Environmental Medicine, Vol. 46 No.1, 2, 2002 (page 101 ~ 102)

Voltage-Dependent Effects of Bepridil on D540K HERG Channels

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Abstract: To address the question of whether depolarization or channel opening facilitates drug binding, we studied the voltage-dependent block by bepridil, a positively charged drug, on D540K HERG channels expressed in Xenopus oocytes. At 0 mV, bepridil caused a concentration-dependent inhibition of D540K HERG current with IC_{50}s of 11 ± 3 μM (n=6). At -150 mV, inhibition by bepridil was decreased by 15-fold (161 ± 31 μM, n=3). These results suggest that 1) membrane depolarization facilitates block of HERG channels; 2) binding of positively charged drug is augmented at depolarization and diminished at hyperpolarization if the channel remains open.

Key words: bepridil, potassium channels, arrhythmia

Preferential channel block in response to membrane depolarization is a common finding for local anesthetics and antiarrhythmic agents, however the mechanism and its structural basis are poorly understood.[1-2] It is difficult to distinguish between voltage-sensitive drug movement and altered drug affinity due to a conformational change of channel structure. D540K HERG channels have a unique ability to open in response to membrane hyperpolarization while retaining activation and inactivation properties in response to depolarization.[3-4] To address the question of whether depolarization or channel opening facilitates drug binding, we studied the voltage-dependent block by bepridil, a positively charged drug, on D540K HERG channels expressed in Xenopus oocytes.

Methods

The isolation, maintenance of Xenopus oocytes and injection with D540K HERG cRNA were performed as described previously.[5] Stage V and VI oocytes were injected with 10 ng cRNA of D540K HERG. Oocytes were cultured in Barth's solution supplemented with 50 μg/L gentamycine and 1 mM pyruvate at 18°C. Currents were recorded at room temperature (22–24°C) by standard two-microelectrode voltage-clamp techniques 2 to 4 days after cRNA injection. Bepridil at concentrations from 1 to 300 μM was tested to observe its effects on these currents. Data were expressed as mean ± SEM.

Results

Properties of the D540K HERG has been described previously.[3-4] Briefly, D540K current activated nearly instantaneously upon depolarization (-80 to +50 mV) from a holding potential of -90 mV. Hyperpolarization to potentials negative to -100 mV induced a slowly activating current that attained a steady state amplitude in 2 sec. The inward current amplitude was much greater than outward current for equivalent electromechanical driving forces. Repolarization to -80 mV after hyperpolarizing test pulse induced a tail current whose magnitude was larger and decay slower than tail currents induced after depolarizing test pulses. Tail currents after depolarizing test pulses initially increased as channels deactivated into the closed state.

Bath application of bepridil at 30 μM for 10 min resulted in substantial decreases in the outward currents during depolarization. In contrast, hyperpolarization in a same oocyte produced a minimum reduction. At 0 mV, bepridil caused a concentration-dependent inhibition of D540K HERG current with IC_{50}s of 11 ± 3 μM (n=6). At -150 mV, inhibition by bepridil was decreased by 15-fold (161 ± 31 μM, n=3).

Discussion

In the present study, bepridil potently blocked D540K HERG currents evoked by depolarization. IC_{50} value (11 μM) is comparable to that we obtained in WT HERG (3.0 μM) previously. At hyperpolarization, the potencies of block on D540K compared to depolarization attenuated 15-fold in same

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This work was supported by Grants-in-Aid from Ministry of Education, Culture, Sports and Science.

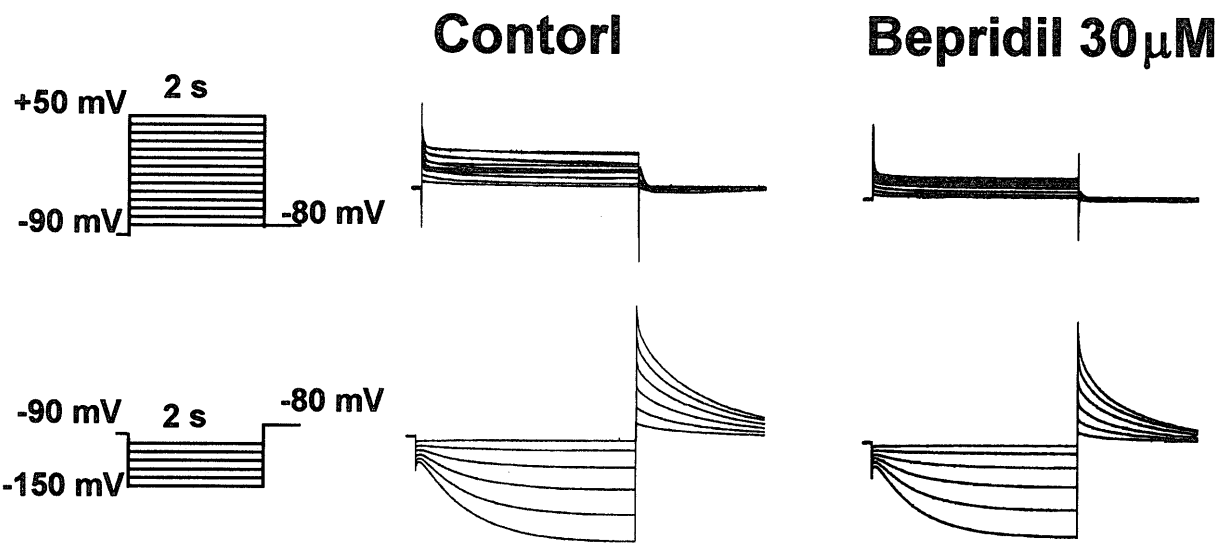

Fig. 1 D540K HERG currents before and after bepridil at 30 μM.
D540K currents were activated by depolarization (-80 to +50 mV) or hyperpolarization (-100 to -150 mV) for 2 sec from a holding potential of -90 mV.

preparations. The less potency of drug block in oocytes compared with mammalian cells is a common finding. Lipophilic yolk sac in oocytes may act to immobilize drug movement. The attenuation of potency at hyperpolarization compared to depolarization might be explained by the polarity of bepridil. In perfusates of physiological pH at 7.40, 40% of bepridil molecules were positively charged (pKa=6.58). Intracellular charged drugs may come and go only while the channel is open. Depolarization-induced open channel drives positively charged molecule to access across the electrical filed to the receptor site in the vestibule. In hyperpolarization, however, intracellular charged molecules were immobilized and prevented to access the binding site.

Useful antiarrhythmic agents have a voltage-time profile such that at normal heart rates and membrane potentials they have little effect, while severely interfering at high rates especially in depolarized tissue.[6] These frequency-dependent blockade by antiarrhythmic drugs have been explained by the idea that affinity of drug to receptor within channel depends on channel state. The transmembrane potential alters the conformation of channels, they can be grouped into three primary states: rested activated and inactivated. At resting membrane potential, channel is in rested state, which usually shows the least affinity to drugs. When membrane is depolarized, channel states transit to activated state and then inactivated state. Questions still remains which of these states between activated and inactivated state have higher affinity and how drug bind channels and transit their states. Reflecting that, modulated recetor[7] theory and guarded receptor theory[8] are two remaining active concepts. Recent introduction of gene technology enables us to mutagenate channels which alter voltage-dependent state transitions of channel states. Mutants which activates during hyperpolarization like D540K HERG are very helpful to clarify the mechanisms of frequency-dependent effects of antiarrhythmic agents and may provide insights into the consecutive arguments on those.

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Received June 3, 2002; accepted June 20, 2002