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Effects of Bepridil on I_{Kr} and I_{Ks} of Rabbit Ventricular Myocytes

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Abstract: Bepridil, a potent antiarrhythmic drug, inhibits a variety of cardiac ion channels. Specific block of bepridil on I_{Kr} or I_{Ks}, however, has been controversial and dependent on species and experimental conditions. To clarify this point, we studied the effects of bepridil on I_{Kr} or I_{Ks} in rabbit ventricular myocytes. Bepridil caused a concentration-dependent inhibition on both currents. IC_{50}s were 0.43 ± 0.20 μM (n=5) for I_{Kr} and 0.82 ± 0.32 μM (n=7) for I_{Ks}. These results suggest that bepridil may thereby cause an antiarrhythmic action through an inhibition of both I_{Kr} and I_{Ks}.

Key words: bepridil, potassium channels, arrhythmia

Bepridil, a potent antiarrhythmic agent, is useful in the treatment of cardiac arrhythmias.[1] For the underlying ionic mechanisms, bepridil has been reported to inhibit various types of cardiac ion channels. Among these ion channels, I_{Kr} and I_{Ks} are two major repolarizing potassium currents which determine action potential duration.[2] The effect of bepridil on I_{Kr} and I_{Ks}, however, is still controversial and dependent on experimental conditions.[3-4] To clarify the differential effects and the mode of inhibitory action on I_{Kr} and I_{Ks}, we studied the effects of bepridil on I_{Kr} or I_{Ks} in rabbit ventricular myocytes.

Methods

Japanese white rabbits (1.5–2.0 kg) were sacrificed under anesthesia with thiamylal sodium after being heparinized. Single myocytes of epicardium were isolated enzymatically from the middle portion of the left ventricular free wall using a procedure as described previously.[5] All animal procedures were approved by the Animal Care and Use Committee, Research Institute of Environmental Medicine, Nagoya University.

I_{Kr} and I_{Ks} were recorded by use of a single-pipette whole-cell patch-clamp method as described previously.[6] I_{Ks} was measured during blockade of I_{Kr} by 10 μmol/L E-4031 added to the superfusate and I_{Kr} was measured during blockade of I_{Ks} by 30 μmol/L chromanol 293B. The temperature in all experiments was 35~37°C. The resistance of the glass pipette was 4~6 MΩ after filling with an internal pipette solution. The cell capacitance was determined by applying a ramp voltage pulse of ±0.5 V/s at a potential ranging between -50 mV and +70 mV. The cell capacitance and series resistance were electrically compensated by about 70%. Current signals (filtered at 2 kHz) were stored on an IBM-PC computer by using PCLAMP software (version 6.0, Axon Instruments, U.S.A.) for analysis. Tyrode's solution used for cell isolation and the recording of action potential was composed of (in mmol/L) NaCl 143, KCl 5.4, $MgCl_2$ 0.5, NaH_2PO_4 0.25, HEPES 5.0, $CaCl_2$ 1.8 and glucose 5.6 (pH=7.35 adjusted with NaOH). The internal pipette solution was composed of (in mmol/L) KOH 60, KCl 80, aspartate 40, HEPES 5.0, EGTA 10, MgATP 5.0, sodium creatinine phosphate 5.0 and $CaCl_2$ 0.65 (pH=7.2 adjusted with NaOH, pCa=8.0). When IK was measured, cells were superfused with a Na^+- and K^+-free solution (NMG solution) composed of (in mmol/L) N-methyl-D-glucamine 149, $MgCl_2$ 5, $CaCl_2$ 0.9, HEPES 5 and nisoldipine 0.003 (pH= 7.35 adjusted with HCl). E-4031 was dissolved in distilled water and chromanol 293B in dimethyl sulfoxide (DMSO) as stock solutions and diluted in superfusates to achieve a final concentration immediately before each application. Data were expressed as mean±SEM. Results were compared using Student's t-test for paired and unpaired data to evaluate statistical significance and differences were considered significant at $P<0.05$.

Results

The I_{Kr} (293B resistent current) and I_{Ks} (E-4031 resistant current) were elicited during 3 sec-depolarizing pulses to potentials ranging from -40 mV to +50 mV from a holding potential of -50 mV. Tail currents were observed at -50 mV. Fig.

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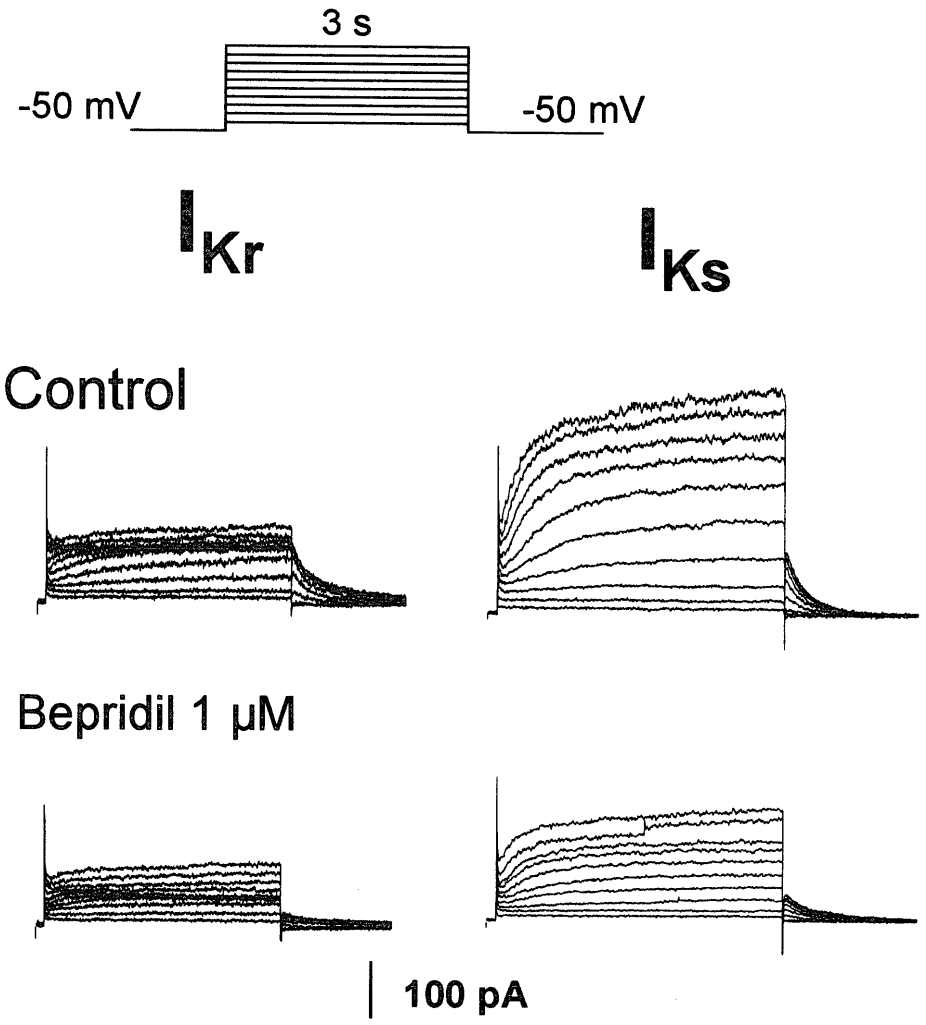

Fig. 1 Effects of bepridil on I_{Kr} and I_{Ks} in rabbit ventricular myocytes.
Voltage protocol and current recordings before and after exposure to bepridil 1 μM on I_{Kr} (left) and I_{Ks} (right).

1 shows representative tracings. Bath application of bepridil (0.03–3 μM) for 10 min resulted in a concentration-dependent decrease in tail currents on repolarization. The concentrations of 50% inhibition on tail currents after 50 mV depolarization were 0.43 ± 0.20 μM (n=5) for I_{Kr} and 0.82 ± 0.32 for I_{Ks} (n=7). These values were not significantly different between two groups.

Discussion

In the present study, bepridil potently and almost equally blocked I_{Kr} and I_{Ks} in rabbit ventricular myocytes. These results suggest that bepridil may thereby cause an antiarrhythmic action through an inhibition of both I_{Kr} and I_{Ks}. This is contrary to the previous reports by Wan et al. on I_{Kr} and I_{Ks} of guinea-pig ventricular myocytes.[4] They showed a preferential inhibition on I_{Ks} rather than I_{Kr}. In contrast, specific block on I_{Kr} compared to I_{Ks} was suggested in heterologously expressed currents. I_{Kr} channel is constructed by HERG subunit and I_{Ks} channel is co-assembled by KVLQT1 and mink subunits.[7-8] We have already shown that bepridil preferentially blocked HERG current rather than KVLQT1/minK current in Xenopus oocytes.[9] Supporting evidence of specific block on HERG channels rather than KVLQT1/mink channels has been also shown by Chouabe et al.[10] in transfected COS7 cells. For the moment, we cannot provide any clear interpretation of these discrepancies.

In addition to I_{Kr} and I_{Ks}, bepridil blocked variable cardiac ion channels including inward currents (I_{Na}, $I_{Ca,L}$, $I_{Ca,T}$) as well as outward currents (I_{K1}, I_{to}, I_{Kr}, I_{Ks}, I_{KATP}, I_{KNa}, I_{KACh}). The antiarrhythmic action of bepridil should be explained by its total effects on ionic currents. Bepridil produces different effects on the action potential duration depending on species and preparations. Bepridil shortened the APD in rabbit ventricular myocardium[11-12] and guinea-pig ventricular myocytes.[13] In contrast, prolongation of APD was reported in canine hearts.[14] Thus, further experiments will be required to clarify the mechanisms underlying the antiarrhythmic action of this drug.

References

1) Hollingshead LM, Faulds D, Fitton A. Bepridil. A review of its pharmacological properties and therapeutic use in stable angina pectoris. Drugs 1992; 44: 835–57.
2) Sanguinetti MC, Jurkiewicz NK. Two components of cardiac delayed rectifier K^+ current. Differential sensitivity to block by class III antiarrhythmic agents. J Gen Physiol 1990; 96: 195–215.
3) Sawanobori T, Tanabe S, Shinohara R, et al. Effects of bepridil on ionic currents. Ther Res 1998; 19: 5–10.
4) Wang JC, Kiyosue T, Kiriyama K, et al. Bepridil differentially inhibits two delayed rectifier K^+ currents, I_{Kr} and I_{Ks}, in guinea-pig ventricular myocytes. Br J Pharmacol 1999; 128: 1733–8.
5) Cheng J, Kamiya K, Liu W, et al. Heterogeneity of the delayed rectifier K^+ current components (I_{Kr} and I_{Ks}) in myocytes isolated from apex and base of rabbit ventricle: an underlying mechanism of the proarrhythmic effects of class III antiarrhythmic agents. Cardiovasc Res 1999; 43: 135–47.
6) Lu Z, Kamiya K, Opthof T, et al. Density and kinetics of I_{Kr} and I_{Ks} in guinea pig and rabbit ventricular myocytes explain different efficacy of I_{Ks} blockade at high heart rate in guinea pig and rabbit: implications for arrhythmogenesis in humans. Circulation 2001; 104: 951–6.
7) Sanguinetti MC, Jiang C, Curran ME, et al. A mechanistic link between an inherited and an acquired cardiac arrhythmia: HERG encodes the I_{Kr} potassium channel. Cell 1995; 81: 299–307.
8) Sanguinetti MC, Curran ME, Zou A, et al. Coassembly of KvLQT1 and minK (IsK) proteins to form cardiac I_{Ks} potassium channel. Nature 1996; 384: 80–3.
9) Kamiya K, Hojo M, Lu Z, et al. Differential effects of bepridil on HERG and KvLQT1/minK channels. Environmental Medicine 2001; 45: 40–1.
10) Chouabe C, Drici MD, Romey G, et al. HERG and KvLQT1/IsK, the cardiac K^+ channels involved in long QT syndromes, are targets for calcium channel blockers. Mol Pharmacol 1998; 54: 695–703.
11) Anno T, Furuta T, Itho M, et al. Effects of bepridil on the electrophysiological properties of guinea-pig ventricular muscles. Br J Pharmacol 1984; 81: 589–97.
12) Gill A, Flaim SF, Damiano BP, et al. Pharmacology of bepridil. Am J Cardiol 1992; 69: 11D–16D.
13) Yatani A, Brown AM, Schwartz A. Bepridil block of cardiac calcium and sodium channels. J Pharmacol Exp Ther 1986; 237: 9–17.
14) Kato R, Singh BN. Effects of bepridil on the electrophysiologic properties of isolated canine and rabbit myocardial fibers. Am Heart J 1986; 111: 271–9.

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