CARDIOVASCULAR BETA-ADRENERGIC RECEPTOR BLOCKING ACTIVITIES OF 1-(7-INDENYLOXY)-3-ISOPROPYLAMINOPROPAINE-2-OL HYDROCHLORIDE (YB-2) AND ITS OPTICAL ISOMERS

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Abstract—The cardiovascular beta-adrenergic receptor blocking activities of YB-2 and its optical isomers were investigated in isolated guinea pig atria and anesthetized dogs, and compared with that of propranolol. In isolated guinea pig atria, the positive inotropic and chronotropic responses to isoproterenol were competitively antagonized by these agents, and the order of beta-adrenergic blockade was as follows: I-YB-2 > dl-YB-2 > propranolol > d-YB-2. In anesthetized dogs, the effects of isoproterenol and cardiac sympathetic nerve stimulations on diastolic blood pressure, heart rate and dLVP/dt were competitively antagonized by the beta-adrenergic blocking agents. The blockade appears to be specific for beta-adrenergic receptors. In these experiments, cardioselective blocking activity was not observed with any agent employed. 1-YB-2 was 1.7-2 times more potent than dl-YB-2 and 50-100 times more potent than d-YB-2 in antagonism against the cardiovascular responses to isoproterenol and nerve stimulations. dl-YB-2 was 1.2-1.7 times more potent than propranolol. The results coincided with those in isolated guinea pig atria. In conclusion, YB-2 and its optical isomers appear to have a similar potency ratio for the blockade of cardiac beta-adrenergic receptor stimulations as is the case with propranolol.

Recently a series of indene derivatives were synthesized (1) and several compounds were found to have a beta-adrenergic blocking effect. Among them, 1-(7-indenyloxy)-3-isopropylaminopropane-2-ol hydrochloride (YB-2) has been reported to possess a potent beta-adrenergic receptor blocking activity with a mild intrinsic beta-sympathomimetic activity and a local anesthetic effect, and to be effective against ouabain-induced and epinephrine-induced arrhythmias (2-4).

It is now generally recognized that a beta-adrenergic receptor blocking activity is always greatest for the levo-rotatory isomer of any given compound. In 1963, Howe reported the l-isomer of pronethalol to be 40 times as active as the d-isomer in its ability to produce beta-adrenergic receptor blockade (5). Similarly the l-isomer of propranolol has been reported to be 60-100 times more potent than the d-isomer as a beta-adrenergic receptor blocking agent (6, 7).

The optically active isomers of YB-2 are now available and it has been reported that the l-isomer is the active form for a beta-adrenergic receptor blocking activity (4). In the present study, the cardiovascular beta-adrenergic receptor blocking activities of YB-2 and its l- and d-isomers were investigated in isolated guinea pig atria and anesthetized dogs and compared with those of dl-propranolol.
MATERIALS AND METHODS

Isolated guinea pig atria

Male guinea pigs, 250 to 350 g, were stunned by a blow on the head. The heart was removed quickly and placed in a dish containing Krebs-Henseleit solution (NaCl 6.92, KCl 0.35, CaCl2 0.28, MgSO4 0.15, NaHCO3 2.1, KH2PO4 0.16 and glucose 2.0 g liter) gassed with a mixture of 95% O2 and 5% CO2 at 25°C. The ventricles and other extraneous tissue were trimmed away leaving only the atria.

For the chronotropic measurements, the atria were suspended in a 25-ml organ bath containing Krebs-Henseleit solution gassed with a mixture of 95% O2 and 5% CO2 at 37°C. Heart rate was measured by means of a cardiotachometer (Nihon Kohden, RT-2) triggered by isometric contractions of the atria and recorded on a polygraph (Nihon Kohden, RM-150).

For the inotropic measurements, the left atrium was suspended in a 25-ml organ bath at 31°C. Supramaximal stimuli 3 msec in duration and 0.5 Hz in frequency were continuously applied to the left atrium using a square-wave stimulator (Nihon Kohden, MSE-20). The force of resulting contractions of the tissue was isometrically measured by means of a force displacement transducer (Nihon Kohden, ST-1) and recorded on a polygraph. The tissues were maintained at a diastolic tension of 0.7 g and allowed to adjust to the bath condition for 60 min prior to drug administration. Concentration-response curves for isoproterenol were determined before and after administration of each beta-adrenergic blocking agent. After control response to increasing cumulative doses of isoproterenol had been obtained, a beta-adrenergic blocking agent was added to the bath (exposure time 30 min) and concentration-response curve for isoproterenol was again determined. Quantitative evaluation of the antagonism against isoproterenol was performed by determinations of pA2 values (8).

Arterial blood pressure, heart rate and cardiac contractile force (dLVP/dt) in anesthetized dogs.

Adult male mongrel dogs, 11 to 22 kg, were anesthetized with sodium pentobarbital, 35 mg kg i.v. The trachea was cannulated and ventilation was maintained using a positive-pressure respirator (Natsume, KN-50) and room air. The cervical vagi were severed bilaterally. The chest was opened by a median sternotomy and the pericardium was incised. Myocardial contractility was measured by the maximal rate of rise of left ventricular pressure (dLVP/dt) which was recorded through a metal cannula inserted into the apical dimple and connected with a pressure transducer (Nihon Kohden, MPU-0.5), carrier amplifier (Nihon Kohden, RP-2), condenser (500PF, time constant 1 msec), DC amplifier (Nihon Kohden, AD3-2) and biophysical amplifier (Nihon Kohden, RB-2) in series. Heart rate was measured by means of a cardiotachometer (Nihon Kohden, RT-2) and arterial blood pressure was measured, in the right femoral artery, by means of a pressure transducer (Nihon Kohden, MPU-0.5). Recordings were made on a polygraph (Nihon Kohden, RM-150). Each drug solution was administered through a catheter inserted into the left cephalic vein and was flushed with 1 ml of 0.9% saline. The right cardioac-
The celerans nerve was exposed between the first intercostal space and ligated where the nerve emerged from the right stellate ganglion. A bipolar platinum electrode was placed distal to the ligature and the nerve was stimulated intermittently for periods of 20 sec, supra-maximal voltage, 10 Hz in frequency and 1 msec in duration, with a square-wave stimulator (Nihon Kohden, MSE-20). Isoproterenol in a dose of 0.2 μg/kg i.v. was injected as an agonist. When the responses to isoproterenol and nerve stimulation were uniform, the first dose of a beta-adrenergic blocking agent was injected i.v. After a 10-min period, the animal was re-challenged with isoproterenol and the nerve stimulation, followed by an injection of the second dose of the beta-adrenergic blocking agent. In this manner, a beta-adrenergic blocking agent was given in increasing doses at 20-min intervals: 0.01, 0.03, 0.1, 0.3 and 1 mg/kg i.v. for dl-propranolol and dl-YB-2; 0.003, 0.01, 0.03, 0.1 and 0.3 mg/kg i.v. for 1-YB-2; 0.1, 0.3, 1, 3 and 5 mg/kg i.v. for d-YB-2.

The dose producing a 50% inhibition of the responses of diastolic blood pressure, heart rate and dLVP/dt to isoproterenol and of the responses of heart rate and dLVP/dt to nerve stimulations was estimated in each experiment from straight lines obtained by plotting the inhibition percentage semi-logarithmically. Calcium chloride, 5 mg/kg i.v., was also injected before the first dose and after the last dose of each beta-adrenergic blocking agent.

**Drugs used**

1-(7-Indenyloxy)-3-isopropylaminopropane-2-ol hydrochloride (YB-2) and its d- and l-isomers (Yamanouchi Pharmaceutical Co.), dl-propranolol hydrochloride (Sumitomo Chemical Co.) and calcium chloride (Wako Pure Chemicals Co.) were freshly dissolved in 0.9% saline. l-Isoproterenol hydrochloride (Kaken Chemical Co.) was freshly dissolved in 0.9% saline containing 0.01% ascorbic acid as a preservative. All doses of the drugs are expressed in terms of the salt.

**RESULTS**

**Effects on isolated guinea pig atria**

In the electrically-driven left atria the concentration-response curves for positive inotropic effects induced by cumulative doses of isoproterenol, 10^{-9} to 10^{-6} M, showed a parallel shift to the right after administrations of dl-YB-2, 1-YB-2 and propranolol, 2.5 \times 10^{-7} M, and of d-YB-2, 2.5 \times 10^{-6} M, (N=6 for each agent) as shown in Fig. 1.

In the spontaneously-beating atria the concentration-response curves for positive chronotropic effects of isoproterenol, 10^{-9} to 10^{-6} M, also caused a parallel shift to the right after administrations of dl-YB-2, 1-YB-2 and propranolol, 3 \times 10^{-7} M, and of d-YB-2, 3 \times 10^{-6} M, (N=6 for each agent) as shown in Fig. 2. Such a parallel shift without a decrease in maximal response is an indication of competitive or surmountable antagonism. The pA2 values for dl-, d- and l-YB-2 in the left atria for inotropism and the atria for chronotropism and their relative potencies are summarized and compared with those of propranolol in Table 1. From these results a similar tendency in the inhibitory effects of four beta-adrenergic blocking agents on positive inotropic and chronotropic responses
to isoproterenol was observed. On the basis of $pA_2$ values the relative activities are as follows: $1$-$YB$-$2 > d$-$l$-$YB$-$2 > propranolol > $d$-$YB$-$2$. $1$-$YB$-$2$ was 1.5–2.2 times and 50–70 times more potent than $d$-$l$-$YB$-$2$ and $d$-$YB$-$2$, respectively. $d$-$l$-$YB$-$2$ was slightly more potent than propranolol.
Effects on arterial blood pressure, heart rate and dLVP/dt in anesthetized dogs

The effects of dl-, l- and d-YB-2 on the changes in diastolic blood pressure, heart rate and dLVP/dt induced by isoproterenol, 0.2 μg/kg i.v., and electrical stimulations of the cardiac sympathetic nerve were observed and compared with those of propranolol in anesthetized, vagotomized dogs. The effects of these beta-adrenergic blocking agents on the positive inotropic responses on calcium chloride, 5 mg/kg i.v., which was injected before and after administrations of the blocking agents, were also observed. The tracings from a typical experiment on dl-YB-2 are shown in Fig. 3. The effects of the beta-adrenergic

### Table 1. pA₂ values and relative potencies of beta-adrenergic blocking agents in isolated guine pig atria.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Atrial contraction pA₂ value (μM)</th>
<th>Relative potency</th>
<th>Atrial rate pA₂ value (μM)</th>
<th>Relative potency</th>
</tr>
</thead>
<tbody>
<tr>
<td>dl-YB-2</td>
<td>7.80±0.07</td>
<td>100</td>
<td>7.96±0.12</td>
<td>100</td>
</tr>
<tr>
<td>l-YB-2</td>
<td>8.14±0.10</td>
<td>220</td>
<td>8.17±0.09</td>
<td>148</td>
</tr>
<tr>
<td>d-YB-2</td>
<td>6.34±0.17</td>
<td>3</td>
<td>6.46±0.12</td>
<td>3</td>
</tr>
<tr>
<td>dl-Propranolol</td>
<td>7.77±0.12</td>
<td>93</td>
<td>7.86±0.05</td>
<td>98</td>
</tr>
</tbody>
</table>

Atrial contraction and rate show the values for isoproterenol-induced positive inotropic and chronotropic responses, respectively. Relative potency is expressed relative to pA₂ values for dl-YB-2=100.

**Fig. 3.** Effects of increasing doses of dl-YB-2 on the cardiovascular responses to isoproterenol, 0.2 μg/kg i.v., cardiac sympathetic nerve stimulations (ES) and calcium chloride, 5 mg/kg i.v., in the anesthetized, vagotomized dog.

Each recording shows systemic blood pressure (BP), heart rate (HR) and dLVP/dt. Arrows indicate points at which drugs were injected. Horizontal bars with ES indicate the period of nerve stimulation.
FIG. 4. Effects of beta-adrenergic blocking agents on the changes in diastolic blood pressure, heart rate and dLVP/dt produced by isoproterenol, 0.2 μg/kg i.v., and nerve stimulations in anesthetized, vagotomized dogs. White columns show the responses on isoproterenol and black ones the responses to nerve stimulations. The flat and pointed ends of the columns show the levels immediately before isoproterenol or nerve stimulations and the maximal responses to each treatment, respectively. Each column represents the averaged value from five separate experiments.

blocking agents on the fall in diastolic blood pressure and on the increases in heart rate and in dLVP/dt produced by isoproterenol and on the increases in heart rate and in dLVP/dt produced by nerve stimulations are shown in Fig. 4 as the averaged results from five dogs for each blocking agent. dl-YB-2, l-YB-2, d-YB-2 and propranolol caused a gradual diminution of the responses to isoproterenol and nerve stimulations. In the dose level used, however, l-YB-2 was most potent and d-YB-2 was least potent among these beta-adrenergic blocking agents. In the case of d-YB-2, an appreciable decline in the responses to isoproterenol was not elicited until the doses in excess of 1 mg/kg i.v. were given. Diastolic blood pressure was significantly lowered by the doses of d-YB-2, i.e. 3 or 5 mg/kg i.v., to produce a sufficient beta-adrenergic blockade. Heart rate and dLVP/dt were also lowered by all agents studied. No significant influence of each beta-adrenergic blocking agent on the positive inotropic responses to calcium chloride, 5 mg/kg i.v., was observed. Fig. 5 shows the dose-response curves for the inhibition of cardiovascular effects of isoproterenol (Fig. 5A) and nerve stimulations (Fig. 5B) by beta-adrenergic blocking agents. Table 2 shows their 50% inhibition doses (ED 50). The relative potencies calculated from ED 50 values (dl-YB-2:100) and the ratios of (ED 50 in heart rate)/(ED 50 in diastolic pressure) and of (ED 50 in dLVP/dt)/(ED 50 in diastolic pressure) as indications of the selectivity of action are shown in Table 3. There was no indication of any
FIG. 5. Dose-response relations for the effects of beta-adrenergic blocking agents on the responses of diastolic blood pressure (DBP), heart rate (HR) and dLVP/dt produced by isoproterenol, 0.2 μg/kg i.v., (A) and cardiac sympathetic nerve stimulations (B) in anesthetized, vagotomized dogs. Each point represents the mean±S.E. from five separate experiments.

TABLE 2. ED50 values of beta-adrenergic blocking agents in antagonizing the cardiovascular effects of isoproterenol, 0.2 μg/kg i.v., and cardiac sympathetic nerve stimulations in anesthetized, vagotomized dogs.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Diastolic blood pressure</th>
<th>Heart rate</th>
<th>dLVP/dt</th>
<th>Heart rate</th>
<th>dLVP/dt</th>
</tr>
</thead>
<tbody>
<tr>
<td>dl-YB-2</td>
<td>0.017±0.004</td>
<td>0.037±0.006</td>
<td>0.046±0.019</td>
<td>0.080±0.027</td>
<td>0.045±0.010</td>
</tr>
<tr>
<td>l-YB-2</td>
<td>0.009±0.002</td>
<td>0.019±0.004</td>
<td>0.026±0.008</td>
<td>0.042±0.010</td>
<td>0.026±0.014</td>
</tr>
<tr>
<td>d-YB-2</td>
<td>0.73±0.08</td>
<td>1.65±0.34</td>
<td>2.29±0.40</td>
<td>1.83±0.23</td>
<td>1.95±0.47</td>
</tr>
<tr>
<td>dl-Propranolol</td>
<td>0.022±0.004</td>
<td>0.042±0.007</td>
<td>0.078±0.017</td>
<td>0.090±0.010</td>
<td>0.076±0.010</td>
</tr>
</tbody>
</table>

Each value represents the mean±S.E. (mg/kg) from five separate experiments.
selectivity of action for either agent, since each antagonist was similarly effective on the depressor responses to isoproterenol and positive inotropic and chronotropic responses to isoproterenol and nerve stimulations.

From the results 1-YB-2 was 1.7-2 times and 50-100 times more potent than dl-YB-2 and d-YB-2, respectively. dl-YB-2 was 1.2-1.7 times more potent than propranolol. These results coincided with those obtained from the experiments in isolated guinea pig atria.

### DISCUSSION

YB-2 has been examined for its antagonistic activity on the responses to beta-adrenergic stimulations in vitro and in vivo test situations. Tachikawa and Takenaka reported that YB-2 was equipotent to propranolol or alprenolol in isolated guinea pig atrial and tracheal preparations and anesthetized dogs (4). In the present study a similar result was obtained from the experiments performed in isolated guinea pig atria: YB-2 was 1.2-1.7 times more potent than propranolol in antagonizing the responses to isoproterenol and cardiac sympathetic nerve stimulations in anesthetized dogs.

Observing the results of various beta-adrenergic agents it has been suggested that beta-adrenergic receptors may be divided into two sub-groups denoted beta-1 and beta-2 (9-12). It seems that YB-2, like propranolol, exhibits no cardioselectivity which has been claimed in the studies on practolol (13-15), H 64/52 (16) and M & B 17803 A (15, 17).

YB-2 and its optical isomers in the doses required to inhibit the positive inotropic and chronotropic responses to isoproterenol and cardiac sympathetic nerve stimulations failed to alter the inotropic response to calcium chloride. The finding appears to be similar to the result obtained from the study on isoproterenol-antagonism of propranolol and its optical isomers (18).

It has been pointed out that many possible potency ratios may exist so far as the ac-

### TABLE 3. Relative potency and selectivity of action of beta-adrenergic blocking agents on the cardiovascular effects of isoproterenol, 0.2 µg/kg i.v., and cardiac sympathetic nerve stimulations in anesthetized, vagotomized dogs.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Relative potency</th>
<th>Selectivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Relative potency</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Diastolic blood pressure</td>
<td>Heart rate</td>
</tr>
<tr>
<td>dl-YB-2</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>l-YB-2</td>
<td>188</td>
<td>195</td>
</tr>
<tr>
<td>d-YB-2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>dl-Propranolol</td>
<td>77</td>
<td>88</td>
</tr>
</tbody>
</table>

Relative potency is expressed relative to ED50 value for dl-YB-2 = 100. HR/DBP = (ED 50 in heart rate)/(ED 50 in diastolic blood pressure). (dLVP/dt)/DBP = (ED 50 in dLVP/dt)/(ED 50 in diastolic blood pressure).
tivities of the d- and l-isomers are concerned. The experiments in anesthetized dogs and cats showed that l-propranolol was 60–100 times more active than the d-isomer in blocking the inotropic, chronotropic and vasodepressor actions of isoproterenol (6). A similar ratio of the activity was found for the optical isomers of pronethalol (5). Tachikawa and Takenaka reported l-YB-2 to be 200 times more active than the d-isomer in antagonizing the positive inotropic response to isoproterenol in isolated guinea pig atria (4). In isolated rabbit atria, the l-isomer of propranolol was 1.7–1.9 times more potent than the racemate and 60–80 times more potent than the d-isomer in blocking isoproterenol-induced inotropic and chronotropic effects (18). Burrett estimated that d-propranolol was 50 times less active than dl-propranolol to isoproterenol-induced responses in heart rate, cardiac contractile force and blood pressure in anesthetized dogs, although the dose-response curves were not parallel (19). In conclusion, YB-2 and its optical isomers appear to have similar potency ratios in blocking cardiac beta-adrenergic receptor stimulations as is the case with propranolol.

Whitsitt and Lucchesi observed no significant difference in the doses of propranolol required to antagonize the pharmacological and neurogenic beta-adrenergic stimulations in both isolated rabbit atria and anesthetized dogs (18). In the present study, a similar tendency was observed not only in the doses of propranolol but in those of YB-2 and its optical isomers in both isolated guinea pig atria and anesthetized dogs.

In isolated guinea pig atria, the pA₂ values of all agents studied were slightly larger in blocking the chronotropic than the inotropic responses to cardiac sympathetic nerve stimulations in anesthetized dogs, while to isoproterenol all agents showed an opposite result. Whitsitt and Lucchesi reported that in both isolated rabbit atria and dog hearts the positive inotropic effects of beta-adrenergic stimulations were antagonized in a lower concentration of propranolol than that required to antagonize the positive chronotropic effects (18). Singh and Vaughan Williams also reported a similar result with d- and l-propranolol to block the isoproterenol-induced responses in isolated rabbit atrium and ventricular muscle preparations (20). The evidence so far accumulated remains, however, inadequate for drawing any conclusions regarding possible differences in the receptor mediating these effects.

REFERENCES


