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Inhibitory effect of bepridil on hKv1.5 channel current: comparison with amiodarone and E-4031

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Abstract

Effects of bepridil on the depolarization-activated outward K^+ currents (I_{out}) in rat atrial myocytes and the human cardiac K^+ (hKv1.5) channel current stably expressed in human embryonic kidney (HEK) 293 cells were examined, and compared with those of amiodarone and *N*-[4-[[1-[2-(6-methyl-2-pyridinyl)ethyl]-4-piperidinyl]carbonyl]phenyl] methanesulphonamide dihydrochloride dihydrate (E-4031). Membrane currents were recorded using patch-clamp techniques in enzymatically isolated rat atrial myocytes and HEK 293 cells expressing hKv1.5 channels. Bepridil potently inhibited I_{out} elicited by depolarization pulses and prolonged the action potential in rat atrial cells. Bepridil also inhibited the hKv1.5 channel current with the IC_{50} value of 6.6 μM. The inhibitory effects of bepridil on the currents in HEK 293 cells were voltage-dependent. Amiodarone weakly inhibited rat atrial I_{out} and hKv1.5 channel current. In contrast, E-4031 at a concentration of 10 μM had little influence on these currents. Thus, bepridil inhibits hKv1.5 channel current and the inhibitory effect may be useful for the treatment of atrial fibrillation.

Keywords: Antiarrhythmic agent; Ion channel; Membrane current; Repolarization

1. Introduction

Atrial fibrillation is the most common persistent cardiac arrhythmia and prevalence of atrial fibrillation is estimated to be 6% in those older than 65 years (Feinberg et al., 1995). Since atrial fibrillation is associated with considerable morbidity and mortality (Braud et al., 1985; Kopecky et al., 1987; Alpert et al., 1988), the insight is growing that it may be necessary to restore and maintain sinus rhythm rather than to control the ventricular rate during atrial fibrillation. Although the precise mechanism of atrial fibrillation is not fully understood, random reentry of coexisting multiple wavelets in the atria may be a likely mechanism (Allessie et al., 1985). As action potential duration is a major determinant of the refractory period, it would be of importance to evaluate the effects of antiarrhythmic drugs on repolarizing K^+ currents in atrial cells. Many K^+ currents including the delayed rectifier K^+ current (I_K), the transient outward current (I_{to}), the inward rectifier K^+ current (I_{K1}) and the muscarinic acetylcholine receptor-operated K^+ current ($I_{K.ACh}$) are involved in the repolarization of the atrial action potential (Roden and George, 1997). One of the primary K^+ currents involved in atrial repolarization is the ultra-rapid delayed rectifier K^+ current (I_{Kur}) because the K^+ current activates very rapidly and inactivates little during the whole plateau phase of the atrial action potential. The I_{Kur}-type outward currents have been recorded from rat (Boyle and Nerbonne, 1991), dog (Yue et al., 1996) and human atrial myocytes (Wang et al., 1993). It has been proposed that human Kv1.5 (hKv1.5) underlies the I_{Kur} found in human atrial myocytes (Wang et al., 1993; Fedida et al., 1993; Snyders et al., 1993). In addition, electrophysiological studies (Konarzewska et al., 1995; Li et al., 1996) have shown that the hKv1.5-like current could not be recoded from human ventricular myocytes. Therefore, antiarrhythmic drugs having inhibitory action on I_{Kur} would be expected to suppress reentrant atrial arrhythmias more effectively.

Bepridil is assumed to be an antiarrhythmic drug possessing classes I and IV properties. Several reports from our and other laboratories have demonstrated that bepridil inhibits the L-type Ca^{2+} current as well as the Na^+ current

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in isolated cardiomyocytes (Yatani et al., 1986; Hara and Nakaya, 1995). In addition, bepridil inhibits several K^+ currents such as the delayed rectifier K^+ current (I_K), $I_{K.ACh}$, I_{to}, the Na^+-activated K^+ current ($I_{K.Na}$) and the ATP-sensitive K^+ current ($I_{K.ATP}$) (Berger et al., 1989; Hara and Nakaya, 1995; Mori et al., 1998; Wang et al., 1999; Li et al., 1999). Such a multichannel blocker has been shown to be effective against not only ventricular arrhythmias but also atrial arrhythmias (Roden, 1995). However, effects of bepridil on I_{Kur} have not been evaluated. Accordingly, this study was undertaken to determine the effects of bepridil on the depolarization-activated outward K^+ currents (I_{out}) in rat atrial myocytes, which may include the I_{Kur}-type current (Boyle and Nerbonne, 1992; Bou-Abboud and Nerbonne, 1999), and the hKv1.5 channel current expressed in human embryonic kidney (HEK) 293 cells using the whole-cell patch-clamp technique. In addition, the effects of bepridil were compared with those of amiodarone, a prototype multichannel blocker, and *N*-[4-[[1-[2-(6-methyl-2-pyridinyl)ethyl]-4-piperidinyl]carbonyl]phenyl] methanesulphonamide dihydrochloride dihydrate (E-4031), a class III antiarrhythmic drug.

2. Methods

2.1. Isolation of single atrial cells

The experiments were performed under the regulations of the Animal Research Committee of Chiba University School of Medicine. This investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Single atrial myocytes were obtained from rats by enzymatic dissociation, as described previously (Mori et al., 1995). The hearts were rapidly removed from the rats (200–300 g) anesthetized with pentobarbital sodium, and mounted on a Langendorff perfusion system for retrograde perfusion with a normal HEPES–Tyrode solution (37 °C). The perfusate was then changed to a nominally Ca^{2+}-free Tyrode solution, and changed to a solution containing 0.04% w/v collagenase (Wako, Osaka, Japan). After digestion, the heart was perfused with high K^+, low Cl^- solution (modified Kraftbrühe (KB) solution) (Mori et al., 1995). Atrial tissue was cut into small pieces in the modified KB solution, and the pieces were gently agitated to dissociate cells. The cell suspension was stored in a refrigerator (4 °C) and used on the same day. The composition of the normal HEPES–Tyrode solution was (mM): NaCl 143, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 0.5, NaH_2PO_4 0.33, glucose 5.5 and HEPES–NaOH buffer 5.0 (pH 7.4). The nominally Ca^{2+}-free Tyrode solution was prepared by simply omitting $CaCl_2$ from the normal HEPES–Tyrode solution. The composition of the modified KB solution was (mM): KOH 70, L-glutamic acid 50, KCl 40, taurine 20, KH_2PO_4 20, $MgCl_2$ 3, glucose 10, ethylene glycol-bis-2-aminoetylether-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA) 1.0 and HEPES–KOH 10 (pH 7.4).

2.2. Transfection and cell culture

Full-length cDNA of human Kv1.5 was ligated to the mammalian expression vector pcDNA-3.1 (Invitrogen). HEK 293 cells were transfected with this plasmid using Lipofect AMINE PLUS (Gibco) followed by selection and propagation in Dulbecco's modified Eagle's medium (DMEM, Gibco) supplemented with 10% fetal bovine serum (Gibco), 100 U/ml penicillin, 100 μg/ml streptomycin and 1 mg/ml Geneticin (G 418, Gibco). The cultures were passed every 3–5 days by use of a brief trypsin treatment. The cells were maintained at 37 °C in 5% CO_2 and plated on glass cover slips 2–3 days before electrophysiological experiments.

2.3. Electrophysiological study

Whole-cell membrane current recordings were performed by the patch-clamp method. Single atrial myocytes or HEK 293 cells were placed in a recording chamber (1 ml volume) attached to an inverted microscope (Olympus IMT-2, Tokyo, Japan), and superfused with the HEPES–Tyrode solution at a rate of 3 ml/min. The temperature of the external solution was kept constant at 36 ± 1 °C. Glass patch pipettes with a tip diameter of 2–3 μm were heat-polished and filled with an internal solution composed of (mM): KOH 110, L-aspartate 110, KCl 20, $MgCl_2$ 1, ATP-K_2 5, phosphocreatine-K_2 5, EGTA 10 and HEPES–KOH 5 (pH 7.4). The free Ca^{2+} concentration in the pipette solution was adjusted to pCa 8. The resistance of the pipette filled with the internal solution was 1–3 MΩ. After the gigaohm seal between the tip and the cell membrane was formed, the membrane patch was disrupted by applying more negative pressure to make the whole-cell voltage-clamp mode. The electrode was connected to a patch/whole-cell clamp amplifier (Nihon Kohden CEZ-2300, Japan). Recording signals were filtered at 1-kHz band width, and series resistance was compensated by 40–70%. Voltage command pulses were generated, and data were acquired by an IBM compatible computer (Compaq Prolinea 4/50 with a 244 Mbyte hard disc, USA) using pCLAMP software (Version 5.5.1, Axon Instruments, Foster City, CA, USA). Current signals were digitized with a sampling interval of 2 kHz and stored on the hard disc of the computer. A liquid junction potential between the internal solution and the bath solution of −8 mV was corrected.

In the experiments using rat atrial myocytes, membrane currents were recorded by delivering 100-ms depolarizing pulses from a holding potential of −60 mV to voltages between −40 and +40 mV in increments of 10 mV at a rate of 0.1 Hz. After the stabilization of the membrane

currents in the control condition, the bath solution was switched from normal HEPES–Tyrode solution to Na^+-free and Co^{2+}-containing Tyrode solution to block Ca^{2+} current and Na^+ current. The composition of the solution was (mM): choline Cl 143, KCl 5.4, KH_2PO_4 0.33, $MgCl_2 \cdot 6H_2O$ 0.5, glucose 5.5, atropine sulphate 0.005, $CaCl_2$ 1.8, $CoCl_2$ 2 and HEPES–KOH 5 (pH 7.4). After the stabilization of the membrane current, rat atrial cells were exposed to antiarrhythmic drugs. After exposure to one of the antiarrhythmic drugs, 4-aminopyridine (3 mM) was applied to the cells since 4-aminopyridine was reported to inhibit the three components of I_{out}, i.e., $I_{K,fast}$, $I_{K,slow}$ and I_{ss}, in rat atrial cells (Boyle and Nerbonne, 1992). It has been reported that the 4-aminopyridine-sensitive I_{ss} (non-activating, steady-state current) resembles I_{Kur} in human atrial myocytes and Kv1.5 underlies the I_{ss} (Bou-Abboud and Nerbonne, 1999). Therefore, effects of antiarrhythmic drugs on the steady-state current of I_{out}, measured at the end of depolarization pulses, were evaluated.

In a part of experiments, effects of bepridil on the action potential were examined in rat atrial cells. The action potential was recorded in the current-clamp mode using the same pipette solution and the normal HEPES–Tyrode solution. The cells were stimulated by passing 2-ms currents through the pipette at a rate of 0.2 Hz.

In patch-clamp experiments using HEK 293 cells, membrane currents were elicited by 200-ms depolarizing pulses from −80 mV to voltages between −60 and 60 mV, and then clamp back to −40 mV for 200 ms in normal Tyrode solution. After the attainment of stable membrane currents, the cells were exposed to one of various antiarrhythmic drugs.

2.4. Drugs

The following drugs were used: bepridil (Sankyo Pharmaceutical, Tokyo, Japan), amiodarone (Taisho Pharmaceutical, Tokyo, Japan), E-4031 (Eisai Tsukuba, Japan), atropine sulfate monohydrate, 4-aminopyridine (Wako). Bepridil and amiodarone were prepared as a stock solution of 10 mM in dimethyl sulphoxide. Test carried out showed that the solvents had no influence on the membrane currents. Other drugs were dissolved in distilled water.

2.5. Statistics

All values are presented in terms of mean ± S.E.M. Student's *t*-test for paired or unpaired observations was used for statistical analysis. *P* values of less than 0.05 were considered significant. The concentration–effect data were fitted and IC_{50} values were obtained using Delta Graph Professional (Delta Point, Polaroid Computing, Tokyo, Japan).

3. Results

3.1. Effects of bepridil, amiodarone and E-4031 on I_{out} in rat atrial cells

Effects of bepridil on the I_{out} recorded from rat atrial myocytes in the Na^+-free and Co^{2+}-containing Tyrode solution were examined. Fig. 1A shows representative

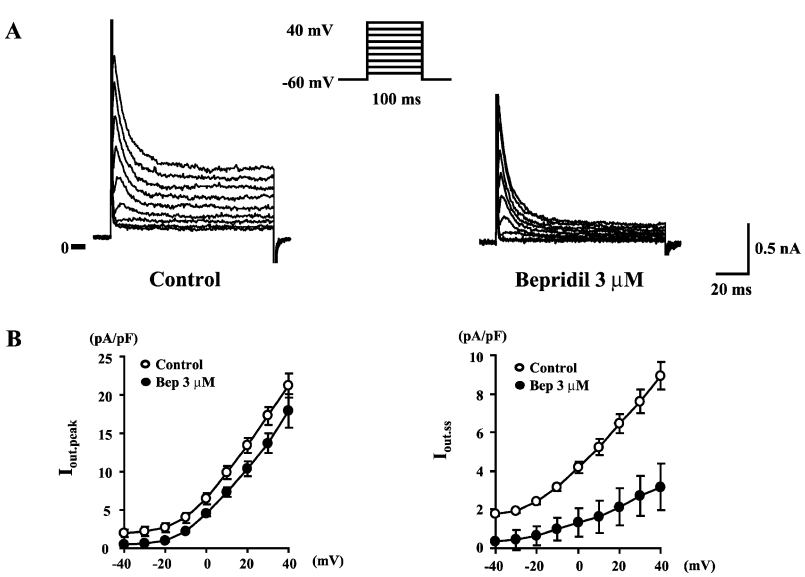

Fig. 1. Effects of bepridil on the depolarization-activated outward K^+ currents (I_{out}) in rat atrial myocytes. Membrane currents were elicited by 100-ms depolarizing pulses from −60 mV to voltages between −40 and +40 mV in steps of 10 mV in control condition and in the absence and presence of 3 μM bepridil (A). The current–voltage (*I–V*) relationships for the peak current ($I_{out.peak}$) and the steady-state current of I_{out} ($I_{out.ss}$) before (Control) and after 3 μM bepridil (Bep) are shown on the lower panel (B). Each point represents the mean ± S.E.M. of five rat atrial myocytes.

tracings of I_{out} before and after 3 μM bepridil. The outward current rose rapidly to a maximal level and then partially inactivated. It has been reported that I_{out} of rat atrial cells are composed of three distinct types K^+ currents referred to as $I_{K,fast}$, $I_{K,slow}$ and I_{ss}: $I_{K,fast}$ is a rapidly activating and inactivating current that resembles I_{to}; $I_{K,slow}$ is a rapidly activating, slowly inactivating current; and I_{ss} is a rapidly activating, non-inactivating (steady-state) current that resembles I_{Kur} (Boyle and Nerbonne, 1992; Barry and Nerbonne, 1996; Bou-Abboud and Nerbonne, 1999). The current–voltage (I–V) relationships for the peak current of I_{out} ($I_{out.peak}$) at the beginning of depolarization pulses and the steady-state current of I_{out} ($I_{out.ss}$) at the end of depolarization pulses are shown in Fig. 1B. Bepridil at a concentration of 3 μM reduced $I_{out.ss}$ (65.9 ± 3.3%) more effectively than $I_{out.peak}$ (16.7 ± 4.8%) at +40 mV.

Amiodarone also inhibited I_{out} in rat atrial cells although the potency was smaller than bepridil on molar basis. Fig. 2A shows representative tracings of I_{out} in the absence and presence of 10 μM amiodarone. The outward current was markedly suppressed by 3 mM 4-aminopyridine. The outward current ($I_{out.ss}$) measured at the end of the 100-ms depolarizing pulses to +40 mV before and after 10 μM amiodarone was 1.2 ± 0.3 and 0.6 ± 0.1 nA ($P < 0.05$, $n = 5$), respectively. In contrast, E-4031 hardly affected I_{out} in rat atrial cells, as shown in Fig. 2C. The $I_{out.ss}$ at +40 mV was insignificantly changed from 1.0 ± 0.2 to 1.1 ± 0.1 nA after 10 μM E-4031 (n.s., $n = 7$).

3.2. Effects of bepridil on the action potential

Effects of bepridil on the action potential of rat atrial cells were examined in the current-clamp mode. The baseline characteristics of action potentials recorded from single atrial myocytes stimulated at 0.2 Hz were as follows: resting membrane potential, −78.6 ± 1.1 mV; action potential amplitude, 139.5 ± 4.8 mV; action potential duration at 0 mV ($APD_{0\ mV}$), 17.9 ± 2.9 ms; action potential duration at −50 mV ($APD_{-50\ mV}$), 59.8 ± 5.3 ms ($n = 5$). Bepridil at a concentration of 1 μM prolonged $APD_{0\ mV}$ and $APD_{-50\ mV}$ by 38.2 ± 7.4% and 53.5 ± 7.8% ($P < 0.05$, $n = 5$), respectively, and the prolongation reverted toward the control after washout (Fig. 3).

3.3. Effects of bepridil, amiodarone and E-4031 on hKv1.5 channel current in HEK 293 cells

Effects of bepridil, amiodarone and E-4041 on the potassium current through hKv1.5 channels stably expressed in HEK 293 cells were examined. Representative current tracings in the absence and presence of bepridil are shown in Fig. 4A. The cell was held at −80 mV and 200-ms depolarizing pulses from −60 to +60 mV in increments of 10-mV steps were applied every 10 s. The hKv1.5 current rose rapidly to a peak and was then slowly and partially inactivated. Bepridil at a concentration of 10 μM reduced the steady-state current at +40 mV by

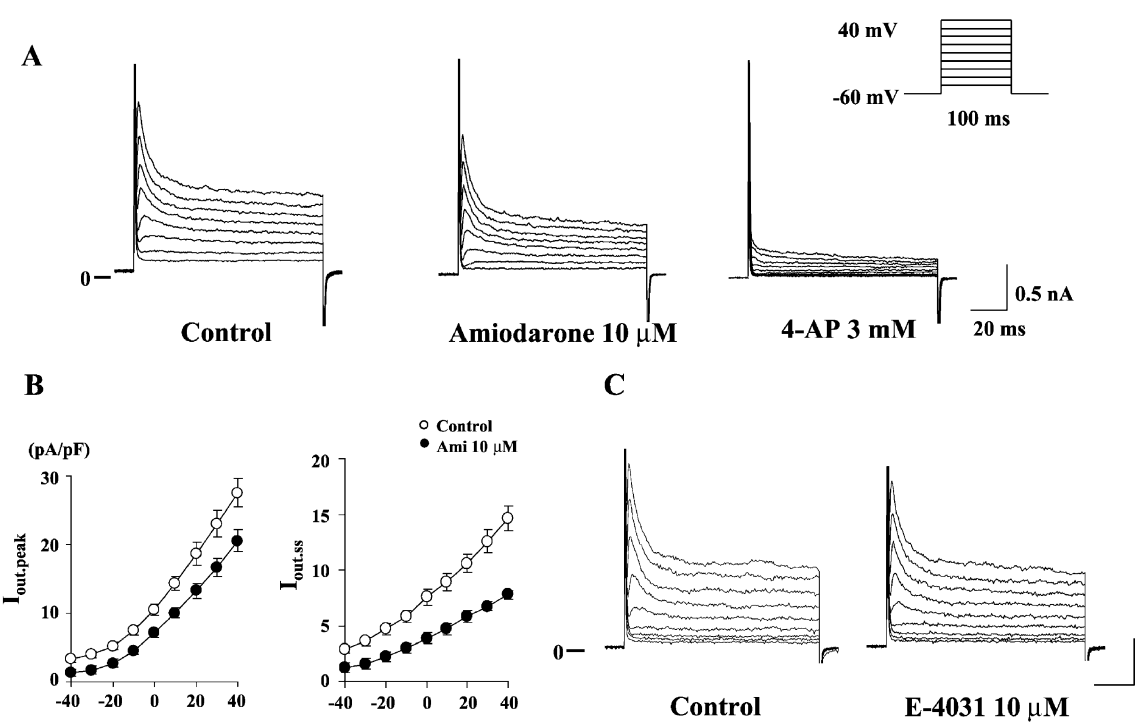

Fig. 2. Effects of amiodarone and E-4031 on I_{out} in rat atrial cells. Effects of amiodarone and 4-aminopyridine (4-AP) on I_{out} are shown in panel A. The current–voltage relationships for the peak current ($I_{out.peak}$) and the steady-state current of I_{out} ($I_{out.ss}$) before and after 10 μM amiodarone (Ami) are shown in panel B ($n = 5$). Representative changes of I_{out} after 10 μM E-4031 are shown in panel C.

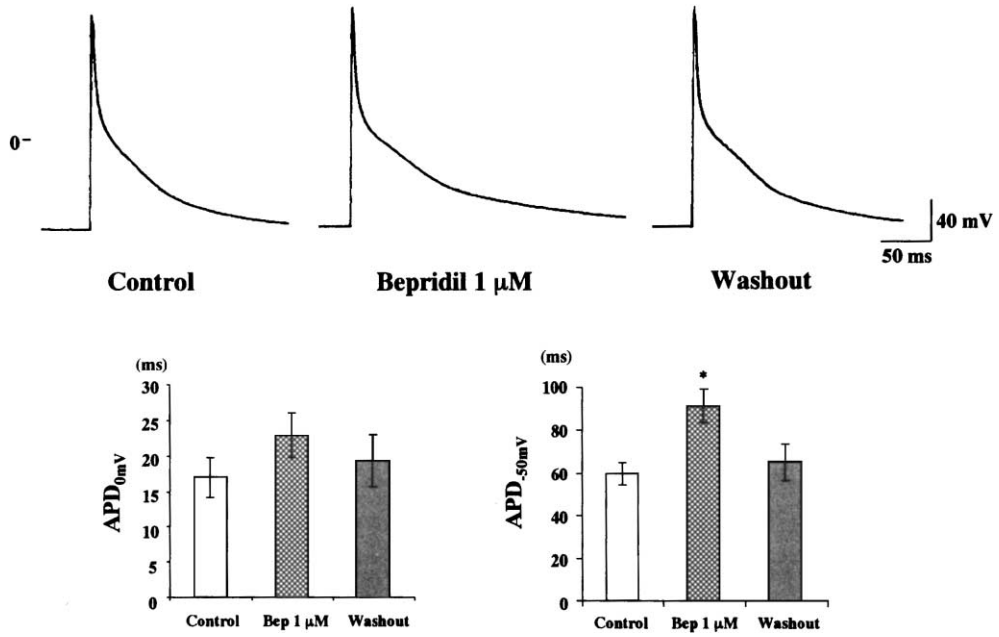

Fig. 3. Effects of bepridil on the action potential recorded from rat atrial cells. Representative changes of the action potential configuration after 1 μM bepridil are depicted in upper panels. Summarized changes of action potential duration at 0 mV ($APD_{0\ mV}$) and −50 mV ($APD_{-50\ mV}$) are shown in lower panel. Values are expressed as mean ± S.E.M. of five cells. $^{*}P < 0.05$ vs. control.

62.3 ± 3.0% (from 5.6 ± 0.3 to 2.1 ± 0.2 nA, $P < 0.05$, $n = 5$), and the peak current by 17.3 ± 2.7% (from 6.3 ± 0.3 to 5.2 ± 0.4 nA, $P < 0.05$). Bepridil inhibited the steady-state current measured at the end of the depolarizing pulses more effectively than the peak current, suggesting that bepridil binds preferentially to the open state of

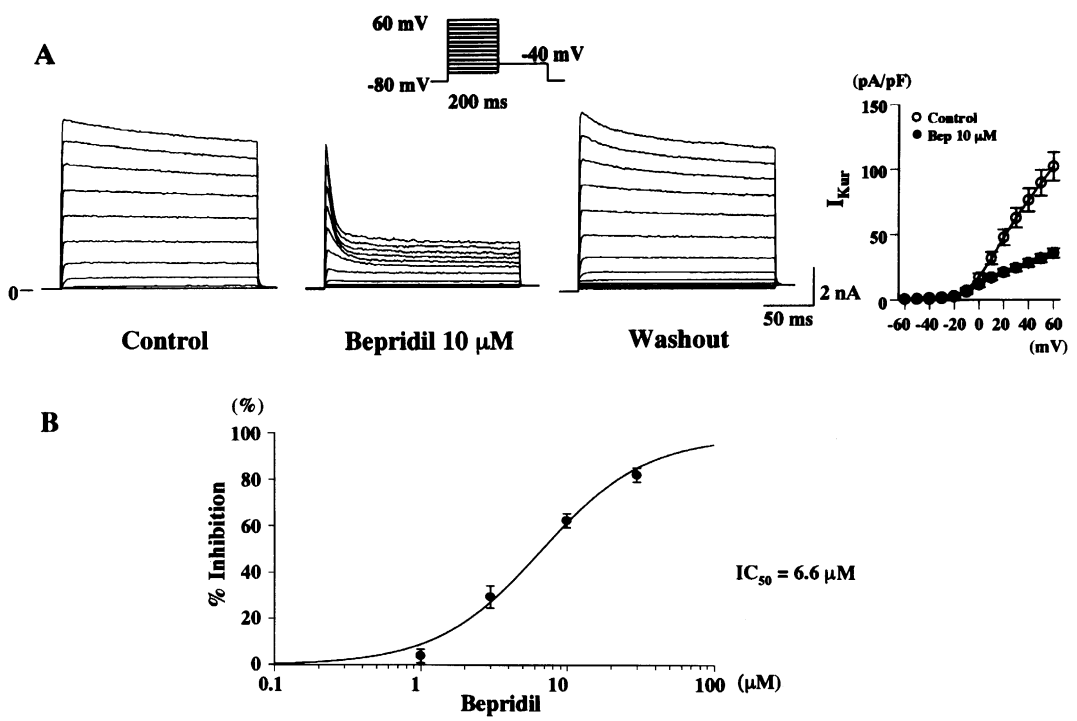

Fig. 4. Effects of bepridil on hKv1.5 channels stably expressed in HEK 293 cells. Representative current traces before, after 10 μM bepridil and after washout are shown in panel A. The steady-state current–voltage (I–V) relationships before and after bepridil (Bep) are shown on the right side of panel A ($n = 5$). Concentration–response curve for the inhibitory effect of bepridil on hKv1.5 channel current is shown in panel B. Percent inhibition of the current after 200-ms depolarizing pulse to +40 mV was used as an index of block, and the calculated IC_{50} value was 6.6 μM. Each point represents the mean ± S.E.M. of five experiments.

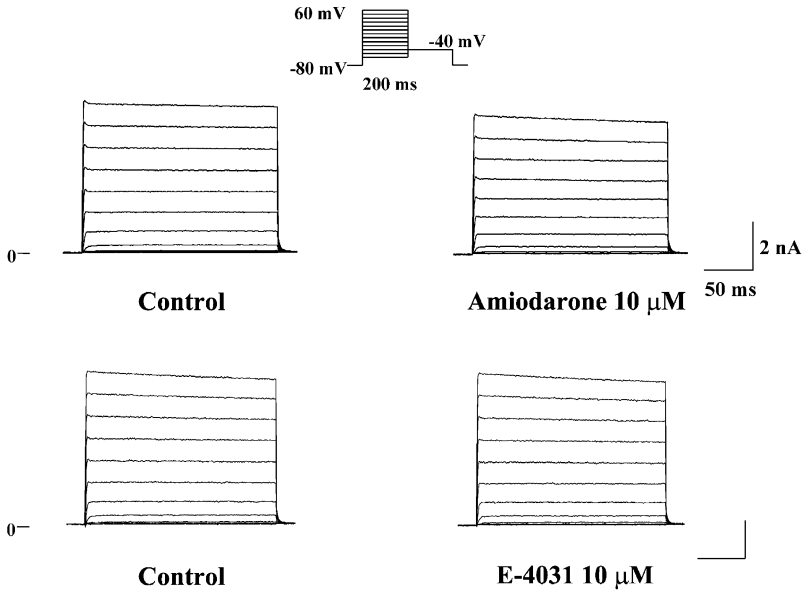

Fig. 5. Representative current traces of hKv1.5 channels expressed in HEK 293 cells after amiodarone (upper panel) and E-4031 (lower panel). Pulse protocol is shown in the upper inset. Note that neither amiodarone nor E-4031 produced a marked inhibition of hKv1.5 channel current in HEK 293 cells at a concentration of 10 μM.

the channel. The current–voltage relationships of the steady-state current before and after 10 μM bepridil are shown in Fig. 4A. Bepridil reduced hKv1.5 channel current over the whole voltage range over which this current was activated, but the inhibitory effect was more prominent at more positive potentials. The fractional block of hKv1.5 channel current by 10 μM bepridil at the end of depolarizing pulses to 0 and +60 mV was 0.31 ± 0.04 and 0.65 ± 0.03, respectively. Fig. 4B shows the concentration-dependent inhibition of the hKv1.5 channel current measured at the end of 200-ms depolarizing pulses to +40 mV by bepridil, and the calculated IC_{50} value was 6.6 μM.

In contrast, amiodarone and E-4031 failed to inhibit the hKv1.5 channel current markedly. Fig. 5 illustrates representative tracings of hKv1.5 channel current in the absence and presence of 10 μM amiodarone or 10 μM E-4031 in HEK 293 cells. The current measured at the end of the 200-ms depolarizing pulses to +40 mV was 4.3 ± 0.4 and 4.0 ± 0.4 nA before and after 10 μM amiodarone, respectively ($P < 0.05$, $n = 12$). The steady-state hKv1.5 channel was insignificantly changed from 4.4 ± 1.1 to 4.2 ± 1.2 nA by 10 μM E-4031 ($n = 4$).

4. Discussion

The I_{Kur}-type currents have been recorded in cardiomyocytes of many animal species such as rat (Boyle and Nerbonne, 1991; Guo et al., 1997; Bou-Abboud and Nerbonne, 1999), dog (Jeck and Boyden, 1992; Yue et al., 1996) and mouse (Fiset et al., 1997). The K^+ current is an outwardly rectifying and highly selective K^+ channel with a rapid time course of activation. The close biophysical correspondence between I_{Kur} identified in human atrial cells and hKv1.5 current suggests that hKv1.5 underlies I_{Kur} channel. Support for this concept derives from the observation that antisense oligodeoxynucleotides directed against the Kv1.5 coding sequence reduced I_{Kur} in cultured human atrial myocytes by 50%, without affecting other currents (Feng et al., 1997b). The I_{Kur} has been postulated to be the predominant delayed rectifier K^+ current responsible for human atrial but not ventricular repolarization (Konarzewska et al., 1995; Li et al., 1996). Therefore, it can be expected that antiarrhythmic drugs inhibiting I_{Kur} may effectively prevent the occurrence of atrial fibrillation without fear of excessive QT prolongation.

In this study, effects of antiarrhythmic drugs on the depolarization-induced outward currents (I_{out}) of rat atrial cells were examined. It has been reported that the I_{out} are composed of three different components of outward K^+ currents, i.e., $I_{K,fast}$, $I_{K,slow}$ and I_{ss} (Boyle and Nerbonne, 1992; Bou-Abboud and Nerbonne, 1999). From the experiments using antisense nucleotides targeted against Kv alpha subunits, Kv4.2, Kv1.2 and Kv1.5 underlie $I_{K,fast}$ (a rapidly activating and inactivating current like I_{to}), $I_{K,slow}$ (a rapidly activating, slowly inactivating current) and I_{ss} (a rapidly activating, steady-state current like I_{Kur}), respectively (Bou-Abboud and Nerbonne, 1999). Since all of these K^+ currents are sensitive to 4-aminopyridine (Boyle and Nerbonne, 1992; Bou-Abboud and Nerbonne, 1999; Nattel et al., 1999), we could not dissect I_{ss} through Kv1.5 channels from other K^+ currents using 4-aminopyridine. Bepridil potently inhibited the steady-state current of I_{out} at the end of depolarization pulses, suggesting that bepridil

might inhibit the K^+ currents through Kv1.5 channels. However, we cannot exclude the possibility that this drug might also block $I_{K,fast}$ and $I_{K,slow}$ through Kv4.2 and Kv1.2 channels, respectively. Indeed, bepridil was shown to inhibit I_{to} in sheep Purkinje fibers (Berger et al., 1989).

Bepridil prolonged APD effectively in rat atrial cells. The prolonging effect was more overt at $APD_{-50\ mV}$ than at $APD_{0\ mV}$. It is unclear why this drug prolonged $APD_{-50\ mV}$ more markedly than $APD_{0\ mV}$. The findings that bepridil inhibited the $I_{out.ss}$ more markedly than the $I_{out.peak}$ in rat atrial cells may explain the difference in the APD prolongation. However, we cannot exclude the possibility that bepridil might inhibit other K^+ current(s) and thereby prolong APD.

It has been reported that several antiarrhythmic drugs inhibit I_{Kur} and/or Kv1.5 current. Quinidine was shown to inhibit I_{Kur} at clinically relevant concentrations (IC_{50}: 5 μM) in human atrial myocytes although flecainide produced little effect on the K^+ current (Wang et al., 1995). Propafenone, a class Ic antiarrhythmic drug, was also reported to inhibit I_{Kur} in human atrial cells and hKv1.5 current at the therapeutic concentrations (Franqueza et al., 1998; Seki et al., 1999). In terms of effects of class III antiarrhythmic drugs on I_{Kur}, ambasilite and clofilium were shown to inhibit I_{Kur} in human atrial myocytes although both d-sotalol and E-4031 failed to alter the K^+ current (Snyders and Yeola, 1995; Feng et al., 1997a). Consistent with the report, E-4031 hardly affected the I_{out} in rat atrial myocytes and hKv1.5 channel current expressed in HEK 293 cells.

Amiodarone is classified as a class III antiarrhythmic drug although the electropharmacological effects are really complex and the drug inhibits many membrane currents including Na^+, Ca^{2+} and K^+ currents (Kodama et al., 1997). Recent studies (Watanabe et al., 1996; Mori et al., 1996; Varro et al., 1996; Kiehn et al., 1999) have suggested that acute and chronic administration of amiodarone inhibit different kinds of K^+ currents. Acute amiodarone inhibits I_{Kr}, I_{K1}, $I_{K.ACh}$ and $I_{K.Na}$ in addition to the inhibition of the Na^+ and Ca^{2+} currents, whereas chronic amiodarone inhibits I_{to} and I_{Ks}. The present study demonstrated that acute administration of amiodarone weakly suppressed hKv1.5 channel current in HEK 293 cells and I_{out} in rat atrial cells. In this context, a recent report (Rolf et al., 2000) showed that a high concentration (100 μM) of amiodarone slightly (by 18%) inhibited Kv1.5 current expressed in *Xenopus* oocytes. However, it was reported that chronic treatment with amiodarone reduced Kv1.5 mRNA level in rat ventricle (Kamiya et al., 1995). Therefore, we cannot exclude the possibility that long-term treatment with amiodarone decreases the density of I_{Kur} in atrial cells and prolongs the action potential duration.

Bepridil is an antiarrhythmic drug possessing multichannel blocking action like amiodarone although the drug does not have antiadrenergic effects on the heart. Bepridil was shown to inhibit many K^+ currents such as I_{Kr}, I_{Ks}, I_{K1}, I_{to}, $I_{K.ACh}$, $I_{K.Na}$ and $I_{K.ATP}$ in addition to its inhibitory action on the Na^+ and Ca^{2+} currents (Yatani et al., 1986; Berger et al., 1989; Hara and Nakaya, 1995; Mori et al., 1998; Li et al., 1999). In this study, bepridil also inhibited I_{out} of rat atrial cells and hKv1.5 channel current at clinically relevant concentrations. Since the therapeutic concentrations of bepridil were reported to be 0.5–4 μM in humans (Benet, 1985), the drug may partly inhibit hKv1.5 channel current in clinical settings.

Bepridil inhibited the steady-state currents more effectively than the peak currents, suggesting that the drug inhibits hKv1.5 channel current as an open channel blocker. The inhibition of these currents by bepridil was greater at more depolarized potentials. Most of the voltage-dependent block might be attributable to voltage dependence of hKv1.5 channel current activation. Thus, bepridil inhibited these currents in a time- and voltage-dependent fashion. Similar mode of blocking action was observed with quinidine (Wang et al., 1995) and propafenone (Franqueza et al., 1998; Seki et al., 1999).

It is well known that electrical remodeling, i.e., alteration of electrophysiological properties, can be induced in the atrial myocardium during repetitive electrical activity (Wijffels et al., 1995). Although initially atrial fibrillation terminates spontaneously within a few seconds, the repetitive induction of atrial fibrillation leads to progressive prolongation of the duration of the induced paroxysms of atrial fibrillation. This phenomenon is called as "atrial fibrillation begets atrial fibrillation" and probably due to the electrical remodeling such as shortenings of atrial action potential and atrial refractoriness (Wijffels et al., 1995). Although the precise mechanism(s) underlying the electrical remodeling have not been well established, the decreased density of the L-type Ca^{2+} current may play an important role in the electrical remodeling in the chronic phase (Yue et al., 1997). More recently, it has been demonstrated that high-rate atrial pacing increased the mRNA level of the Kv1.5 channel within a few hours in rat hearts (Yamashita et al., 2000). Therefore, the antiarrhythmic drugs possessing Kv1.5 channel blocking action may be useful to prevent or terminate the recurrence of paroxysmal atrial fibrillation within a short period.

Bepridil has been shown to inhibit I_{Kr}, I_{Ks}, (Li et al., 1999) and $I_{K.ACh}$ (Hara and Nakaya, 1995) in addition to Na^+ and Ca^{2+} channel blockade. This study has demonstrated that bepridil can also inhibit the I_{Kur}-type current ($I_{out.ss}$) in rat atrial cells and hKv1.5 channel current expressed in HEK 293 cells. These electrophysiological effects of bepridil may be useful for the antiarrhythmic effects, especially for the prevention or termination of atrial fibrillation. However, we have to extrapolate such experimental data to clinical settings with great caution because there may be some differences in the sensitivity to such a drug between rat and human atrial cells. In addition, only alpha-subunit of Kv1.5 channels was expressed in HEK 293 cells without coexpression of the beta-subunit in

this study. Therefore, the sensitivity of hKv1.5 channel to bepridil might be altered in native human atrial cells. Further studies are needed to determine whether bepridil is really effective to prevent the recurrence of atrial fibrillation in clinical settings.

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