British Journal of Pharmacology (1999) 128, 1733–1738
http://www.stockton-press.co.uk/bjp

Bepridil differentially inhibits two delayed rectifier K^+ currents, I_{Kr} and I_{Ks}, in guinea-pig ventricular myocytes

[1]Jin-Cheng Wang, *,[1]Tatsuto Kiyosue, [1]Kuninori Kiriyama & [1]Makoto Arita

[1]Department of Physiology, Oita Medical University, Hasama, Oita 879-5593, Japan

1 We investigated the effects of bepridil on the two components of the delayed rectifier K^+ current, i.e., the rapidly activating (I_{Kr}) and the slowly activating (I_{Ks}) currents using tight-seal whole-cell patch-clamp techniques in guinea-pig ventricular myocytes, under blockade of L-type Ca^{2+} current with nitrendipine (5 μM) or D600 (1 μM).

2 Bepridil decreased I_{Ks} under blockade of I_{Kr} with E4031 (5 μM), in a concentration-dependent manner. The concentration-dependent inhibition of I_{Ks} by bepridil was fitted by a curve, assuming one-to-one interactions between the channel and the drug molecule. The concentration of half-maximal inhibition (IC_{50}) was found to be 6.2 μM.

3 The effect of bepridil on I_{Kr} was assessed using an envelope-of-tails test. In the control condition, a ratio of the tail current to the time-dependent current measured during depolarization was large (>1) at shorter pulses (<200 ms), and it decreased to a steady state value of ~ 0.4 with increases in the pulse duration. Bepridil at a concentration of 2 μM did not decrease this ratio at shorter pulses.

4 In a short-pulse (duration = 50 ms) experiment that largely activates I_{Kr}, the drug was found to block I_{Kr} in a cooperative manner (Hill coefficient = 3.03) and the IC_{50} was 13.2 μM.

5 These results suggest that bepridil at a clinical therapeutic concentration (~ 2 μM) selectively blocks I_{Ks} but does not inhibit I_{Kr}. This may relate to the characteristic frequency-dependent effects of bepridil on the action potential duration (APD), e.g., the non-reverse use-dependent prolongation of APD.

Keywords: Bepridil; I_{Kr}; I_{Ks}; antiarrhythmic drug; ventricular myocytes

Abbreviations: APD, action potential duration; I_{Ca}, the L-type Ca^{2+} current; I_K, the delayed rectifier K^+ current; I_{Kdepo}, time-dependent current during depolarizing pulse; I_{Kr}, the rapidly activating delayed-rectifier K^+ current; I_{Ks}, the slowly activating delayed-rectifier K^+ current; I_{Ktail}, tail current of the delayed rectifier K^+ current

Introduction

Bepridil is a diarylaminopropylamine derivative with both anti-anginal and anti-arrhythmic effects: it dilates coronary vessels, limits consumption of oxygen, decreases the heart rate and prevents arrhythmias (Cosnier *et al*., 1977; Duchene-Marullaz *et al*., 1983; Pelleg *et al*., 1985; Marshall *et al*., 1983). Bepridil blocks the TTX-sensitive Na^+ current (Yatani *et al*., 1986; Nawada *et al*., 1995; Sato *et al*., 1996), the T-type Ca^{2+} current (Cohen *et al*., 1992), and the L-type Ca^{2+} current (Yatani *et al*., 1986). It has also been shown that bepridil blocks the outward K^+ currents including the inward rectifier (I_{K1}) and delayed rectifier K^+ current (I_K) in sheep cardiac Purkinje fibres (Berger *et al*., 1989). The effects of bepridil on the action potential duration (APD) differ depending on species and preparations. Bepridil shortened the APD in rabbit ventricular myocardium (Anno *et al*., 1984; Gill *et al*., 1992), and in guinea-pig ventricular myocytes (Yatani *et al*., 1986; Nawada *et al*., 1995). In contrast, it prolonged the APD and the effective refractory period of the ventricular muscle in canine hearts (Kato & Singh, 1986). This difference may be related to the fact that the APD is determined by a critical balance between inward and outward ionic currents, both of which are affected by bepridil. Another possibility would be differential effects of this drug on outward K^+ currents. The I_K in mammalian ventricles consists of at least two different K^+ channels, the rapidly activating I_{Kr} and the slowly activating I_{Ks} channels (Sanguinetti & Jurkiewicz, 1990). Each component has a distinct physiological role and expression levels differ from one species to another (Nair & Grant, 1997). If bepridil selectively suppresses either I_{Ks} or I_{Kr}, this may cause the differential effects on the APD, especially at different stimulation frequencies. Thus, the aim of the present study is to elucidate the effects of bepridil on the two components of I_K, namely I_{Kr} and I_{Ks}, which are known to exist in guinea-pig ventricular myocytes. A part of this study has appeared elsewhere in abstract form (Wang *et al*., 1998).

Methods

Single ventricular myocytes were isolated from guinea-pig hearts using an enzymatic dissociation procedure described previously (Wang *et al*., 1996). The dissociated myocytes were allowed to settle in a chamber on an X-Y stage of an inverted microscope (TMD, Nikon, Tokyo, Japan). The cells were superfused with an external bathing solution containing (in mM): NaCl 137, KCl 5.4, $CaCl_2$ 1.8, NaH_2PO_4 0.16, $NaHCO_3$ 3, N-[2-hydroxyethyl] piperazine-N′-[2-ethanesulfonic acid] (HEPES) 5, glucose 5.5, (pH 7.4). All the experiments were performed at 35 ± 0.5°C.

The whole-cell patch-clamp technique (Marty & Neher, 1983) was used to record the transmembrane ionic current by using a patch-clamp amplifier (CEZ-2100, Nihon Kohden, Japan). Patch pipettes were fabricated from borosilicate

*Author for correspondence; E-mail: kiyosue@oita.med.ac.jp

capillary-glass tubes (Narishige, Tokyo, Japan) using a puller (P-97, Sutter Instrument Co., Novato, CA, U.S.A.) and a heat-polisher (MF-83, Narishige, Tokyo, Japan). The electrodes were filled with an internal solution containing (in mM): KCl 140, EGTA 11, $CaCl_2$ 1, $MgCl_2$ 2, HEPES 10, ATP 5, CP 5, pH 7.2 by KOH. The tip resistances ranged from 1 to 3 MΩ. All experiments were performed in accordance with the Guidelines and Principles for Animal Experiments stipulated by the Animal Ethics Committee of the Oita Medical University and the Physiological Society of Japan.

Bepridil hydrochloride was a gift from Sankyo Pharmaceutical Co. (Tokyo, Japan); E4031 from Eisai Pharmaceutical Co. (Tokyo, Japan); and D600 and chromanol 293B from Hoechst Marion Roussel Deutschland GmbH (Frankfurt, Germany). Nitrendipine was purchased from Sigma Co. (St. Louis, MO, U.S.A.). Bepridil, nitrendipine and chromanol 293B were dissolved in dimethylsulphoxide (DMSO) to make the stock solution at concentrations of 10, 10 and 50 mM, respectively. E4031 was dissolved in distilled water at a concentration of 1 mM. D600 was dissolved in ethanol at a concentration of 1 mM. Each drug was stored at 4°C and diluted to desired final concentrations with Tyrode's solution just before use. The effect of each drug was assessed 4–5 min after application of the test solution.

The amplitude of I_K tail current (I_{Ktail}) was defined as a difference current between the holding current recorded just before initiation of depolarizing pulse and the peak tail current evoked on return to a holding potential (−40 mV). The amplitude of the time dependent current during depolarization (I_{Kdepo}) was measured from an initial minimal current after depolarization to a terminal current at the end of the pulse.

The current signals were stored on video cassette tapes using a Pulse-Code-Modulation Processor (VR-10B, Instrutech Co., Elmont, NY, U.S.A.) with a band width of DC-15 kHz, and were digitized using a personal computer (NEC PC-9801RS, Tokyo, Japan) equipped with an analogue-digital converter (ADX98, Canopus Co, Kobe, Japan). All values were expressed as mean ± s.e.mean. Student's *t*-test was used to determine the statistical significance between the means obtained before and after addition of the drug. A *P*-value of 0.05 or less was considered significant.

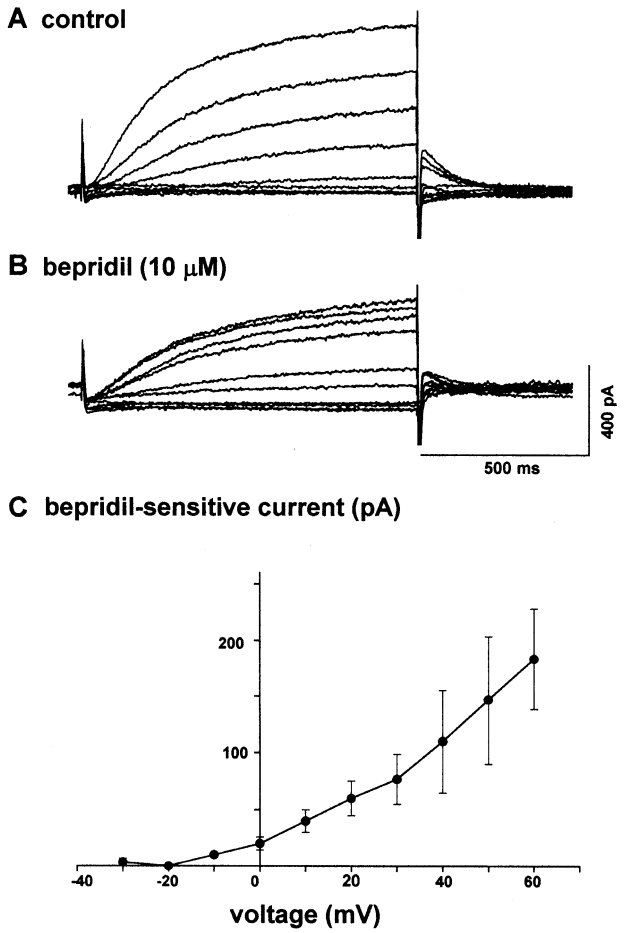

Figure 1 (A,B) Effects of bepridil on the outward current of a guinea-pig ventricular myocyte. Current families in the absence (A) and presence (B) of 10 μM bepridil. (C) Current-voltage relationship of bepridil-sensitive time-dependent current. The ordinate shows the difference in the time-dependent current at the end of the depolarizing pulses (abscissa), measured between corresponding traces in panels A and B. The lack of an apparent inward-going rectification in the I–V curve suggests that bepridil blocked the slowly activating component of the delayed-rectifier K^+ current, i.e., I_{Ks}. The L-type Ca^{2+} current was blocked by 1 μM D600. Temperature, 35°C.

Results

We examined first the effects of bepridil on the delayed rectifier K^+ current consisting of both I_{Kr} and I_{Ks} components. The L-type Ca^{2+} current was suppressed by nitrendipine (5 μM) or D600 (1 μM) and the Na^+ current was inactivated by holding the membrane potential at −40 mV. Depolarizing voltage steps with a duration of 1 s were applied in 10 mV increments. Figure 1 shows representative current traces obtained before (Figure 1A) and after application of 10 μM bepridil (Figure 1B). It is apparent that bepridil decreased the amplitudes of both time-dependent outward currents that appeared during depolarizing pulses and the tail currents evoked on return to the holding potential. Figure 1C shows the current-voltage relationship of the bepridil-sensitive time-dependent current. The current was increased with increases in membrane potential, thereby suggesting that bepridil might have suppressed I_{Ks}, which is reported to possess a property of no inward-going rectification (Sanguinetti & Jurkiewicz, 1990), as it is observed in Figure 1C.

Thus, we examined the effect of bepridil on I_{Ks}, more selectively, after blockade of I_{Kr} with 5 μM E4031 (Sanguinetti & Jurkiewicz, 1990). In order to activate I_{Ks}, we applied various levels of depolarizing pulses with a duration of 3 s at an interpulse interval of 10 s (Figure 2A). Bepridil (10 μM) decreased both the time-dependent outward current during depolarization (I_{Kdepo}) and the tail current (I_{Ktail}) evoked on clamping back to the holding potential (−40 mV). In Figure 2B, the bepridil-induced suppression of I_{Ktail} (evoked after depolarization to +60 mV) was plotted against various drug concentrations used. The data were well fitted by an equation assuming one-to-one interactions between the receptors (channels) and the drug molecules. The concentration of half-maximal inhibition (IC_{50}) was calculated to be 6.2 μM.

To examine whether or not bepridil blocks I_{Ks} in a voltage-dependent manner, the per cent current inhibition caused by 10 μM bepridil was plotted against voltage for either the tail current (open circles) or the time-dependent current measured at the end of depolarization (filled circles) in Figure 3. The data indicate that the bepridil-induced block of I_{Ks} is not voltage-dependent.

We then examined the effects of bepridil on I_{Kr} using the envelope-of-tails test (Noble & Tsien, 1969; Sanguinetti & Jurkiewicz, 1991). Depolarizing voltage steps from −40 mV to a test potential of +50 mV were applied with stepwise

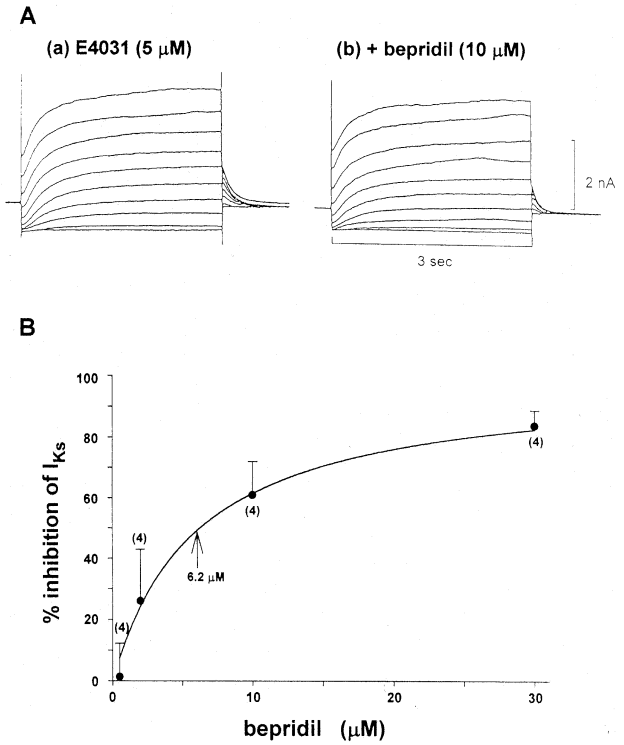

Figure 2 (A) Effects of 10 μM bepridil on the slowly activating delayed rectifier K^+ current, I_{Ks}, which was recorded after blockade of the rapidly activating component of the current, I_{Kr}, with 5 μM E4031. (B) Concentration-dependent effect of bepridil on I_{Ks}. The per cent inhibition of the tail current evoked after depolarization to +60 mV (duration of 1 s) was plotted against the drug concentrations tested. The data were fitted by the following equation, by assuming one-to-one interaction between the channel and the drug molecule: Fractional inhibition (per cent inhibition of I_{Ks}) = I_{max} *C/(C+K_d), where I_{max} is the maximal inhibition and K_d is a dissociation constant. I_{max} and K_d were calculated to be 100% and 6.2 μM.

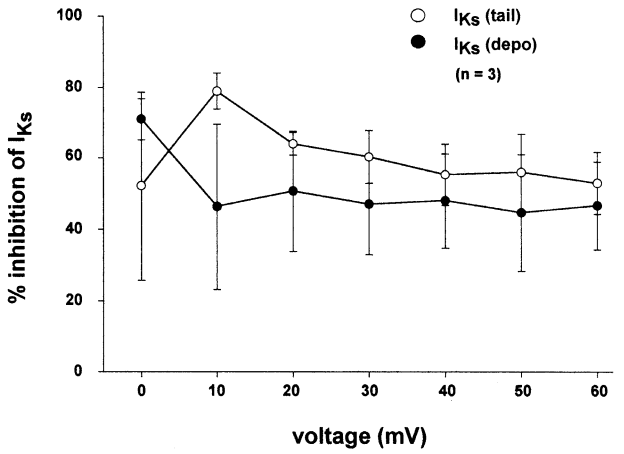

Figure 3 Voltage-independent effects of bepridil on I_{Ks}. The experiments were conducted in the presence of 5 μM E4031. Each data point and vertical bar indicate the mean and s.e.mean from three experiments.

increases in the pulse duration from 50 ms to 3 s. Figure 4A shows representative current traces obtained before (Figure 4A-a) and after application of 2 μM bepridil (Figure 4A-b). The ratio of the peak of I_{Ktail} to the amplitude of the time-dependent current (I_{Kdepo}) was plotted against the duration of

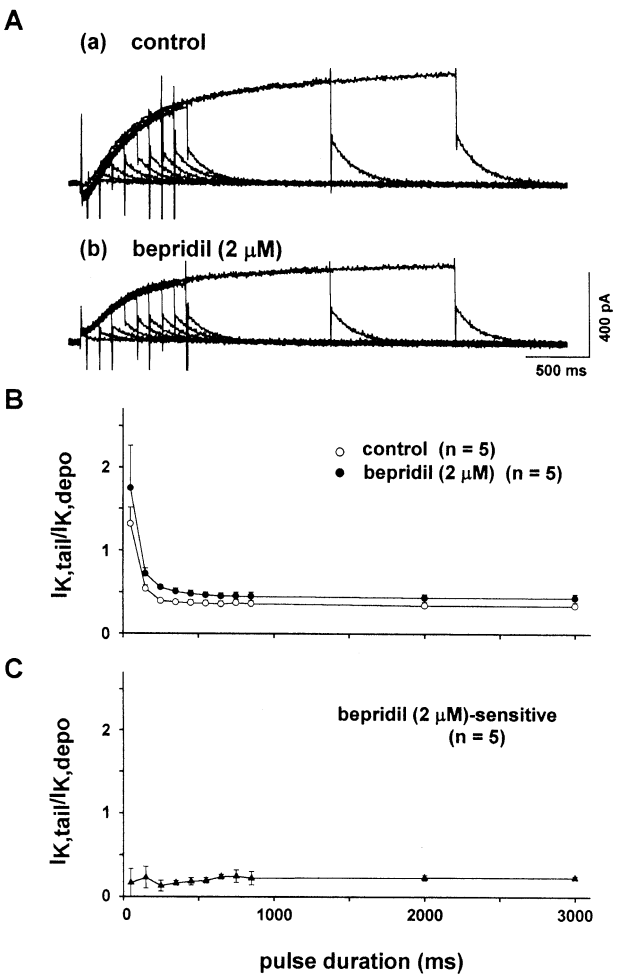

Figure 4 (A) Envelope-of-tails test in the absence (a) and presence of 2 μM bepridil (b). Depolarizing pulses to +50 mV from −40 mV were applied by increasing the pulse duration. (B) The ratio of the tail current (I_{Ktail}) to the time-dependent current during the pulse (I_{Kdepo}) was plotted against the pulse duration. Bepridil shifted the curve upward, preserving a large value in the I_{Ktail}/I_{Kdepo} ratio at the short pulse duration of $\leqslant$200 ms. (C) The I_{Ktail}/I_{Kdepo} ratio of the bepridil-sensitive current, which was obtained by subtracting the current in the presence of bepridil from the current in the absence of bepridil. The absence of a large I_{Ktail}/I_{Kdepo} ratio at shorter pulse durations suggests that bepridil at 2 μM did not block I_{Kr}, but did block I_{Ks} (*cf.* Figure 7C).

the depolarizing pulses (Figure 4B). Under the control condition (open circles), the ratio (I_{Ktail}/I_{Kdepo}) for the short pulses (<200 msec) was much larger than that for longer pulses, indicating the presence of two types of I_K (Sanguinetti & Jurkiewicz, 1991). Application of 2 μM bepridil shifted the curve upward over all pulse durations tested, without decreasing the I_{Ktail}/I_{Kdepo} ratio at shorter pulses, indicating that the drug at this concentration does not inhibit I_{Kr} (*cf.* Discussion). To obtain further support for this notion, we conducted the envelope-of-tails test on the bepridil-sensitive current, as shown in Figure 4C. The bepridil-sensitive current was obtained by subtraction of the current recorded in the presence of bepridil from that in the absence of the drug. It is apparent that the I_{Ktail}/I_{Kdepo} ratio was almost constant regardless of the pulse duration. In other words, the 2 μM bepridil-sensitive current represents only a single component of the delayed rectifier K^+ current, that is I_{Ks}.

Using the same protocol, the effect of bepridil on I_{Kr} was then examined using a higher drug concentration of 10 μM (Figure 5A). Bepridil again shifted the curve upward,

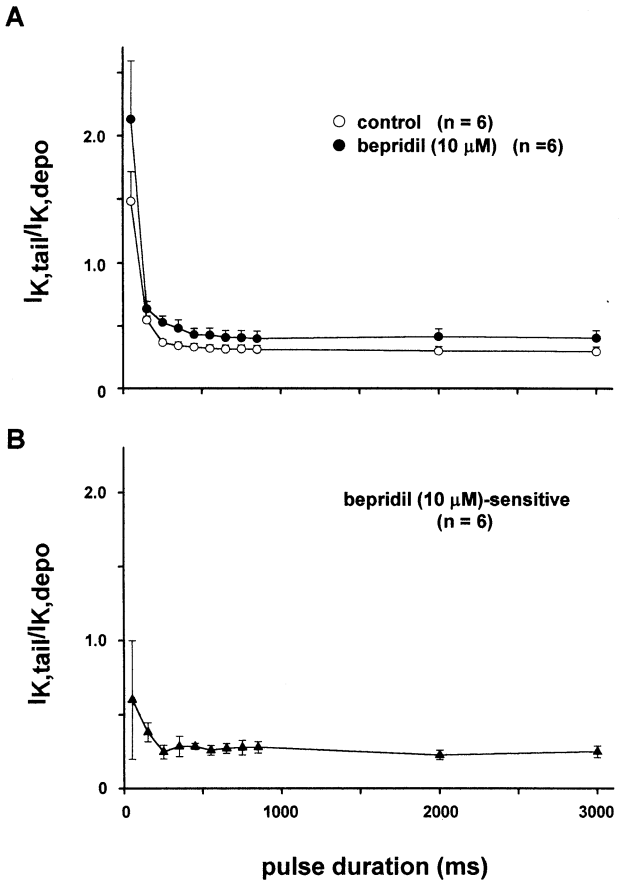

Figure 5 (A) Envelope-of-tails test in the absence and presence of 10 μM bepridil. (B) Envelope-of-tails test of the current blocked by 10 μM bepridil. A slight increase in the I_{Ktail}/I_{Kdepo} ratio at shorter pulses suggests that a higher concentration of bepridil ($\sim$10 μM) blocks both I_{Kr} and I_{Ks}.

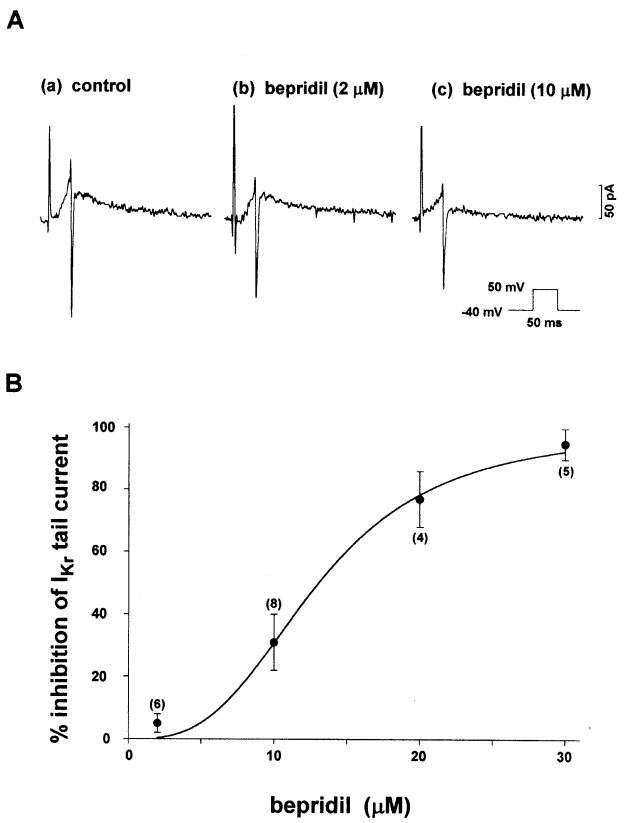

Figure 6 Effects of bepridil on I_{Kr} tail current. (A) Typical examples of the effect of bepridil at 2 and 10 μM on I_{Kr} evoked by short depolarizing pulses (duration = 50 ms; see inset). (B) The concentration-dependent inhibition of bepridil on I_{Kr}, examined using a short-pulse protocol. The data were fitted by a Hill equation: Fractional inhibition (per cent inhibition of I_{Kr}) = $\{K \cdot C^h/(1 + K \cdot C^h)\} \cdot 100$, where C, K, and h are the drug concentration, dissociation constant, and Hill coefficient, respectively. Best fit was obtained when K = 0.0004 and h = 3.03. The figures in parentheses indicate the number of cells tested.

indicating the suppression of I_{Ks} by the drug. However, the envelope-of-tails test of the current blocked by 10 μM bepridil (the drug-sensitive current) showed a slight increase in the I_{Ktail}/I_{Kdepo} ratio for short pulses (<0.1 s) as compared to the longer ones. These results suggest that 10 μM bepridil inhibits some fraction of I_{Kr} in addition to the blockade of I_{Ks}.

To study the effect of bepridil on I_{Kr} more quantitatively, we employed a short test-pulse protocol (duration = 50 ms) during which a negligible amount of I_{Ks} was activated, but there was a sufficient activation of I_{Kr}. The tail current evoked on returning to -40 mV was not influenced by 50 μM chromanol 293 B, a specific blocker of I_{Ks} (34.7 $\pm$ 5.6 pA for the control vs 35.1 $\pm$ 6.5 pA in the presence of 50 μM 293 B, n = 4). In contrast, the amplitude of the tail current was markedly decreased from 86.7 $\pm$ 14.5 pA to 18.3 $\pm$ 6 pA, or by 80% (n = 3) after application of E4031 (5 μM). This finding lends support to the notion that under the experimental condition, the tail current was composed of only one component of I_K, namely I_{Kr} alone. Figure 6A shows the effects of 2 and 10 μM bepridil on the tail currents (I_{Kr}) thus obtained; bepridil produced little (2 μM) or considerable (10 μM) depression on these tail currents. The concentration-dependent inhibition of the drug on the tail current, i.e., I_{Kr}, is summarized and plotted in Figure 6B. The inhibition was nil or small at low concentrations (2–10 μM) but was augmented rather steeply at higher concentrations (>20 μM). As the overall contour of the inhibition-concentration relationship seemed somewhat different from that for I_{Ks} (Figure 2B), we fitted the data with the following Hill equation: Per cent inhibition of $I_{Kr} = K \cdot C^h/(1 + K \cdot C^h) \cdot 100$, where C is the drug concentration, K is the apparent dissociation constant and h is the Hill coefficient. The best fit was obtained when K = 0.0004 and h = 3.03, indicating that the drug interacts with the channel in a highly cooperative manner. The concentration of half-maximal inhibition of I_{Kr} was found to be 13.2 μM.

Discussion

The major findings in the present study are as follows: (1) Bepridil decreased the I_{Ks} measured after blockade of I_{Kr} with the use of E4031 (5 μM). The concentration of half-maximal inhibition, IC_{50} was 6.2 μM. (2) In an envelope-of-tails test, 2 μM bepridil did not decrease, but rather increased the $I_{K\ tail}/I_{Kdepo}$ ratio at short pulses (<0.2 s), suggesting that the drug does not inhibit I_{Kr} at this concentration. However, a higher concentration (10 μM) of the drug slightly depressed I_{Kr}. (3) The concentration-dependent effect of bepridil on I_{Kr} was studied using the tail current activated by short depolarizing pulses (duration = 50 ms) which largely represented I_{Kr}. High concentrations ($\geq$10 μM) of bepridil depressed I_{Kr}, with an IC_{50} of 13.2 μM and the blockade occurred in a cooperative manner (Hill coefficient = 3.03).

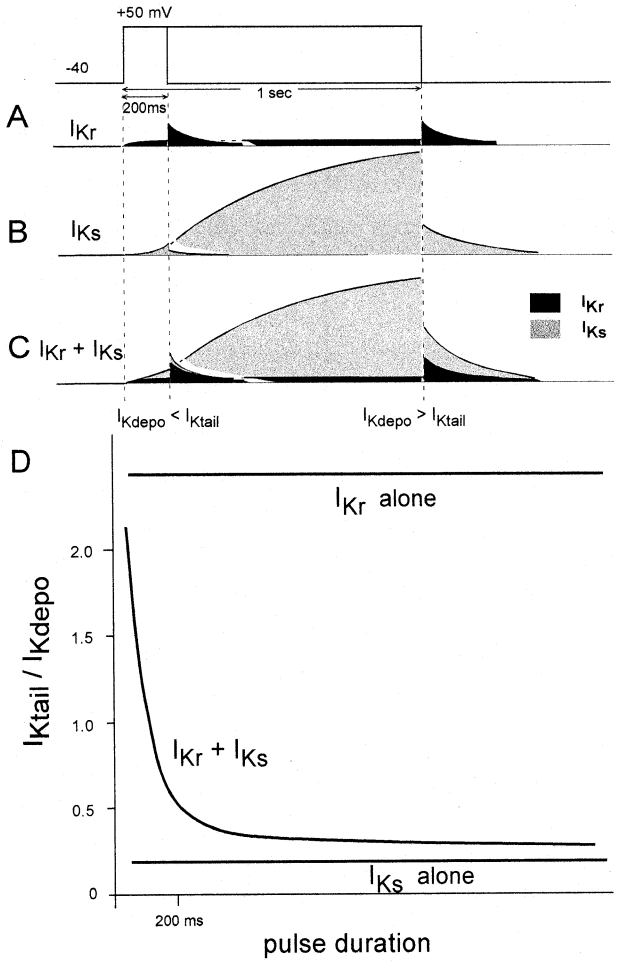

Figure 7 A scheme explaining the bepridil-induced changes in the profile of an envelope-of-tails test. The ordinate unit in panel D is arbitrary. See text for details.

Effects of bepridil on the delayed rectifier K^+ current

We found that bepridil at concentrations relevant to the clinical therapeutic concentrations suppressed I_{Ks}. This was confirmed by two different sets of experiments. In the envelope-of-tails test (Noble & Tsien, 1969), bepridil at a concentration of 2 μM did not change the shape of the curve, but shifted the curve upward (Figure 4B). This parallel shift can be interpreted from the diagram illustrated in Figure 7. When the pulse duration is short (<200 ms), the amplitude of I_{Kr} seen during the depolarizing pulse is small due to its strong inward-going rectification. On return to -40 mV, a large tail current of I_{Kr} appeared as the channel quickly recovered from rectification (Sanguinetti & Jurkiewicz, 1990) (left, Figure 7A). In contrast, as I_{Ks} is activated very slowly, its contribution to either I_{Kdepo} or I_{Ktail} is very small when the duration of the depolarizing pulse is short (<200 ms) (left, Figure 7B). Consequently, the amplitude of the I_{Ktail} evoked by a short pulse is much larger than that of I_{Kdepo} (left, Figure 7C). On the other hand, the longer depolarization (~ 1000 ms) activates I_{Ks} considerably (Figure 7B) (in addition to the activation of I_{Kr}) and increases the contribution of I_{Ks} to the overall current of either I_{Kdepo} or I_{Ktail} (right, Figure 7C) and causes the I_{Ktail}/I_{Kdepo} ratio to converge to a small certain constant value (~ 0.4, '$I_{Kr}+I_{Ks}$' curve of Figure 7D). In the plots of I_{Ktail}/I_{Kdepo} vs pulse duration (Figure 7D), the idealistic relationship of I_{Kr} alone (i.e. under nearly complete blockade of I_{Ks}) could be a straight line, as shown at the top of Figure 7D, the ratio of which is large and determined mostly by the rectification property of I_{Kr}. However, in the presence of I_{Kr} alone (i.e. under nearly complete blockade of I_{Ks}) the I_{Ktail}/I_{Kdepo} ratio would be fairly small and constant as shown at the bottom of Figure 7D, which could be determined mostly by the difference in the driving force for potassium ions at the different potentials (-40 and $+50$ mV). In the presence of both I_{Kr} and I_{Ks}, the ratio was large for short pulses and became smaller as the pulse duration was increased ($I_{Kr}+I_{Ks}$, Figure 7D). In the present study, 2 μM bepridil shifted the relationship upward (Figure 4B), suggesting that the relative contribution of I_{Kr} to total I_K was slightly increased as the drug partially suppressed I_{Ks}.

Selective block of I_{Ks} by 2 μM bepridil was further supported in the plot of the I_{Ktail}/I_{Kdepo} ratio of the bepridil-sensitive current (Figure 4C). The ratio was almost constant regardless of the pulse duration, and the bepridil (2 μM)-sensitive component of the I_{Ktail}/I_{Kdepo} ratio shown in Figure 4C shared the characteristic common to the 'I_{Ks} alone' curve schematically drawn at the bottom of Figure 7D. These results strongly suggest that 2 μM bepridil selectively inhibits I_{Ks}. In contrast, a much higher concentration of bepridil (~ 10 μM) may inhibit both I_{Kr} and I_{Ks}, since the relationship of the 10 μM bepridil-sensitive current showed a biphasic pattern (Figure 5B). These results suggest that at concentrations relevant to the plasma therapeutic concentrations in clinical use (2–3 μM, Hollingshead *et al*., 1992), bepridil selectively blocks I_{Ks} with no practical effect on I_{Kr}.

In an alternative experiment, we studied the concentration-inhibition relationships of the I_{Kr} block by bepridil using a short-pulse protocol. The duration of depolarization for 50 ms was too short to activate I_{Ks}, because the tail current elicited on return to -40 mV was insensitive to chromanol 293 B (50 μM), a putative I_{Ks} blocker (Busch *et al*., 1996). The tail current was almost completely abolished, however, by E4031 (5 μM). This apparently I_{Kr}-selective tail current was suppressed by bepridil in a highly cooperative manner (Figure 6). Such findings imply that the I_{Kr} channel does not have a single binding site, but rather multiple ($\cong 3$) sites for bepridil. Due to this cooperation observed in the bepridil action on I_{Kr}, the blocking effect of this drug on the I_{Kr} at low concentrations might have become much smaller than expected from the case when there is no cooperation. Indeed 2 μM bepridil inhibited I_{Kr} only less than 5% (Figure 6B).

Bepridil on action potential duration

Most class III drugs selectively block I_{Kr}. The resulting reverse-use-dependent effect, i.e. marked prolongation of the APD at low heart rates and lack of prolongation at higher heart rates, hampered the effectiveness of these drugs as antiarrhythmic agents (Nair & Grant, 1997). Thus agents that selectively block the I_{Ks} may have a potential usefulness in effectively suppressing tachyarrhythmias due to re-entry (Nair & Grant, 1997). The present study demonstrated that bepridil at therapeutic concentrations (2–3 μM) preferentially blocked I_{Ks}. This effect may underlie at least in part the previous finding that bepridil prolonged the APD in a use-dependent manner in guinea-pig ventricular papillary muscles (Nobe *et al*., 1993). In the latter report, bepridil significantly lengthened the APD only at the highest stimulation frequency of 5 Hz.

Multiple effects of bepridil

Bepridil has multiple inhibitory effects on sarcolemmal ionic currents including the L-type (Yatani *et al*., 1986) and T-type

Ca^{2+} currents (Cohen *et al*., 1992), the delayed-rectifier K^+ current, the transient outward current (Berger *et al*., 1989) as well as the K^+ current activated by intracellular Na^+ (Mori *et al*., 1998). The drug also modulates contractile proteins and increases the Ca^{2+} sensitivity (Solaro *et al*., 1986; Ozaki *et al*., 1999). The main target of bepridil is believed to be the L-type Ca^{2+} current, because the concentration of half-maximal inhibition was as low as 0.5 μM (Yatani *et al*., 1986). Bepridil has multiple intracellular and sarcolemmal targets and these effects in concert may form a characteristic profile of the drug action. For example, the drug enhances the Ca^{2+} sensitivity of the contractile proteins, which may counteract the depression of contractile tension due to a decrease in I_{Ca} (Solaro *et al*., 1986; Ozaki *et al*., 1999).

We demonstrated that bepridil has differential effects on the two components of the delayed-rectifier K^+ current, I_{Kr} and I_{Ks}: the IC_{50} for inhibition of I_{Kr} and I_{Ks} was 13.2 and 6.2 μM, respectively. In addition, since the inhibition of I_{Kr} occurred in a cooperative manner (Hill coefficient = 3.03), the inhibition of I_{Kr} by low concentrations of bepridil is much attenuated. Therefore, we conclude that bepridil at therapeutic concentrations (2–3 μM, Hollingshead *et al*., 1992) blocks I_{Ks}, with no practical effect on I_{Kr}. The differential inhibition of two delayed-rectifier K^+ currents by bepridil may be important in understanding the antiarrhythmic effects of this drug.

We thank Dr Hans-J Lang for the gift of chromanol 293 B and Ms K Moriyama for her secretarial services.

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(Received June 21, 1999
Revised September 17, 1999
Accepted September 24, 1999)