Effects of Beta-Adrenergic Blocking Drug Pindolol (LB46) on Cardiac Fibers in Relation to Its Membrane Effects

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SUMMARY

Direct membrane effects and beta-adrenergic blocking action of pindolol were studied comparatively on isolated perfused cardiac fibers of dogs. In Purkinje fibers, the duration of transmembrane action potential decreased in concentrations higher than 0.5 mg/L. Maximum rate of depolarization also decreased in higher concentrations (5.0 mg/L or more). The functional refractory period decreased to a lesser degree compared with the decrease of the action potential duration. Nevertheless, slow-rising action potentials could not be abolished in premature responses, probably because of membrane depressant effects of this drug. In ventricular muscle fibers, these changes were minimum in the same range of concentrations. On the other hand, 0.05 mg/L of pindolol which was close to the therapeutic plasma concentrations and had no effect on action potentials of both Purkinje and ventricular muscle fibers, minimized the increase in automaticity of Purkinje fibers induced by 0.2 mg/L of isoproterenol. It is rationally speculated that the mechanism of antiarrhythmic action of pindolol is mainly due to its beta-adrenergic blocking action.

Additional Indexing Words:
Canine Purkinje fibers Ventricular muscle fibers Intracellular microelectrode Action potential duration Repolarization time dV/dt

It has been widely known clinically1)–3) and experimentally4)–6) that beta-adrenergic blocking agents have the depressant action against a variety of cardiac arrhythmias. The mechanism of the antiarrhythmic activity of these drugs has been explained by the virtue of beta-blocking action itself, by the non-specific membrane effects, or by a combination of the two. However, its latter action has become recently debatable, because clinical blood levels of beta-blocking agents have been proved to be much lower than their experimentally-used concentrations needed to exert the membrane effect.7)–9)
In the present experiment, the effects of pindolol (4-(2-hydroxy-3-isopropylaminopropoxy)-indole) which had been reported to have selective beta-blocking action and relatively weak membrane effects\(^{10,11}\) were studied electrophysiologically. We investigated its effect on the transmembrane action potential of canine Purkinje fibers and ventricular muscle fibers, as well as its effect on the response of Purkinje fibers to isoproterenol. Through these investigations, we tried to elucidate the mechanism of antiarrhythmic action of this drug.

**Methods**

Hearts were removed from mongrel dogs anesthetized with intravenous thiamylal sodium of approximately 30 mg/Kg, and the false tendon with attached ventricular muscle was dissected from the right ventricle. Preparations were then fixed in a tissue bath and were perfused with modified Tyrode's solution. Oxygenation was achieved by bubbling the perfusate in the tissue bath with 100% O\(_2\).

The chemical composition of Tyrode's solution used are as follows; NaCl 147, KCl 2.7, CaCl\(_2\) 1.8, MgCl\(_2\) 0.5, Dextrose 5.5, Na\(_2\)HPO\(_4\) 4.5, and NaH\(_2\)PO\(_4\) 1.5, in mM.

For the electrical stimulation of the specimen, rectangular pulses of 2 msec in duration and twice the diastolic threshold were delivered through the contiguous bipolar electrodes made of Teflon coated silver wires (0.2 mm in diameter), at a basic cycle length of 600 msec. Transmembrane action potentials were recorded through glass microelectrodes filled with 3 M KCl and having tip resistances of 10 to 30 megohms. Action potentials displayed on an oscilloscope through the amplifier were photographed on 35 mm film.

For the determination of the duration of action potential, time intervals were measured from the onset of a rapid depolarization phase to the points when the repolarization proceeded to 50% and 90%. The maximum rate of depolarization of the action potential was determined by electronic differentiation.

For the measurement of the refractory period, stimulating electrodes and a recording microelectrode were placed very close in distance less than 0.5 mm, and premature stimuli were given every 5 to 7 beats of basic stimuli through the same electrodes. The coupling interval between the driving and test stimuli was gradually prolonged and the interval at which the first premature response was elicited by the test stimuli was taken as the functional refractory period.

To determine the effects of the drug on action potential characteristics, the control features were first recorded. Then, keeping the recording microelectrode within the same cell, perfusion with test solution containing the lowest concentration of pindolol was started and maintained until the observed change in the action potential became stable (approximately 15 min). After the necessary measurements were carried out, experiments were repeated by changing the perfusate to that with higher concentration of the drug. In the experiments to evaluate the antagonistic effect of pindolol against isoproterenol, the control action potential was first recorded, then the changes that occurred with isoproterenol solution were
measured, and then the pindolol was added into the isoproterenol solution.

At the end of the experiment, the preparation was again perfused with control solution to confirm that there was no change in action potential characteristics before and after the experiment.

The concentration of pindolol used in the present experiment varied from 0.5 to 5.0 mg/L in most instances. Concentrations of 0.05 mg/L and 0.1 mg/L were also used in some cases in which the effect of pindolol in antagonism with isoproterenol was examined.

**RESULTS**

*Effects of pindolol on action potential characteristics and functional refractory period of Purkinje fibers*

Over a range of concentrations from 0.5 to 5.0 mg/L, pindolol caused dose-dependent change in the action potential configuration of Purkinje fibers (Table I and Fig. 1). In a concentration of 0.5 mg/L, 50% repolarization time, 90% repolarization time, and the functional refractory period were decreased, while little change were observed in the amplitude of action potential and the maximum rate of depolarization. The action potential duration was further decreased in a concentration of 1.0 mg/L and this tendency was more prominent in 50% repolarization time than in 90% repolarization time. The maximum rate of depolarization also showed slight decrease at this concentration. At a higher concentration of the drug (5.0 mg/L), 50% repolarization time decreased significantly, while little change was observed in the functional refractory period as compared with that in 1.0

| Table I. Effect of Pindolol on the Transmembrane Action Potentials of Purkinje Fibers |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                                 | Magnitude of Action Potential (mV) | Maximum Rate of Depolarization (V/sec), | 90% Repolarization Time (msec), | 50% Repolarization Time (msec), | Functional Refractory Period (msec), |
|                                 | n=6      | n=6             | n=6             | n=6             | n=5             |
| Control                         | 133.0    | 598.5           | 328.0           | 247.5           | 273.0           |
| Pindolol 0.5 mg/L              | 130.3    | 540.3           | 306.7           | 225.8           | 248.8           |
| Mean difference ±S.D.          | -2.7±9.0 | -58.2±64.5      | -21.3±10.4      | -21.7±11.8      | -24.2±6.7       |
| Pindolol 1.0 mg/L              | 126.7    | 508.6           | 298.5           | 215.8           | 239.0           |
| Mean difference ±S.D.          | -6.3±4.8 | -89.8±81.2      | -29.5±8.5       | -31.7±8.8       | -34.0±6.0       |
| Pindolol 5.0 mg/L              | 118.3    | 341.7           | 292.5           | 179.2           | 236.0           |
| Mean difference ±S.D.          | -14.7±9.0| -256.8±70.8     | -35.5±11.4      | -68.3±15.7      | -37.0±7.1       |

* p<0.05,  ** p<0.01,  *** p<0.001,

Values of mean difference are in comparison with the initial control values.
Fig. 1. Effects of pindolol on transmembrane action potential of a Purkinje fiber. A: control. B: pindolol, 0.5 mg/L. C: pindolol, 1.0 mg/L. D: pindolol, 5.0 mg/L.

Fig. 2. Effect of pindolol on maximum rate of depolarization of a Purkinje fiber. A: control. B: pindolol, 0.5 mg/L. C: pindolol, 1.0 mg/L. D: pindolol, 5.0 mg/L. The upper trace of each panel shows the initial part of the transmembrane action potentials. The bottom trace represents the differentiated records of the corresponding action potentials and the height of the spike indicates the maximum rate of depolarization. Vertical calibration of 300 V/sec is for the differentiated records.
Fig. 3. Effect of pindolol on the maximum rate of depolarization of the premature response in a Purkinje fiber. A: control. B: pindolol, 5.0 mg/L. The premature response in each panel is the one obtained at the shortest coupling interval under each experimental condition (278 msec in control state and 242 msec in pindolol solution). The top trace of each panel shows the zero potential lines. A pair of middle traces show the initial parts of the last response during a regular basic drive (first upstroke) and the premature response (second upstroke). Two bottom traces show the differentiated records of the middle action potentials. The first and the second spikes correspond to the basic and the premature response, respectively. Note that the take off potential of the premature response (shown by arrows) is more negative in panel B than in A; nevertheless, its maximum rate of depolarization is slower in panel B compared with that in A.

mg/L. Considerable decrease was also observed in maximum rate of depolarization as well as in the amplitude of action potential (Fig. 2 D).

As a result of greater decrease in the action potential duration than in the functional refractory period, earliest premature response arose from a more negative level of membrane potential. However, as shown in Fig. 3, an expected increase in maximum rate of depolarization of the earliest premature response was not obtained. This would be explained by the concomitantly seen depressed membrane responsiveness at this concentration as indicated by reduced maximum rate of depolarization of basic responses; i.e., the maximum rate of depolarization at a given level of membrane potential became so depressed that even the take off potential of the premature response became more negative than in the control state, the depolarization rate at this concentration could not exceed the control value.

Effects of pindolol on action potential characteristics and the functional refractory period of ventricular muscle fibers

Compared with the effect on Purkinje fibers, the same concentration of pindolol exerted lesser effect on ventricular muscle fibers (Table II and Fig. 4). Fifty % repolarization time, 90% repolarization time, and refractory period became slightly shortened in a concentration of 0.5 mg/L, and 90% repolarization time showed minimum prolongation in a concentration of
Table II. Effect of Pindolol on the Transmembrane Action Potentials of Ventricular Muscle Fibers

<table>
<thead>
<tr>
<th></th>
<th>90% Repolarization Time (msec), n=5</th>
<th>50% Repolarization Time (msec), n=5</th>
<th>Functional Refractory Period (msec), n=3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>240.0</td>
<td>174.0</td>
<td>232.7</td>
</tr>
<tr>
<td>Pindolol 0.5 mg/L</td>
<td>229.0</td>
<td>163.8</td>
<td>223.7</td>
</tr>
<tr>
<td>Mean difference ± S.D.</td>
<td>-11.0±9.8</td>
<td>-10.2±10.2</td>
<td>-9.0±16.1</td>
</tr>
<tr>
<td>Pindolol 1.0 mg/L</td>
<td>235.2</td>
<td>171.8</td>
<td>223.7</td>
</tr>
<tr>
<td>Mean difference ± S.D.</td>
<td>-4.8±12.8</td>
<td>-2.2±12.4</td>
<td>-9.0±18.4</td>
</tr>
<tr>
<td>Pindolol 5.0 mg/L</td>
<td>251.4</td>
<td>185.0</td>
<td>236.7</td>
</tr>
<tr>
<td>Mean difference ± S.D.</td>
<td>+10.6±19.6</td>
<td>+11.0±13.6</td>
<td>+4.0±29.8</td>
</tr>
</tbody>
</table>

* Values of mean difference are in comparison with the initial control values.

5.0 mg/L. However, these changes were not statistically significant.

**Effects of pindolol on the response of Purkinje fibers to isoproterenol**

In regularly driven Purkinje fibers, isoproterenol of 1.0 mg/L, induced significant shortening of 50% repolarization time, 90% repolarization time, and the functional refractory period (Table III). After the observed change in transmembrane action potential induced by isoproterenol became stabilized (in 15 to 20 min), pindolol of 0.5 to 5.0 mg/L was added to the isoproterenol solution. By administering 1.0 mg/L of pindolol, slight but signifi-
Table III. Effect of Pindolol on the Response of Purkinje Fibers to Isoproterenol

<table>
<thead>
<tr>
<th></th>
<th>90% Repolarization Time (msec), n=5</th>
<th>50% Repolarization Time (msec), n=5</th>
<th>Functional Refractory Period (msec), n=5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>336.4</td>
<td>267.0</td>
<td>269.8</td>
</tr>
<tr>
<td>Isoproterenol 1.0 mg/L</td>
<td>303.6</td>
<td>231.0</td>
<td>246.6</td>
</tr>
<tr>
<td>Mean difference±S.D.</td>
<td>−30.8±10.2</td>
<td>−36.0±11.7</td>
<td>−23.2±11.6</td>
</tr>
<tr>
<td>Isoproterenol 1.0 mg/L + Pindolol 0.5 mg/L</td>
<td>306.0</td>
<td>229.0</td>
<td>246.6</td>
</tr>
<tr>
<td>Mean difference±S.D.</td>
<td>+0.4±8.6</td>
<td>−3.0±11.0</td>
<td>0.0±9.9</td>
</tr>
<tr>
<td>Isoproterenol 1.0 mg/L + Pindolol 1.0 mg/L</td>
<td>318.0</td>
<td>231.6</td>
<td>255.8</td>
</tr>
<tr>
<td>Mean difference±S.D.</td>
<td>+12.4±8.4</td>
<td>+0.6±7.0</td>
<td>±9.2±7.3</td>
</tr>
<tr>
<td>Isoproterenol 1.0 mg/L + Pindolol 5.0 mg/L</td>
<td>320.6</td>
<td>217.0</td>
<td>248.2</td>
</tr>
<tr>
<td>Mean difference±S.D.</td>
<td>+15.0±19.0</td>
<td>−13.0±13.0</td>
<td>+1.6±11.2</td>
</tr>
</tbody>
</table>

a p<0.05, b p<0.01.
c Values of mean difference in isoproterenol alone are in comparison with control values.
d Values of mean difference in isoproterenol plus pindolol are in comparison with the values in isoproterenol alone.

cant increase in both 90% repolarization time and the functional refractory period were observed, whereas there were no significant changes by adding pindolol of 0.5 or 5.0 mg/L.

In the spontaneously beating Purkinje fibers, isoproterenol in concentrations from 0.2 to 1.0 mg/L markedly accelerated their automaticity. The effect of pindolol on this positive chronotropic action of isoproterenol was
striking. The representative data are shown in Figs. 5 and 6. On the accelerated automaticity induced by 1.0 mg/L of isoproterenol, pindolol in 0.1 mg/L did not show any observable effects (Fig. 5 C). By increasing the concentration of pindolol to 0.5 mg/L, however, the positive chronotropic effect of isoproterenol became less prominent (Fig. 5 D) and, by adding 5.0 mg/L of pindolol, the effect of isoproterenol was completely antagonized (Fig. 5 E). Fig. 6 shows one of the results of the similar experiments using a lower concentration of isoproterenol (0.2 mg/L) in which the accelerated automaticity was fully antagonized by 0.05 mg/L of pindolol which had no effects on the action potential configurations of both Purkinje fibers and ventricular muscle fibers.

These findings suggest that pindolol acts as a specific antagonist against the positive chronotropic action of isoproterenol.

DISCUSSION

Our experimental results indicate that, besides its beta-blocking action, pindolol in relatively high concentrations has definite membrane effects on isolated Purkinje fibers. The changes observed such as decrease in both the action potential duration and the functional refractory period, and reduced maximum rate of depolarization resemble those induced by propranolol.\(^{12,13}\)
PINDOLOL EFFECT ON CARDIAC FIBERS

The shortening of the action potential duration was disproportionately greater than that of the refractory period. This tendency was especially evident, when a comparison was made between 50% repolarization time and the functional refractory period in a concentration of 5.0 mg/L. This relative prolongation of the refractory period, which was also reported with propranolol, is usually regarded as an important antiarrhythmic mechanism, because, as a result of this, the take off potential of the earliest possible premature response would be expected to increase, thus inhibiting slow or abortive conduction which leads to reentry.\textsuperscript{14,15} However, pindolol was proved not to increase but decrease the upstroke velocity of the premature response because of its concomitant depressant effect on membrane responsiveness (Fig. 3). Thus, the membrane effect of pindolol which accelerates repolarization would not be expected to exert antiarrhythmic activity.

Pindolol had little effect on the action potential characteristics of ventricular muscle fibers in the same range of concentrations. This finding is in accord with the results of Maruyama\textsuperscript{11} on pindolol and other reports on various beta-blocking agents.\textsuperscript{13,16,17}

We also studied on its beta-blocking action by evaluating its effects on decreased action potential duration and accelerated automaticity of Purkinje fibers normally induced by isoproterenol. It is generally believed that the shortening of the action potential duration observed with isoproterenol administration is mediated through beta-receptors and can be antagonized by beta-blocking agents.\textsuperscript{18-20} In our experiments, the decrease in the action potential duration induced by 1.0 mg/L of isoproterenol was little affected by pindolol in concentrations from 0.5 to 5.0 mg/L, except the slight increase in 90\% repolarization time seen in a concentration of 1.0 mg/L. On the contrary, the accelerated automaticity produced by isoproterenol was drastically antagonized by pindolol in lower concentrations. The inability of pindolol in antagonizing the effect of isoproterenol to decrease the action potential duration is difficult to explain, but it may be due to an excess amount of isoproterenol. Giotti \textit{et al}\textsuperscript{20} reported that isoproterenol of 0.02 to 0.08 mg/L showed definite shortening of the action potential duration of sheep Purkinje fibers which was antagonized by 0.1 mg/L of propranolol. It might be inferred that the expected increase in the action potential duration could not be observed because the dose of pindolol antagonizing 1.0 mg/L of isoproterenol inevitably exerted its own membrane effect which also acted to decrease the action potential duration. The fact that pindolol in the same range of concentrations did antagonize the positive chronotropic action of isoproterenol (Fig. 5) provides support for this explanation. Furthermore, pindolol in a concentration below one tenth of that required to produce the membrane
effect suppressed the accelerated automaticity (Fig. 6). This implies the specificity of pindolol as a beta-blocker is high. Consequently, pindolol is expected to show antiarrhythmic activity by a suppression of the ectopic impulse formation and also by preventing the occurrence of conduction disturbances based on the decreased membrane potential due to an enhanced diastolic depolarization.\(^{31,22}\)

The mechanism of antiarrhythmic activity of beta-blocking agents still remains controversial. According to experimental studies using optical isomers of propranolol, arrhythmias induced by catecholamine were selectively inhibited by \(l\)-form or \(dl\)-form of the drug which have beta-blocking action, whereas on ouabain-induced arrhythmias, \(d\)-propranolol which was lacking the beta-blocking activity was as equally effective as \(l\)- or \(dl\)-form.\(^{61,23}\) Lucchesi\(^{4}\) stressed the important role played by the membrane effect of beta-blocking agents, reporting that there were no difference in effectiveness between 2 optical isomers, \(d\)- and \(l\)-form, of pronethalol on ouabain-induced arrhythmias or even on arrhythmias provoked by epinephrine. Koerpel\(^{21}\) also raised the question as to the contribution of beta-blocking activity to the antiarrhythmic action.

However, the dose of beta-blocking agents required to prevent ouabain-induced arrhythmias has been reported to be 10 to 20 times greater than that needed to suppress the catecholamine-induced arrhythmias.\(^{51,23,24}\) Also, the concentrations in which some beta-blocking agents exert their membrane effects on isolated Purkinje fibers are several to 10 times larger than that exhibiting beta-blocking action alone,\(^{11,13}\) and much higher concentrations are usually required in ventricular muscle fibers.\(^{13,16}\) These concentrations are far beyond the effective plasma levels of beta-blockers, and this raised a question as to whether the membrane effect of these drugs was actually responsible for their antiarrhythmic action.\(^{7,9}\) The dextro-isomers of some beta-blockers were found to show very weak antiarrhythmic action in clinical doses, though they did exert some actions in much higher concentrations in which dangerous side effects were frequently observed.\(^{25}\)

Similarly, the concentrations of pindolol required to show the membrane effect in our experimental study were more than 0.5 to 1.0 mg/L which correspond 15 to 30 times of the therapeutic plasma level of this drug.\(^{9}\) On the other hand, 0.05 mg/L of pindolol which was close to the clinical plasma level actually antagonized the positive chronotropic effect of isoproterenol. These findings in the present study support the view that the greater part of the antiarrhythmic effect of this drug is due to its beta-blocking action.
Conclusion

Pindolol in concentrations higher than 0.5 mg/L was shown to exert its membrane effects on canine Purkinje fibers. It decreased the action potential duration, the functional refractory period and the maximum upstroke velocity of phase 0. While these concentrations are much higher than that achieved in the clinical use, pindolol of 0.05 mg/L which is close to the therapeutic plasma concentrations antagonized the positive chronotropic effect of isoproterenol. It seems that beta-blocking effects are more essential for antiarrhythmic actions of this drug than its membrane effects.

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References