histamine may be comparable to that caused by KCl and smaller than the stimulus caused by acetylcholine.

However, it is questionable that the structure-activity relationship estimated from the present study in vitro can be established by in vivo experiments. Kubo et al. (14) confirmed the potent inhibitory effect of tulobuterol on the bronchoconstriction induced by acetylcholine or histamine in vivo.

Figure 8 shows the correlation between percent increases in contractile force and percent increases in heart rate at the same concentration of test drugs. Both actions of drugs with chloro-substituted ring were weak. The other test drugs can be classified into two groups. The one group includes the drugs indicated by the closed symbols. These drugs are N-t-butylamino derivatives and showed stronger positive chronotropic action than positive inotropic action. The remaining drugs indicated by the open symbols were those having the isopropylaminoethyl chain and showed higher potencies in both actions.

The chronotropic and inotropic actions of test drugs were significantly inhibited by propranolol. The dissociation constant \( (K_B) \) of propranolol-receptor complex against positive inotropic action lay between \( 3.3 \times 10^{-8} \) M and \( 2.5 \times 10^{-7} \) M. However, since Farmer et al. (19) reported that trimetoquinol was not a pure \( \beta \)-stimulant and possessed a papaverine-like action, trimetoquinol should be omitted from the following discussion. The remaining \( K_B \) values, \( 7.1 \times 10^{-8} \) M for isoproterenol and \( 2.5 \times 10^{-7} \) M for metaproterenol, were larger than those obtained in the antagonistic effect of propranolol against positive chronotropic action of test drugs, \( 2.6 \times 10^{-9} - 4.8 \times 10^{-8} \) M. These results support Farmer's hypothesis (19) that \( \beta_1 \)-receptors for positive inotropic action differ from \( \beta_1 \)-receptors for positive chronotropic action.

Acknowledgement: The authors are indebted to Hokuriku Seiyaku Co., Ltd. for the generous supply of test drugs.
REFERENCES


