Lipophilic β-Adrenoceptor Blocking Drugs Have Strong Membrane Stabilizing Actions on Atrioventricular Nodal and Intraventricular Conduction: Comparison with Their β-Adrenoceptor Antagonizing Actions

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Abstract: The membrane stabilizing actions (MSA) of eight β-blockers were compared with their β-adrenoceptor antagonizing action in relation to their lipophilicity. The MSA on atrioventricular (AV) nodal and intraventricular conduction were determined as the doses of ID_{25}(AH) and ID_{50}(HV), which produced 25% and 50% increases in atrio-His (AH) and His-ventricle (HV) intervals by their injection into the posterior or the anterior septal artery of the canine isolated, blood-perfused AV node preparations. The basal AH and HV intervals were 83±3 and 40±2 msec (n=52). ID_{25}(AH), the doses producing a 21 msec prolongation of the AH interval, were 71 μg for propranolol (P), 108 μg for alprenolol (A), 129 μg for oxprenolol (O), 397 μg for pindolol (PIN), 385 μg for bunitrolol (B), 648 μg for timolol (T), 3.2 mg for atenolol (AT) and more than 10 mg for carteolol (C). ID_{50}(HV), the doses producing a 20 msec prolongation of the HV interval, were 533(P), 428(A), 361(O), 471(PIN), 598(B), 1700(T), and >10,000 μg (AT, C). The β-adrenoceptor antagonizing actions were estimated as the doses of ED_{20}(NE), the minimum doses of β-blockers which completely suppress the 20 msec shortening of the AH interval by norepinephrine. ED_{20}(NE) were 4.4(P), 3.0(A), 4.3(O), 0.40(PIN), 0.37(B), 1.9(T), 3.3(AT) and 0.29(C). As a result, the ratio of ID_{25}(AH)/ED_{20}(NE) were 16(P), 36(A), 30(O), 990(PIN), 1040(B), 348(T), 970(AT) and >50000(C). Similarly, the ratio of ID_{50}(HV)/ED_{20}(NE) were 120(P), 142(A), 83(O), 1180(PIN), 1620(B), 930(T) and >30000(AT,C). These results indicate that 1) lipophilic β-blockers (P,A,O,PIN,B) directly suppress intraventricular conduction, but approximately 100 times larger doses than β-adrenoceptor antagonizing doses were required; 2) in addition, highly lipophilic β-blockers (P,A,O) preferentially suppress AV nodal conduction, but a hydrophilic β-blocker (AT) barely suppresses AV nodal and intraventricular conduction; and 3) the lipophilicity (or hydrophilicity) of β-blockers is not related to their β-adrenoceptor antagonizing potency. These results suggest that the MSA of highly lipophilic β-blockers are harmful as cardiodepressants, but might be therapeutic as antiarrhythmics.

Key words: Atrioventricular node, β-Adrenoceptor antagonizing action, Intraventricular conduction, Lipophilicity, Membrane stabilizing action

INTRODUCTION

When β-adrenoceptor blocking drugs (β-blockers) are used as antiarrhythmics, their β-adrenoceptor antagonizing actions are expected to eliminate excess tonic adrenergic influences1). Based on this action, β-blockers are grouped as class II antiarrhythmics2). In addition, however, some β-blockers are expected to be class I antiarrhythmics for their membrane stabilizing action (MSA), i.e., local
anesthetic action\(^2\)). In particular, highly lipophilic β-blockers such as propranolol blocked cardiac sodium (Na) channels\(^3\text{--}^5\) and inhibited the binding of \(^{[3H]}\)batrachotoxinin A 20-α-benzoate (BTX-B) to the site of voltage-sensitive Na channels\(^6\text{--}^7\). In contrast, hydrophilic β-blockers such as atenolol have only weak Na channel blocking action\(^3\text{--}^7\).

Much has already been published on the effects of β-blockers on atrioventricular (AV) conduction\(^8\text{--}^18\). The negative dromotropic effects of β-blockers on AV conduction are induced by two major causes; β-adrenoceptor antagonizing and MSA. The latter has been described as having antiarrhythmic properties on one hand and as having adverse effects on the other\(^19\text{--}^21\). Both properties are important for the highly lipophilic β-blockers, although they become evident when large doses are used\(^21\text{--}^22\). It is still unclear, however, what dose is required for producing the MSA on AV nodal and intraventricular conduction. Furthermore, in order to exactly estimate the MSA, any adrenergic influence should be eliminated. In this sense, it is still unclear, too, what the β-adrenoceptor antagonizing dose in AV nodal conduction is, and if the lipophilicity of β-blockers is related to their β-adrenoceptor antagonizing actions.

The canine, blood-perfused AV node preparation used in the present experiments has been isolated from tonic nervous activities\(^23\text{--}^29\). As shown in Fig. 1, in the dog heart the atrial margin and the upper and middle parts of the AV node, where the slow calcium inward current, in addition to the fast Na inward current, is highly responsible for the AV nodal conduction, are supplied arterial blood through the posterior septal artery (PSA, the AV node artery)\(^23\text{--}^26\text{--}^28\). The lower part of the AV node, the His bundle, and the distal conduction system in the ventricular septum (His-Purkinje-ventricular system), where the fast Na inward current is mostly responsible for the intraventricular conduction, are supplied arterial blood through the

![Fig. 1. The canine isolated atrioventricular node preparation. ASA; anterior septal artery, AVN; atrioventricular node, CS; coronary sinus, IVC; inferior vena cava, LAD; left anterior descending artery, LCX; left circumflex artery, PA; pulmonary artery, PM; papillary muscle, PSA; posterior septal artery, RCA; right coronary artery, RA; right atrium.](image-url)
anterior septal artery (ASA)\(^{23,26,28,29}\). Thus, the effects of the drug on AV nodal and intraventricular conduction can be obtained when the drug is selectively administered into the PSA or into the ASA\(^{23,28,29}\).

In the present experiments, the MSA of eight \(\beta\)-blockers were compared with their \(\beta\)-adrenoceptor antagonizing action in relation to their lipophilicity. The MSA on AV nodal and intraventricular conduction were determined as the doses of \(\beta\)-blockers which directly cause a 25% or 50% prolongation of the AH and HV intervals when injected intraarterially into the PSA and ASA of the AV node preparation. The \(\beta\)-adrenoceptor antagonizing doses on AV nodal conduction were estimated as the minimum doses of \(\beta\)-blockers administered intraarterially into the PSA of the AV node preparation, which completely suppress the 20 msec shortening of the AH interval by norepinephrine injected into the same artery. Finally, the ratios of the \(\beta\)-adrenoceptor antagonizing doses and the membrane stabilizing doses on AV conduction are quantitatively compared among eight \(\beta\)-blockers.

**METHODS**

The fifty-two isolated, blood-perfused AV node preparations (Fig. 1)\(^ {23-29}\) were prepared from mongrel dogs of either sex weighing 9–14 kg, anesthetized with pentobarbital sodium (50 mg/kg i.v.), given heparin sodium (500 U/kg, i.v.) and exsanguinated. The AV node preparation consisted of the right atrium and the interventricular septum. The posterior septal (PSA), anterior septal (ASA) and right coronary (RCA) arteries were cannulated. The AV node preparation was placed in a double-wall glass jacket maintained at 38°C by circulating warm water. The AV node preparation was cross-circulated with the heparinized arterial blood of the support dog through three cannulated arteries at a constant perfusion pressure of 120 mmHg with a Cole-Parmer Masterflex peristaltic pump (Cole-Parmer Instruments Co., Chicago) and a Starling pneumatic resistance placed parallel to the perfusion system. Venous blood from the preparation and excess blood passing through the pneumatic resistance was collected in a blood reservoir and returned to the support dog through the jugular vein. The blood flow in cannulated arteries was continuously measured with each electromagnetic flow meter (Nihon Kohden, Tokyo, MFV-1100) using a 2-mm cannulating flow probe\(^ {24,26}\).

The crista terminalis of the right atrium was electrically driven by a stimulator and an isolation unit (Dia Medical, Tokyo, DHM-226-3 and DSP-110) with rectangular pulses of 1–3 V (twice the threshold voltage) at 5 msec duration at a fixed rate of 2.5 Hz (150 stimuli/min) through bipolar stimulating silver electrodes. Bipolar atrial (A) electrograms obtained from the right atrial free wall, His bundle (H) electrograms obtained from the His bundle region, and ventricular (V) electrograms obtained from the base of the anterior papillary muscle of the right ventricular septum, were fed to an automated AH interval meter (Dia Medical, Tokyo, DHM-226-1), which individually measures AV, AH, and HV intervals with an analysis pitch of 1 msec. The basal AV conduction time was 123±2 msec, and AH and HV intervals were 83±3 and 40±2 msec (n=52), respectively.

Adult mongrel dogs of either sex weighing 14–22 kg, used as support dogs, were anesthetized with pentobarbital sodium, 30 mg/kg i.v. and given an additional 4–5 mg/kg every hour. The animals received an initial dose of 500 U/kg heparin sodium, followed by 200 U/kg every hour. Respiration was controlled using an animal respirator (Shinano, Tokyo, SN-480-3). Systemic blood pressure at the femoral artery, and heart rate triggered by the R wave of the lead II EKG were monitored continuously with a polygraph (NEC San-ei Instruments, Tokyo, 361–6).

Drugs used were alprenolol hydrochloride
(Hassle), atenolol (ICI), bunitrol hydrochloride (Boehringer Ingelheim), carteolol hydrochloride (Otsuka), oxprenolol hydrochloride (Ciba Geigy), pindolol (Sandoz), propranolol hydrochloride (Sumitomo) and timolol maleate (Merck). The drug was given as a 30 μl bolus intraarterially into each nutrient artery over a period of 4 sec with a microsyringe (Terumo Co.).

The experiment was performed in accordance with Guidelines for Animal Experiments, Yamanashi Medical College.

RESULTS

Direct effects of β-blockers on atrioventricular nodal and intraventricular conduction

A typical experiment of propranolol injected i.a. into the PSA (upper panel) and into the ASA (lower panel) is shown in Fig. 2. By either administration, dose-related increases in AV conduction time were produced (the top record in each panel). By the injection into the PSA, however, the increases in AV conduction time consisted entirely of the prolongation of the AH interval (Fig. 2. upper panel). Meanwhile, the increases in AV conduction time

Fig. 2. A typical experiment on the negative dromotropic effects of propranolol injected into the posterior septal artery (PSA, upper panel) and into the anterior septal artery (ASA, lower panel) on atrioventricular (AV) conduction time, atrio-His (AH) interval and His-ventricle (HV) interval.
induced by the injection into the ASA were predominantly due to the prolongation of the HV interval. The dose-response curves for negative dromotropic effects of propranolol on AH and HV intervals are shown in Figs. 3 and 4, respectively. The second degree AV conduction block was produced in 7 out of 9 AV node preparations at 300 µg of propranolol injected into the PSA, while the AV block was not produced even in a dose of 600 µg of propranolol injected into the ASA.

Similar to propranolol, when alprenolol,
oxprenolol, pindolol, bunitrolol and timolol were injected either into the PSA or the ASA, they caused dose-related prolongations of AH or HV intervals (Figs. 3 and 4), although the negative dromotropic effects of these β-blockers were weaker than those of propranolol. Second degree AV block was produced in 3 of 8 and 2 of 8 AV node preparations at 300 μg of alprenolol and oxprenolol, respectively, injected into the PSA (Fig. 3). On the other hand, pindolol, bunitrolol, and timolol did not induce the AV block up to doses of 600 μg or 1 mg injected into the PSA. Nonetheless, atenolol and carteolol had little effect on AH and HV intervals up to 300 μg. At 1 and 3 mg, both β-blockers only slightly increased the AH and HV intervals.

When the β-blockers were injected i.a. into the PSA, all drugs except propranolol initially decreased the AH interval at lower doses (not shown as results). The degrees of shortening of the AH interval were variable and not dose-related for each drug. Carteolol caused only positive dromotropic effects up to a dose of 300 μg and the maximum shortening was 13 msec at 100 μg. In contrast to the injection into the PSA, these β-blockers did not cause any shortening of the HV interval when injected into the ASA.

The doses producing 50% and 25% increases in the AH interval (approximately 41 and 20 msec) were obtained from each dose-response curve in Fig. 3, as ID50(AH) and ID25(AH), respectively. The ID50(AH) of pindolol, bunitrolol, and timolol, and the ID25(AH) of atenolol and carteolol were obtained by extrapolating each dose-response curve. The extrapolation of the dose-response curves of atenolol and carteolol did not reach the level of ID50(AH). Similarly, the doses producing 50% and 25% increases in the HV interval (approximately 20 and 10 msec), were obtained from each dose-response curve in Fig. 4, as ID50(HV) and ID25(HV), respectively. The ID50(HV) of timolol and carteolol were obtained by extrapolating each dose-response curve. Extrapolating the dose-response curve of atenolol did not reach the level of ID50(HV). All these values are shown in Table 1.

Estimation of β-adrenoceptor antagonizing doses of β-blockers on AV nodal conduction

As an agonist to stimulate β-adrenoceptors on the AV node, norepinephrine was injected intrarxterially into the PSA. In a dose range of 0.03–0.3 μg, norepinephrine dose-relatedly

<table>
<thead>
<tr>
<th>Drug</th>
<th>ED20(NE)</th>
<th>ID50(AH)</th>
<th>ID25(AH)</th>
<th>ID50(AH)</th>
<th>ID25(AH)</th>
<th>ID50(HV)</th>
<th>ID25(HV)</th>
<th>ED20(NE)</th>
<th>ED25(NE)</th>
</tr>
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<tr>
<td>propranolol</td>
<td>4.4</td>
<td>1.0</td>
<td>187</td>
<td>71</td>
<td>42</td>
<td>16</td>
<td>533</td>
<td>163</td>
<td>120</td>
</tr>
<tr>
<td>alprenolol</td>
<td>3.0</td>
<td>1.5</td>
<td>229</td>
<td>108</td>
<td>79</td>
<td>36</td>
<td>428</td>
<td>123</td>
<td>142</td>
</tr>
<tr>
<td>oxprenolol</td>
<td>4.5</td>
<td>1.0</td>
<td>283</td>
<td>129</td>
<td>65</td>
<td>30</td>
<td>361</td>
<td>108</td>
<td>83</td>
</tr>
<tr>
<td>pindolol</td>
<td>0.40</td>
<td>11.1</td>
<td>(2030)</td>
<td>397</td>
<td>(5080)</td>
<td>900</td>
<td>471</td>
<td>146</td>
<td>1180</td>
</tr>
<tr>
<td>bunitrolol</td>
<td>0.37</td>
<td>12.0</td>
<td>(2080)</td>
<td>385</td>
<td>(5620)</td>
<td>1040</td>
<td>598</td>
<td>194</td>
<td>1620</td>
</tr>
<tr>
<td>timolol</td>
<td>1.9</td>
<td>2.4</td>
<td>(3410)</td>
<td>648</td>
<td>(1890)</td>
<td>348</td>
<td>(1728)</td>
<td>540</td>
<td>(930)</td>
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<tr>
<td>atenolol</td>
<td>3.5</td>
<td>1.3</td>
<td>(--)</td>
<td>(3192)</td>
<td>(--)</td>
<td>(970)</td>
<td>(--)</td>
<td>(1970)</td>
<td>(--)</td>
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<tr>
<td>carteolol</td>
<td>0.29</td>
<td>15.3</td>
<td>(--)</td>
<td>(10000)</td>
<td>(--)</td>
<td>(56000)</td>
<td>(10500)</td>
<td>(1710)</td>
<td>(36200)</td>
</tr>
</tbody>
</table>

Values in brackets were obtained by extrapolating each dose-response curve.

(--) Extrapolating line did not reach the values within the dose scale.

Abbreviations are in text.
Fig. 5. Dose-response curves for the positive dromotropic effects on the AH interval of norepinephrine injected into the PSA in the absence (C) and in the presence of various doses of β-blockers.

decreased the AH interval, while the HV interval was not affected. Dose-response curves for the positive dromotropic effects of norepinephrine obtained just before injection of each β-blocker were almost the same as shown in each panel of Fig. 5. The shortening of the AH interval with i.a. norepinephrine was suppressed by a prior injection of β-blockers into the same artery (the PSA). The dose-response curve of norepinephrine was shifted to the right after treatments of β-blockers in a dose-dependent manner (Fig. 5).

The doses producing a 15 msec decrease in the AH interval with i.a. norepinephrine were obtained in the absence and in the presence of several doses of β-blockers. In the case of propranolol, for example, the doses were 0.068 µg at control, and 0.62 and 2.8 µg in the presence of 3 and 10 µg of propranolol, respectively. From these doses, the dose-ratio (DR) was calculated as 9.1 and 41 and expressed as log [DR] of 0.96 and 1.62 at doses of 3 and 10 µg of propranolol. In Fig. 6, the log [DR] were plotted against the doses of each β-blocker. For comparison, the β-adrenoceptor antagonizing doses on AV nodal conduction were estimated as the minimum doses of β-blockers injected i.a. into the PSA, which completely suppress the 20 msec shortening of the AH interval by norepinephrine injected
into the same artery, and expressed as ED$_{20}$(NE). The shortening of 20 msec is compatible with the 50% of the HV interval, which was used for estimation of the membrane stabilizing action in the first experiments. The ED$_{20}$(NE) were obtained as the doses producing the log [DR] of approximately 1.2 (Fig. 6).

Finally, the ratios of the β-adrenoceptor antagonizing doses and the membrane stabilizing doses on AV conduction are quantitatively compared among eight β-blockers, as shown in Table 1.

**Discussion**

Much work has been published on the effects of β-blockers on AV nodal conduction$^{8-18}$. β-Blockers depress AV nodal conduction by β-adrenoceptor antagonizing effects when the sympathetic nervous tones and adrenergic influences are elevated$^{19-21}$. It has been very difficult, however, to determine precisely the β-adrenoceptor antagonizing doses on AV nodal conduction in either *in vivo* or *in vitro* experiments$^{9,12,15,16,19,21,22}$. The potencies of β-blockers for antagonizing β-adrenoceptors are usually expressed as the pA$_2$-values obtained from *in vitro* experiments$^{15,20}$. The pA$_2$-values are useful for comparison of the potencies among individual β-blockers$^{15}$. Furthermore, they are occasionally expressed as relative potencies to propranolol$^{15,20}$. In the present study, the β-adrenoceptor antagonizing dose was estimated as the minimum dose completely antagonizing a 20 msec shortening of the AH interval by norepinephrine, expressed as ED$_{20}$(NE). The rank of the β-adrenoceptor antagonizing doses obtained in the present study is compatible with those in the literature$^{15,20}$, suggesting that the doses could be useful for comparison with the membrane stabilizing dose. The 20 msec was derived from the value of the 50% of the basal HV interval, from which each ID$_{50}$(HV) was determined as the membrane stabilizing dose.

In previous *in vitro* experiments$^{3-5}$, the membrane stabilizing dose determined as local anesthetic potencies for depressing the maximum rate of rise in the action potential of the neuronal or cardiac excitation was obtained as a molar concentration, which could be compared with the pA$_2$-values. It is very difficult to estimate, however, to what extent the depressant effect on the maximum rate of rise in the action potential is therapeutic as an antiarrhythmic$^{21}$ or harmful as a cardiac depressant and/or an arrhythmogenic$^{20,21}$. In the present study, the ID$_{50}$(HV) was determined as the membrane stabilizing dose producing the 20 msec prolongation of the HV interval. Since the fast Na inward current is most responsible for the intraventricular conduction (the HV interval), the ID$_{50}$(HV) accurately represents the membrane stabilizing dose as a Na channel blocker. In addition, however, the Na channel blockers grouped as class I antiarrhythmics prominently impair AV nodal
conduction. In the present experiments, the \( ID_{50}(AH) \) and/or \( ID_{25}(AH) \) were obtained as direct depressant (membrane stabilizing) doses and compared with the \( \beta \)-adrenoceptor antagonizing doses.

The ratios of the \( \beta \)-adrenoceptor antagonizing dose versus the membrane stabilizing dose were quantitatively shown in the present experiments (Table 1). The larger the ratio, the smaller the adverse cardiac depressant effect\(^{19-22,29}\). In this sense, when the \( ID_{50}(HV) \) are used as the membrane stabilizing doses of \( \beta \)-blockers, propranolol, alprenolol, oxprenolol, pindolol and bunitrolol were at least 350-500 times larger than the \( ED_{20}(NE) \) estimated as the \( \beta \)-adrenoceptor antagonizing doses on AV nodal conduction. However, when the \( ID_{50}(AH) \) and \( ID_{25}(AH) \) are used as the membrane stabilizing doses, the ratio fell to 16-42, suggesting that these \( \beta \)-blockers have low safety margins to produce an AV conduction block.

It has been reported that the MSA of \( \beta \)-blockers is closely related to the lipophilicity of drugs\(^{6,7,9,21}\). In the present study, too, the direct depressant effects on the AH interval of highly lipophilic \( \beta \)-blockers, propranolol (Logarithm partition coefficient of octanol/water = 3.65), alprenolol (2.61) and oxprenolol (2.18) are markedly strong, while that of atenolol (0.23), which is hydrophilic, is very weak. The prolongation of the AH interval by pindolol (1.75), bunitrolol, and timolol (2.10) are moderate. In the HV interval, the direct depressant actions of propranolol, alprenolol, and oxprenolol are similar to those of pindolol and bunitrolol. These results are very compatible with the evidence that the lipophilicity of \( \beta \)-blockers was very closely related to the inhibition of BTX-B binding to the sites of Na channels\(^{6,7}\), resulting in depression of the fast Na currents\(^{3-5}\).

Nevertheless, in addition to the Na current, the slow Ca currents are responsible for the AV nodal conduction\(^{2,25,28}\). Similarly, the author recently reported that the higher the pKa values of Ca channel blockers, the stronger the negative dromotropic effects even by the dihydropyridines like nicardipine (pKa = 7.2)\(^{25,27}\). The binding site of verapamil (pKa = 8.2) in the Ca channel is located and exposed to intracellular space\(^{30}\), to which the drug could thereby have access from the intracellular space. Since the pKa values of most \( \beta \)-blockers are over 9.3, they were entirely ionized at physiological pH of 7.4\(^{13,7,19,21}\). Thus, the lipophilic \( \beta \)-blockers could have access to the binding site of the Na channel, and possibly to that of the Ca channel, from the intracellular space with ionized forms after penetration through the cell membrane\(^{13,7,19,21}\). By this mechanism, the strongest depressant effect of propranolol on the AH interval, i.e., AV nodal conduction, might be explained by its most lipophilic property. However, as shown in Fig. 2B, the direct depressant effect of propranolol on the HV interval seems to be entirely due to the Na channel blocking, which is entirely responsible for intraventricular conduction\(^{28,29}\). Thus, the prolongation of the AH interval by propranolol, too, seems to be entirely due to a blocking of the Na channels even in the AV node.

In conclusion, (1) lipophilic \( \beta \)-blockers directly suppress intraventricular conduction, but approximately more than 100 times larger doses than the \( \beta \)-adrenoceptor antagonizing doses were required; (2) in particular, highly lipophilic \( \beta \)-blockers such as propranolol, alprenolol and oxprenolol more preferentially suppress AV nodal conduction, but a hydrophilic \( \beta \)-blocker such as atenolol barely suppresses AV nodal and intraventricular conduction; and (3) the lipophilicity (or hydrophilicity) of \( \beta \)-blockers is not related to their \( \beta \)-adrenoceptor antagonizing potency. Since \( \beta \)-blockers are usually used chronically in the treatment of cardiac disease\(^{11,9,21}\), the highly lipophilic \( \beta \)-blockers can easily accumulate in the cardiac tissues\(^{19,21}\). Thus, the highly lipophilic \( \beta \)-blockers are harmful as cardio-depressants, but might be therapeutic as anti-
arrhythmics, since the ratios of the β-adrenoceptor antagonizing dose against the membrane stabilizing dose in AV nodal conduction are small.

References


