Bepridil Blunts the Shortening of Action Potential Duration Caused by Metabolic Inhibition via Blockade of ATP-Sensitive K$^+$ Channels and Na$^+$-Activated K$^+$ Channels

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ABSTRACT

The effects of bepridil, a potent antiarrhythmic drug, on the activity of ATP-sensitive K$^+$ (K$_{ATP}$) channels and Na$^+$-activated K$^+$ (K$_{Na}$) channels were examined in isolated patches from guinea pig ventricular myocytes. In inside-out membrane patches, K$_{ATP}$ channel currents were recorded with 140 mM [K$^+$], and 140 mM [K$^+$]$_o$ solutions, and K$_{Na}$ channel currents were recorded by increasing [Na$^+$] to 100 mM with 40 mM [K$^+$]$_o$, respectively. Bepridil (1–100 μM) inhibited the K$_{ATP}$ channel current in a concentration-dependent manner. The IC$_{50}$ value of bepridil was estimated to be 10.5 μM for outward K$_{ATP}$ channel currents (holding potential, +60 mV) and 6.6 μM for inward K$_{Na}$ channel currents (holding potential, −60 mV). Bepridil (0.1–30 μM) also inhibited K$_{Na}$ channel currents measured at the holding potential of −60 mV, in a concentration-dependent manner with an IC$_{50}$ value of 2.2 μM. In coronary-perfused guinea pig right ventricular preparations, the metabolic inhibition (MI) achieved with the application of 0.1 μM carbonyl cyanide p-(trifluoromethoxy)phenylhydrazone shortened the action potential duration (APD) in a time-dependent manner. When bepridil (10 μM) was applied 5 min after the introduction of MI, the APD shortening was significantly blunted. The concomitant application of a K$_{ATP}$ channel antagonist (glibenclamide, 1 μM) and a K$_{Na}$ channel antagonist (RS6865, 10 μM) could mimic the effect of bepridil and attenuated the shortening otherwise produced by MI. These results suggest that bepridil inhibits both K$_{ATP}$ channels and K$_{Na}$ channels and blunts the shortening of APD during MI. These effects of bepridil may partly account for the alleged antiarrhythmic action of this drug during ischemia.

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ABBREVIATIONS: K$_{ATP}$, ATP-sensitive K$^+$; APD, action potential duration; FCCP, carbonyl cyanide p-(trifluoromethoxy)phenylhydrazone; IC$_{50}$, concentration of half-maximum inhibition; NPo, open probability; K$_{Na}$, Na$^+$-activated K$^+$; MI, metabolic inhibition; RS6865, N[1-(4-(4-fluorophenoxoy)butyl)-4-piperidinyl]-N-methyl-2-benzothiazolamine.
that bepridil inhibits both K$_{ATP}$ and K$_{Na}$ channels and blunts the APD shortening during metabolic inhibition.

**Materials and Methods**

All procedures conformed to the guidelines stipulated by the Physiological Society of Japan and the Animal Ethics Committee of Oita Medical University.

**Chemicals**

Bepridil (a kind gift from Sankyo, Tokyo, Japan), glibenclamide (a kind gift from Hoechst Japan, Tokyo, Japan), R56865 (N-[1-(4-fluorophenoxo)butyl]-4-piperidinyl-N-methyl-2-benzoazolamine; a kind gift from Janssen Research Foundation, Beerse, Belgium), FCCP [carbonyl cyanide p-(trifluoromethoxy)phenylhydrazone; Sigma Chemical Co., St. Louis, MO] were dissolved in dimethyl sulfoxide as stock solutions. Each stock solution was added to the experimental solution immediately before use to produce the final concentration given in the text. Control experiments were performed with 0.08% dimethyl sulfoxide, which was the maximal concentration used and had no effect on the ionic currents, action potentials, and contraction.

**Isolated Patches from Single Ventricular Myocytes**

**Cell Isolation.** Single guinea pig ventricular myocytes were isolated enzymatically using a modified procedure described by Taniguchi et al. (1981). In brief, guinea pigs weighing 250 to 300 g were stunned by a blow on the neck, and the heart was quickly dissected and perfused through the coronary arteries with modified Tyrode’s solution. The composition of modified Tyrode’s solution was 137 mM NaCl, 3 mM NaHCO$_3$, 5.4 mM KCl, 0.16 mM NaH$_2$PO$_4$, 0.5 mM MgCl$_2$, 1.8 mM CaCl$_2$, 5.5 mM glucose, and 5 mM HEPES (pH 7.4 with NaOH). After 5 min of perfusion, the hearts were perfused without Ca$^{2+}$ for an additional 5 min. The perfusate was switched to Ca$^{2+}$-free modified Tyrode’s solution containing 0.005% collagenase (Type I; Yakult, Tokyo, Japan). After a 3- to 5-min perfusion, the heart was immersed in KB solution composed of 5 mM KCl, 70 mM glutamic acid, 10 mM taurine, 10 mM oxalic acid, 5 mM KH$_2$PO$_4$, 5 mM HEPES, 11 mM glucose, and 0.5 mM EGTA (pH 7.4 with KOH). The temperature of these perfusates was maintained at 35–36°C. A small piece of tissue was detached from the ventricles, and the cells were dispersed by stirring in the recording chamber (0.8 ml in volume) and mounted on the stage of an inverted microscope (TMD; Nikon, Tokyo, Japan). Rod-shaped cells with a clear margin and striation were used for the experiments.

**Electrophysiological Measurements.** Conventional patch-clamp techniques (Hamill et al., 1981) were used to record K$_{ATP}$ and K$_{Na}$ channel currents from inside-out membrane patches. The resistance of the patch electrodes ranged from 3 to 5 MΩ. The composition of the pipette solution (extracellular medium) was 140 mM KCl, 1.8 mM CaCl$_2$, 0.53 mM MgCl$_2$, 5.5 mM glucose, and 5 mM HEPES (pH 7.4 with KOH). After a gigahm-seal formation, the patch membrane was excised to make inside-out patches in bath solution (intracellular medium). For K$_{ATP}$ channel current recording, the composition of the bath solution was 140 mM KCl, 5 mM HEPES, 5 mM EGTA, and 5.5 mM glucose (pH 7.3 with KOH). For K$_{Na}$ channel current recordings, 2 mM ATP was added to the bath solution to block the K$_{ATP}$ channel activity completely after recording this channel current; then, the bath solution was switched to a high-sodium solution to evoke K$_{Na}$ currents, which contained 100 mM NaCl, 40 mM KCl, 2 mM MgCl$_2$, 2 mM Na$_2$ATP, 5 mM HEPES, 5 mM EGTA, and 5.5 mM glucose (pH 7.4 with KOH). All experiments were carried out at room temperature (~22°C).

Single channel currents were recorded using a patch-clamp amplifier (EPC-7; List) and stored on magnetic tape using a PCM data recording system (RP-880; NF Electronic Instruments, Tokyo, Japan). The data were replayed and processed with a computer (Macintosh LC III; Apple Japan) equipped with an analog-to-digital converter (MacLab 2e; AD Instruments, Tokyo, Japan). In our preliminary experiments, when the single channel currents were digitized at 1 to 5 kHz, the open probability (NPO) of the K$_{ATP}$ and K$_{Na}$ channels was not affected. Therefore, the current signals were filtered at 3 kHz and digitized at 1 to 2 kHz. The channel activity was measured as NPO, which was calculated from the equation $P_{o} = I/(NI)$, where $I$ is the mean current carried by all K$_{ATP}$ or K$_{Na}$ channels activated in a particular patch for a certain period of time, $N$ is the number of functioning channels in the patch, and $i$ is the unitary current of the K$_{ATP}$ or K$_{Na}$ channel. The mean current ($I$) was obtained over 20 s as time-averaged K$_{ATP}$ or K$_{Na}$ currents, measured as the difference between the baseline (a current level where all channels are in the closed state) and the current levels where some channels are in the open state. The NPO that was obtained from the current trace record for 20 s during the application of bepridil (NPO$_{initial}$) was normalized relative to the NPO of the predrug control (NPO$_{control}$). This relative NPO (NPO$_{before}$/NPO$_{control}$) was plotted against various concentrations of bepridil, and the data were fitted by the following Hill equation with the use of the least-squares method:

$$\text{Relative NPO} = \frac{1}{1 + ((C/C_0)^{1/h})^1}$$

where [C] is the concentration of bepridil, C$_{50}$ is the bepridil concentration at the half-maximum inhibition of the channel current, and $h$ is the Hill coefficient. With all these patches, the NPO was restored to >80% of the predrug (control) NPO after removal of bepridil from the perfusate.

**Coronary-Perfused Right Ventricular Myocardium**

**Preparations.** The isolated coronary-perfused guinea pig right ventricular free wall was prepared as described previously (Shigematsu et al., 1995). In brief, the isolated right ventricular free wall preparation, in which the coronary artery was cannulated via the aorta, was mounted in a chamber, with the aortic end of the preparation attached to the floor of the chamber. The coronary artery was perfused with oxygenated Tyrode’s solution composed of 136.7 mM NaCl, 11.9 mM NaHCO$_3$, 5.4 mM KCl, 0.42 mM NaH$_2$PO$_4$, 0.5 mM MgCl$_2$, 1.8 mM CaCl$_2$, and 11 mM glucose (pH 7.35–7.40 when gassed with 97% O$_2$/3% CO$_2$). The flow rate was maintained at 1.0 ± 0.2 ml/min/g wet weight using a roller pump (MP-3; Tokyo Rikakikai, Tokyo, Japan), with an intra-aortic pressure ranging from 40 to 50 mm H$_2$O. The surface of the preparation was superfused with glucose-free hypoxic Tyrode’s solution (10 ml/min) to minimize direct O$_2$ diffusion from the surface of the preparations into the muscles. The composition of the hypoxic Tyrode’s solution was the same as above, except that it contained no glucose and was gassed with 97% N$_2$ and 3% CO$_2$. The temperatures of these solutions were maintained at 37 ± 0.5°C.

**Electromechanical Measurements.** The basal portion of the preparation was stimulated at 3 Hz throughout the experiment with the use of a pair of platinum electrodes connected to the isolation unit of an electrical stimulator (SS-302J; Nihon Kohden, Tokyo, Japan). Action potentials were recorded from the epicardial site of the ventricular muscle fiber that was located deep (usually five or six cell layers) in the subepicardial surface with the use of a flexibly mounted microelectrode. Microelectrodes (tip resistance, 20–30 MΩ) were filled with 3 M KCl. A direct current preamplifier (MEZ-7101; Nihon Kohden) was used to record the transmembrane potential. Contractile tension was recorded using a force transducer (TB-612T; Nihon Kohden) connected to the apical end of the preparation. Resting tension was adjusted to obtain the optimal developed tension. The membrane potential and contractile tension were monitored on a multichannel thermal arrayorderer (WT-645G; Nihon Kohden).

**Data Analysis**

All data are expressed as mean ± S.E., and the number of cells tested was given (n) for patch-clamp experiments (all individual
patches were taken from different cells; the number of patches used is equivalent to the number of cells used) and the number of preparations for the experiments with coronary-perfused ventricular muscles. ANOVA with the Fisher post hoc test and paired or unpaired \( t \) tests were used to assess statistical significance. A value of \( P \leq .05 \) was considered statistically significant.

### Results

**Effect of Bepridil on \( K_{ATP} \) Channels.** We first examined the effects of bepridil on \( K_{ATP} \) channel currents recorded in inside-out membrane patches. Figure 1A shows the representative effects of bepridil on outward \( K_{ATP} \) channel currents. After the inside-out patch was prepared from ventricular myocytes, the membrane potential was held first at +60 mV to generate the outward current through the \( K_{ATP} \) channel. Multiple channels with a unitary channel conductance of \( \sim 80 \) pS were opened. Bepridil at a concentration of 10 \( \mu \)M decreased the \( K_{ATP} \) channel current without affecting the unitary current amplitude. After removal of the drug from the perfusate, the current was restored. Concentration-dependent effects of bepridil on the outward \( K_{ATP} \) channel current are summarized in Fig. 2. The relative channel activity (relative NPo) measured 1.5 min after the application of bepridil was plotted against the drug concentrations tested (1–100 \( \mu \)M). The IC\(_{50}\) value of NPo was attained at a bepridil concentration of 10.5 \( \mu \)M with a Hill coefficient of 1.01.

The membrane potential was then held at -60 mV to generate the inwardly directed \( K_{ATP} \) channel current. As shown in Fig. 3A, the application of bepridil (10 \( \mu \)M) decreased the \( K_{ATP} \) channel activity. The IC\(_{50}\) value and Hill coefficient of bepridil for inhibition of the inward \( K_{ATP} \) channel currents were 6.6 \( \mu \)M and 0.86, respectively (Fig. 4). Glibenclamide at a concentration of 1 \( \mu \)M blocked both out-
the outwardly directed KNa channel current. We verified that we did not systematically examine the effect of bepridil on more positive than 20 mV as reported by Wang et al. (1991), (Fig. 6). Because the slope conductance of K Na channels was concentration dependent, and the IC50 value for the outward current was estimated to be 6.6 μM.

![Fig. 4](image)

**Fig. 4.** Concentration-response relationship for bepridil inhibition of inward KATP channel currents at -60 mV. The relative NPo of the channels was plotted against the bepridil concentration. Data are mean ± S.E. with the number of patches tested indicated in parentheses. The curve was drawn to fit the Hill equation. The IC50 value for the outward current was estimated to be 6.6 μM.

ward (Fig. 1B) and inward KATP channel currents (Fig. 3B) and decreased the mean relative NPo by 94 ± 3% (n = 4) and 96 ± 2% (n = 5), respectively.

**Effect of Bepridil on KNa Channels.** To test the effect of bepridil on KNa channels, a KNa channel current was elicited by exposing an inside-out patch to a high-sodium solution. As shown in Fig. 5A, after the KATP channel current was recorded in the high-potassium solution (Fig. 5A-a), ATP at a concentration of 2 mM was added to the bath solution to block the KATP channel activity, where the inward rectifier K+ current (IK1) remained unaffected (Fig. 5A-b). When the bath solution was switched to the high-sodium solution ([Na+]i = 100 mM), the KNa channel current was eventually activated (Fig. 5B-a). The KNa channel current was observed in ~25% of the patches excised under our experimental conditions (n = 108). The mean slope conductance of the KNa channel was 216 ± 15 pS (n = 5), a value consistent with a previous report (Wang et al., 1991). As shown in Fig. 5, B-b and B-c, bepridil (10 μM) reversibly inhibited the KNa channel currents. The blocking effect of bepridil on KNa channel currents was concentration dependent, and the IC50 value and the Hill coefficient were 2.2 μM and 0.85, respectively (Fig. 6). Because the slope conductance of KNa channels was considerably decreased (to 123 ± 17 pS; n = 5) at potentials more positive than 20 mV as reported by Wang et al. (1991), we did not systematically examine the effect of bepridil on the outwardly directed KNa channel current. We verified that R56865 (10 μM), an alleged KNa channel inhibitor (Carmeliet and Tytgat, 1991), blocked the inwardly directed KNa channel currents (Fig. 5B-d) and decreased the mean relative NPo by 92 ± 3% (n = 4).

**Effects of Bepridil on APD and Contractile Tension during Metabolic Inhibition (MI).** In the next series of experiments, with coronary-perfused right ventricular myocardium, we examined the effects of bepridil on the APD and the contractile tension during MI and compared the results with those for glibenclamide and R56865. MI was achieved by the addition of 0.1 μM FCCP, a mitochondrial uncoupler of oxidative phosphorylation, and by omitting glucose from the coronary-perfused Tyrode’s solution. As shown in Fig. 7, MI induced the shortening of the APD in a time-dependent manner. After a 20-min exposure to MI (control MI), the APD was shortened to 14.3 ± 2.0% of the pre-MI value (from 179.3 ± 2.7 to 26.4 ± 3.6 ms, n = 5). When the application of bepridil (10 μM) was started 5 min after the introduction of MI, the shortening of the APD was dramatically blunted and remained as high as 84.5 ± 1.2% of the pre-MI values after 20 min of MI. Based on our observations mentioned above, it is reasonable to speculate that bepridil inhibited both KATP channels and KNa channels and attenuated the MI-induced shortening of the APD. We therefore examined whether glibenclamide and/or R56865 mimics the effect of bepridil. Glibenclamide at a concentration of 1 μM (started also 5 min after introduction of MI) mitigated the APD shortening, albeit this effect was limited to only the initial 6 to 8 min of MI. In contrast, R56865 at a concentration of 10 μM significantly attenuated the shortening of the APD only in the later phase (10–20 min) of MI. Concomitant application of glibenclamide (1 μM) with R56865 (10 μM) prevented the APD shortening most effectively and significantly during almost the entire period of MI (from 8 to 20 min), although the effect was still apparently smaller in comparison with that seen in the presence of bepridil alone.

Using the same preparations for APD recordings, we compared the time course of changes in contractile tension during MI. There was no significant difference in the developed
tension between control and drug-treated preparations (Fig. 8). However, as shown in Fig. 9, the resting tension measured after 20 min of MI was significantly greater in the R56865-and/or glibenclamide-treated groups than in the control (MI) group. However, there was no significant difference recognized between the control (MI) and the MI-plus-bepridil group.

Discussion

The results of the present study demonstrate that: 1) in inside-out membrane patches from guinea pig ventricular cells, bepridil inhibited both $K_{ATP}$ and $K_{Na}$ channels in a concentration-dependent manner, and 2) in coronary-perfused right ventricular preparations, bepridil significantly blunted the shortening of the APD caused by MI.

The $K_{ATP}$ channels are activated by a decrease in intracellular ATP concentration (Noma, 1983) and selectively inhibited by antidiabetic sulfonylureas (Fosset et al., 1988). Recent molecular-biological studies reveal that cardiac $K_{ATP}$ channels are heteromultimers of inwardly rectifying potassium channel subunits (Kir6.2) and sulfonylurea receptors (SUR2A; Aguilar-Bryan et al., 1998). Other than intracellular ATP and sulfonylureas, several modulators of $K_{ATP}$ channels have been documented (Edwards and Weston, 1993). We previously reported that class Ia antiarrhythmic drugs such as cibenzoline, disopyramide, and procaainamide inhibit the $K_{ATP}$ channel current in guinea pig ventricular cells (Wu et al., 1992; Sato et al., 1993). In addition, class Ic antiarrhythmic drugs such as flecainide inhibit $K_{ATP}$ channels when the
Currents are directed outward but not when they are directed inward (Wang et al., 1995). In the present study, we found that bepridil, a class IV antiarrhythmic drug, blocked both outward and inward K<sub>ATP</sub> current in a concentration-dependent manner. On the other hand, the K<sub>Na</sub> channel is activated by an increase in intracellular Na<sup>+</sup> concentration (Kameyama et al., 1984; Wang et al., 1991; Mistry et al., 1997). The molecular structure of this channel is still unknown. R56865 is reported to be a potent inhibitor of K<sub>Na</sub> channels (Carmeliet and Tytgat, 1991), which we verified in the patch-clamp study. More recently, it has been reported that several antiarrhythmic drugs inhibit K<sub>Na</sub> channels in guinea pig ventricular myocytes (Mori et al., 1996). In the present study, we found that bepridil inhibited K<sub>Na</sub> channels with an IC<sub>50</sub> value of 2.2 μM, and K<sub>ATP</sub> channels with an IC<sub>50</sub> value of 6.6 to 10 μM; these concentrations are within or close to the therapeutic concentrations for humans of ~3 μM (Hollingshead et al., 1992).

Since the discovery of K<sub>ATP</sub> channels in heart cells by Noma (1983), it has been accepted that K<sub>ATP</sub> channels can be opened via hypoxic and ischemic conditions and that they shorten the APD in cardiac myocytes (Fairey and Findlay, 1990; Deutsch et al., 1991; Nakaya et al., 1991). In contrast, the physiological and pathophysiological significance of the K<sub>Na</sub> channel is poorly understood. Nevertheless, it is reasonable to speculate that the activation of K<sub>Na</sub> channels occurs during cardiac ischemia or digitalis intoxication and contributes to the shortening of the APD (Luk and Carmeliet, 1990; Veldkamp et al., 1994). In the present study, the MI (achieved with 0.1 μM FCCP plus glucose removal) shortened the APD in a time-dependent manner in coronary-perfused right ventricular preparations. When glibenclamide (a K<sub>ATP</sub> channel blocker) alone was applied 5 min after the introduction of MI, the APD shortening was significantly attenuated only during the early phase (6–8 min) of MI. In contrast, R56865 (a K<sub>Na</sub> channel inhibitor) alone attenuated the APD shortening only during the relatively late phase (10–20 min) of MI. Based on these results, it is reasonable to assume that 1) the decrease in the subsarcolemmal ATP concentration results in the activation of K<sub>ATP</sub> channels before the inhibition of Na<sup>+</sup>-K<sup>+</sup>-ATPase and 2) further depletion of ATP inhibits Na<sup>+</sup>-K<sup>+</sup>-ATPase, thereby leading to eventual increases in the intracellular Na<sup>+</sup> concentration that activates K<sub>Na</sub> channels. Consistent with this notion, Abe et al. (1999) reported that the inhibition of Na<sup>+</sup>-K<sup>+</sup>-ATPase preserves ATP, and leads to blockade of the K<sub>ATP</sub> channels. In fact, dihydro-ouabain (an Na<sup>+</sup>-K<sup>+</sup>-ATPase inhibitor) attenuated the MI-induced shortening of the APD via inhibition of the K<sub>ATP</sub> channels, when the drug application was started 5 min, but not 10 min, after the introduction of MI, using the same experimental design as used in the present study. These results imply that the Na<sup>+</sup>-K<sup>+</sup> pump is still operating 5 min after introduction of MI, whereas it is inhibited after a relatively longer period of MI. These findings taken together suggest that K<sub>Na</sub> channels are activated during the late phase of MI and principally contribute to the APD shortening in this phase.

In the present study, bepridil blunted the MI-induced shortening of the APD, when application was started 5 min after introduction of MI. Furthermore, the attenuation of the APD shortening was observed in early as well as late phases of MI. The concomitant application of glibenclamide with R56865 also attenuated the shortening of the APD during both early and late phases of MI. Although glibenclamide and R56865 only partially mimicked the effect of bepridil, it is reasonable to consider that the inhibition of K<sub>ATP</sub> and K<sub>Na</sub> channels by these agents contributes to the attenuation of the APD shortening during MI.

Activation of K<sub>ATP</sub> and K<sub>Na</sub> channels accelerates repolarization, shortens the effective refractory period, and confers deleterious consequences (e.g., provocation of reentrant arrhythmias; Rosen, 1995). Therefore, blockade of these channels by bepridil and subsequent prolongation of the APD (i.e., prolongation of the effective refractory period) could reduce the incidence of reentrant arrhythmias. On the other hand, it can be postulated that the prolongation of the APD by bepridil may enhance myocardial damage during ischemia and reperfusion (Shigematsu et al., 1995). However, unlike glibenclamide or R56865, bepridil did not increase the resting tension during MI (Fig. 9), presumably because of its inhibitory effect on L-type Ca<sup>2+</sup> channels (Yatani et al., 1986). Indeed, in common with other Ca<sup>2+</sup> channel antagonists, the cardioprotective effects of bepridil have been reported (Reifart et al., 1986; Watts et al., 1987; van Amsterdam et al., 1990).

In conclusion, bepridil blocks K<sub>ATP</sub> and K<sub>Na</sub> channels in cardiac ventricular cells in its therapeutic concentrations and blunts the APD shortening during MI. This property, along with its alleged cardioprotective effect, may be useful for the management of tachyarrhythmias encountered during ischemia.

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