Environmental Medicine, 47, 2003 (page 51 ~ 52)

Voltage-Dependent Effects of Amiodarone 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 amiodarone, a positively charged drug, on D540K HERG channels expressed in Xenopus oocytes. At 0 mV, amiodarone caused a concentration-dependent inhibition of D540K HERG current with IC_{50} of 15 ± 5 μM (n=4). At -150 mV, inhibition by amiodarone was almost abolished (IC_{50}: >10 mM, n=4). These results suggest that binding of positively charged drug is augmented at depolarization and diminished at hyperpolarization if the channel remains open.

Key words: amiodarone, potassium channels, arrhythmia

Amiodarone is a potent antiarrhythmic agent and inhibits various types of cardiac ion channels. The molecular mechanism of channel inhibition by amiodarone has been extensively studied on sodium and calcium channels but not for potassium channels, especially on its voltage-dependency (Kamiya et al, 2001 a). 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 (Johnson and McKinnon, 1957; Strichartz, 1973). 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 (Sanguinetti and Xu, 1999; Mitcheson et al, 2000). To address the question of whether depolarization or channel opening facilitates drug binding, we studied the voltage-dependent block by amiodarone, 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 (Kamiya et al, 2001 b). 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. Amiodarone at concentrations from 0.56 to 100 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 (Sanguinetti and Xu, 1999; Mitcheson et al, 2000). 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.

Bath application of amiodarone at 56 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, amiodarone caused a concentration-dependent inhibition of D540K HERG current with IC_{50} of 15 ± 5 μM (n=4). At –150 mV, inhibition by amiodarone was decreased by >600-fold (IC_{50}: >10 mM, n=4).

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

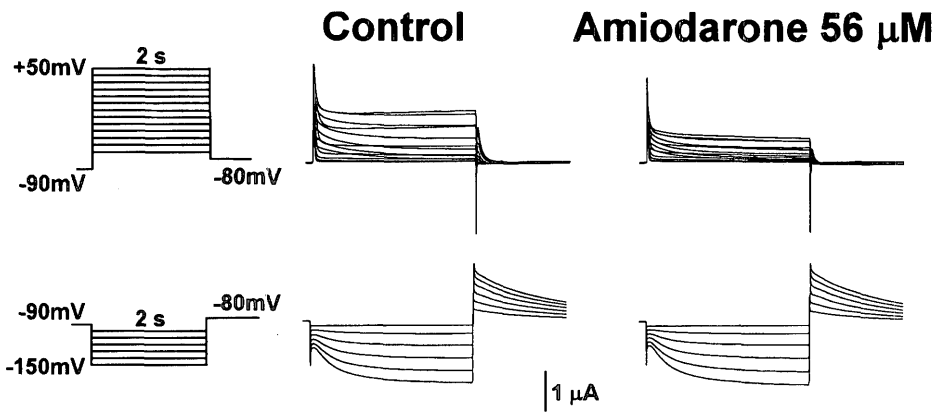

Fig.1 D540K HERG currents before and after amiodarone at 56 μM.
D540K currents were activated by depolarization (–80 to +50 mV) or hyperpolarization (–100 to –150 mV) for 2 sec from a holding potential of –90 mV.

Discussion

In the present study, amiodarone potently blocked D540K HERG currents evoked by depolarization. IC_{50} value (15 μM) is comparable to that we obtained in WT HERG (5.6 μM) previously. At hyperpolarization, amiodarone hardly blocked D540K HERG currents and the potencies compared to depolarization attenuated by more than 600-fold in same preparations. The attenuation of block at hyperpolarization compared to depolarization might be explained by the polarity of amiodarone. In perfusates of physiological pH at 7.40, 99% of amiodarone molecules were positively charged (pKa=8.97). 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.

The depolarization-dependent block by amiodarone may lead to beneficial pharmacological profile as an antiarrhythmic agent. In atrial and ventricular tachyarrhythmias, caridiac myoctes depolarized frequently. Consequently, during tachyarrhythmias, amiodarone has more chances to block potassium channels thereby leading to antiarrhythmic action.

In addition to binding during depolarization, drugs usually unbind from channels to release block in return to resting potential from depolarization (Hondeghem and Katzung, 1977; Starmer et al, 1984). Reflecting that, the frequency-dependent blockade by antiarrhythmic drug to ion channels is determined by the affinity and speed of both drug binding during depolarization and unbinding during hyperpolarization. Thus, binding and unbinding kinetics during depolarization and hyperpolarization should be further clarified to understand antiarrhythmic action by amiodarone on atrial and ventricular tachyarrhythmias.

References

Hondeghem LM, Katzung BG. Time- and voltage-dependent interactions of antiarrhythmic drugs with cardiac sodium channels. Biochim Biophys Acta 1977; 472: 373–398.

Johnson EA, McKinnon MG. The differential effect of quinidine and pyrilamine on the myocardial action potential at various rates of stimulation. JPET 1957; 120: 460–468.

Kamiya K, Nishiyama A, Yasui K, et al. Short- and long-term effects of amiodarone on the two components of cardiac delayed rectifier K^+ current. Circulation 2001a; 103: 1317–1324.

Kamiya K, Mitcheson JS, Yasui K, et al. Open channel block of HERG K^+ channels by vesnarinone. Mol Pharmacol 2001b; 60: 244–253.

Mitcheson JS, Chen J, Sanguinetti MC. Trapping of a methanesulfonanilide by closure of the HERG potassium channel activation gate. J Gen Physiol 2000; 115: 229–240.

Sanguinetti MC, Xu QP. Mutations of the S4-S5 linker alter activation properties of HERG potassium channels expressed in Xenopus oocytes. J Physiol 1999; 514: 667–675.

Starmer CF, Grant AO, Strauss HC. Mechanisms of use-dependent block of sodium channels in excitable membranes by local anesthetics. Biophys J 1984; 46: 15–27.

Strichartz GR. The inhibition of sodium currents in myelinated nerve by quaternary derivatives of lidocaine. J Gen Physiol 1973; 62: 37–57.

Received June 16, 2003; accepted June 24, 2003