Electrophysiological Effects of the Antiarrhythmic Drug Bepridil on the Guinea-Pig Pulmonary Vein Myocardium

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We compared effects of the antiarrhythmic drug bepridil on the electrophysiological parameters in the isolated pulmonary vein preparation from guinea pigs with those in the left atrium. Three pairs of bipolar electrodes were attached to the left atrium, pulmonary vein and junctional region of left atrium and pulmonary vein to measure intra-atrial and intra-pulmonary vein conduction velocity and effective refractory period. Bepridil at 10 μM prolonged the effective refractory period with little effect on the conduction velocity in the pulmonary vein, whereas the drug failed to affect the electrophysiological parameters in the left atrium. Using the conventional microelectrode technique, action potential of the isolated pulmonary vein preparation and left atrium were measured. Bepridil prolonged the action potential duration of the pulmonary vein more potently than that of the left atrium. These results suggest that antiarrhythmic effects of bepridil on reentry within the pulmonary vein are estimated to be greater than within the left atrium, which may be one of the key considerations to understand its antiarrhythmic mechanisms.

Key words bepridil; pulmonary vein myocardium; conduction velocity; effective refractory period; action potential

The pulmonary vein has a myocardial layer, which is known as an origin of the onset of atrial fibrillation in patients. Of note, the pulmonary vein myocardium has different electrophysiological properties from those of the working myocardium, such as a less negative resting membrane potential, which makes it possible to easily generate arrhythmogenic substrates such as abnormal automaticity and triggered activity.

Bepridil is an antiarrhythmic drug possessing a multi-ion channel blocking action. A recent multicenter, randomized, placebo-controlled, double blind study in Japan (J-BAF Study) has demonstrated that bepridil effectively converted to sinus rhythm in patients with persistent atrial fibrillation. To date, electrophysiological effects of bepridil on the sinus node, atrium and ventricle have been investigated in experimental studies. However, information is limited regarding effects of bepridil on electrophysiological parameters of the pulmonary vein myocardium itself. In this study, we recorded the conduction velocity, effective refractory period and action potential of the isolated pulmonary vein preparation from the guinea pig, and compared effects of bepridil on these parameters in the pulmonary vein with those in the left atrium to better understand the antiarrhythmic action of bepridil.

MATERIALS AND METHODS

All experiments were approved by the Ethics Committee of Toho University, and performed in accordance with the Guiding Principles for the Care and Use of Laboratory Animals approved by The Japanese Pharmacological Society. The heart and adjunct lungs were isolated from male or female Hartley guinea pigs weighing 350–450 g and incubated with the Krebs–Henseleit solution of the following composition (in mm): NaCl 118.4, KCl 4.7, CaCl2 2.5, MgSO4 1.2, KH2PO4 1.2, NaHCO3 24.9, glucose 11.1, gassed with 95% O2–5% CO2 (pH 7.4 at 37°C). Electrophysiological parameters were recorded as previously described.

Left atrium and adjunct pulmonary veins were mounted in the organ bath. Bipolar stimulating electrodes were attached onto the left atrial appendage and right inferior pulmonary vein, whereas three sets of bipolar recording electrodes were attached on the left atrial appendage, left atrium-pulmonary vein junction region and right inferior pulmonary vein. Electrogrograms were amplified with a bioelectric amplifier (AB-621G, Nihon Kohden, Tokyo, Japan) and fed into a waveform analysis system (PowerLab, ADInstruments, Castle Hill, Australia). The preparation was electrically driven using an electrical stimulator (SEN-7203, Nihon Kohden) and an isolator (SS-1041, Nihon Kohden) with rectangular pulses (about 1.5 times of the diastolic threshold voltage and 3-ms width). The effective refractory period was assessed by a pacing protocol consisted of ten beats of basal stimuli in a cycle length of 100, 300, 500 or 1000 ms followed by an extra stimulus of various coupling intervals. All experiments were performed at 36.5±0.5°C.

The pulmonary veins were separated from the left atrium and lung at the end of the pulmonary vein myocardium sleeve. Luminal side of the pulmonary vein at the middle region between the ostium and the distal end of myocardial sleeve or endocardial surface of the left atrium was impaled with glass microelectrodes filled with 3 M KCl to record transmembrane potential using a microelectrode amplifier (Intra 767, World Precision Instruments, Sarasota, FL, U.S.A.). The preparation was electrically driven using an electrical stimulator (SEN-7203, Nihon Kohden) and an isolator (SS-1041, Nihon Kohden) with rectangular pulses (about 1.5 times of the diastolic threshold voltage and 3-ms width). The action potential signals were monitored by an oscilloscope (CS-5135, Kenwood, Tokyo, Japan) and fed into a waveform analysis system (DSS98-type IV, Canopus, Tokyo, Japan). All experiments were performed at 36.5±0.5°C.

Bepridil hydrochloride (molecular weight=403.00, Sigma-Aldrich, St. Louis, MO, U.S.A.) was dissolved in distilled water and prepared as stock solutions in 100% ethanol.

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water and small aliquots were added to the organ bath to obtain the desired final concentration. All other chemicals were commercial products of the highest available quality. The statistical significances within a parameter were evaluated by one-way repeated-measures analysis of variance (ANOVA) followed by Dunnett’s test. A p value less than 0.05 was considered significant.

RESULTS

Left Atrium-Pulmonary Vein Conduction Figure 1 shows typical tracings of effects of bepridil on the electrograms obtained from the left atrial appendage (LA), left atrium-pulmonary vein junction region (LA-PV junction) and right inferior pulmonary vein (PV). Figure 2 summarizes the effects of bepridil on the conduction velocity and effective refractory period in the pulmonary vein and left atrium (n=5). In the presence of bepridil at a concentration of 1 µM, no significant change was detected in the conduction velocity or effective refractory period. Thirty minutes after application of 10 µM, the effective refractory period significantly increased only in the pulmonary vein, whereas the conduction velocity was not affected in the pulmonary vein or left atrium.

Effects of Bepridil on the Action Potential Configuration In the pulmonary vein preparation, bepridil at a concentration of 100 µM significantly decreased overshoot and maximum rate of phase 0 depolarization (\( V_{\text{max}} \)) and prolonged action potential duration at 50% (APD\(_{50} \)) and APD\(_{90} \). In the left atrial preparation, same concentration of bepridil significantly decreased the resting membrane potential and prolonged APD\(_{90} \) (Table 1).

DISCUSSION

Previous studies using the isolated canine cardiomyocytes from the pulmonary vein myocardium have demonstrated that inward rectifier current density (\( I_{K1} \)), transient outward \( K^+ \) current (\( I_{\text{to}} \)) and l-type \( Ca^{2+} \) current were significantly smaller than those in the left atrial cells. Slow and rapid delayed rectifier currents (\( I_{Ks} \) and \( I_{Kr} \), respectively) were greater in the pulmonary vein, whereas Na\(^+\) current density under voltage-clamp conditions was similar in the pulmonary vein and left atrium. These electrophysiological properties are potentially accounting for the less negative resting membrane potentials as well as longer action potential duration and effective refractory period in the pulmonary vein myocardium, as observed in this experiment (Table 1) and in our previous study. Furthermore, it is presumed that electrophysiological effects of antiarrhythmic drugs on the pulmonary vein are quantitatively or qualitatively different from those in the left atrium. Indeed, in our previous study using the same preparation, the class Ic antiarrhythmic drug pilsicainide prolonged the effective refractory period more potently in the left atrium than in the pulmonary vein, whereas its effects on the conduction velocity in the pulmonary vein was relatively greater than those in the left atrium.
In this study, bepridil at 10 \( \mu \)M prolonged the effective refractory period with little effect on the conduction velocity in the pulmonary vein, whereas the drug failed to affect the electrophysiological parameters in the left atrium. These results suggest that bepridil more potently suppresses the electrical activity during the repolarization phase in the pulmonary vein, which may partly explain its antiarrhythmic mechanism to suppress reentry involved in the pulmonary vein. The prolongation of effective refractory period by bepridil in the pulmonary vein may be closely associated with the delay of the repolarization phase, as shown in Table 1. In addition, its \( \text{Na}^+ \) channel-blocking property, as reflected by the reduction of the overshoot and maximum rate of phase 0 depolarization (\( V_{\text{max}} \)) in the pulmonary vein, may also contribute to prolongation of the effective refractory period. Previous electrophysiological studies have shown that bepridil blocks various types of \( \text{K}^- \) channels, including \( I_{\text{Kr}} \), ultra-rapid component of delayed rectifier \( \text{K}^- \) current (\( I_{\text{Kur}} \), \( I_{\text{Ks}} \), \( I_{\text{to}} \), \( I_{\text{K1}} \) and muscarinic acetylcholine receptor-operated \( \text{K}^- \) current (\( I_{\text{K,ACH}} \)). In our previous study, pharmacological blockade of \( I_{\text{Kr}} \), \( I_{\text{K1}} \) or \( I_{\text{K,ACH}} \) has been shown to prolong the action potential duration of the pulmonary vein myocardium. Thus, such \( \text{K}^- \) channel-blocking properties of bepridil may contribute to the prolongation of effective refractory period and action potential duration in the pulmonary vein. In this study, bepridil hardly affected the conduction velocity, which may not enlarge the cycle length of atrial fibrillation, in contrast to the effect that class I antiarrhythmic drugs such as pilsicainide potently reduce the conductivity. Although the effect of bepridil on the effective refractory period was moderate in comparison with that of class I antiarrhythmic drugs, the electrophysiological actions of bepridil may be expected to contribute to antiarrhythmic action based on drug’s effects on the balance of the effective refractory period and the cycle length during atrial fibrillation.

In this study, bepridil suppressed the \( V_{\text{max}} \) in the pulmonary vein preparation more potently than the left atrium. A previous study has suggested that \( \text{Na}^+ \) current density under voltage-clamp conditions was similar in the pulmonary vein

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Table 1. Effects of Bepridil on the Action Potential Parameters of the Pulmonary Vein and Left Atrium

<table>
<thead>
<tr>
<th></th>
<th>Pulmonary vein</th>
<th>Left atrium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Bepridil</td>
</tr>
<tr>
<td>( r_{\text{P}} ) (mV)</td>
<td>(-73.8\pm0.9)</td>
<td>(-69.7\pm2.0)</td>
</tr>
<tr>
<td>( \text{OS} ) (mV)</td>
<td>(30.1\pm0.6)</td>
<td>(23.8\pm0.8^{**})</td>
</tr>
<tr>
<td>( \text{APD}_{90} ) (ms)</td>
<td>(31.5\pm2.8)</td>
<td>(44.2\pm4.3^*)</td>
</tr>
<tr>
<td>( \text{APD}_{90} ) (ms)</td>
<td>(80.9\pm3.1)</td>
<td>(114.4\pm8.9^*)</td>
</tr>
<tr>
<td>( V_{\text{max}} ) (V/s)</td>
<td>(183.7\pm20.9)</td>
<td>(91.8\pm15.1^{**})</td>
</tr>
</tbody>
</table>

The preparations were electrically driven at 1Hz. Parameters were obtained before (Control) and 20 min after application of 10 \( \mu \)M of bepridil. Resting potential (\( r_{\text{P}} \)), overshoot (\( \text{OS} \)), action potential duration at 50% (\( \text{APD}_{90} \)) and 90% (\( \text{APD}_{90} \)) repolarization, and maximum rate of phase 0 depolarization (\( V_{\text{max}} \)). Data are means\( \pm \)S.E.M. of 5 experiments. \(^*p<0.05, ^{**}p<0.01\), compared with the corresponding control values.
and left atrium, whereas the membrane potential in the pulmonary vein was significantly smaller than that in the left atrium. Importantly, class I antiarrhythmic drugs generally inhibit $V_{\text{max}}$ or Na$^{+}$ currents of the cardiomyocytes in a voltage-dependent manner; namely, the drugs cause a greater $V_{\text{max}}$ reduction at less negative conditioning membrane potential\(^{15,16}\) which may be associated with the current results of the relatively greater inhibitory effect of bepridil on the $V_{\text{max}}$ in the pulmonary vein than in the left atrium.

The extent of prolongation of action potential duration by bepridil in the pulmonary vein was greater than that in the left atrium, as shown in the Table 1. Similar results have been reported in the studies with the hypertrophied heart, where some K$^{+}$ channels such as $I_{\text{Ks}}$ or $I_{\text{Kx}}$ were down-regulated, showing that the effects of K$^{+}$ channel blockers including nifekalant or barium more potently prolonged the action potential duration.\(^{7-10}\) Since the current densities of the $I_{\text{Ks}}$ and $I_{\text{Kx}}$ channels were smaller in the pulmonary vein myocardium than the left atrium,\(^{2}\) we speculated that such electrophysiological properties in the pulmonary vein myocardium sensitize the myocardium to the multi-K$^{+}$ channel blocker bepridil.\(^{10-12}\)

The electrophysiological features of the pulmonary vein myocardium in the diseased condition may be different from those in the normal one, since our previous study has shown that chronic volume overload to the heart abbreviated the action potential duration of the canine pulmonary vein myocardium,\(^{20}\) which is in accordance with a clinical report that showed shorter effective refractory period of the pulmonary vein myocardium in patients with paroxysmal and persistent atrial fibrillation.\(^{21}\) Thus, further investigations will be effective to predict clinical action of bepridil with higher accuracy using diseased hearts.

In conclusion, bepridil prolonged the effective refractory period in the pulmonary vein. The currently observed electrophysiological property of bepridil suggests that its effects on reentry within the pulmonary vein are estimated to be greater than within the left atrium, which may be one of the key considerations to understand its antiarrhythmic mechanisms.

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REFERENCES

20) Nouchi H, Takahara A, Nakamura H, Namekata I, Sugimoto T,