Absence of Effects of Class III Antiarrhythmic Agents on Cloned Cardiac K Channels

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ABSTRACT—We investigated the effects of class III antiarrhythmic agents, d-sotalol, E-4031 and MS-551, on the currents of two cloned K channels, Kv1.2 (IKvl.2) and Kv1.4 (IKvl.4), by using the Xenopus oocyte expression system. Both IKvl.2 and IKvl.4 were sensitive to 4-aminopyridine and quinidine, but insensitive to tetraethylammonium, d-sotalol, E-4031 and MS-551. The results suggest that some types of structural proteins may be necessary for class III agents to inhibit the cardiac cloned K channels.

Keywords: K channel (cloned), Antiarrhythmic agent (class III), Xenopus oocyte

Class III antiarrhythmic agents are known to prolong cardiac action potential duration (APD) by inhibiting one or more types of K currents; and the targets for these agents are believed to be the delayed rectifier K current (IK), the transient outward current (Ito) and the inward rectifier K current (IK1). Electrophysiological experiments have been carried out to evaluate the ionic mechanisms by which each agent prolongs APD. However, it is not easy to isolate a single type of current from the others even if single cells are used.

Recently, we cloned two types of K channels from rat heart, RH1 (1) and RH10 (2) (Kv1.2 and Kv1.4, respectively, according to the nomenclature by Chandy (3)) . When expressed in Xenopus oocytes, the delayed rectifier type current (IKvl.2) and the transient outward type current (IKvl.4) were observed. Therefore, the present study was designed to evaluate the effects of class III agents, d-sotalol, E-4031 and MS-551, on these cloned cardiac K channels.

Messenger RNA was synthesized in vitro from plasmid DNA containing clones of Kv1.2 or Kv1.4 with T7 RNA polymerase (1, 2). The transcribed RNA specific for Kv1.2 or Kv1.4 was dissolved in water at a final concentration of 0.2 μg/μl for oocyte injection.

Xenopus laevis oocytes were treated for 2 hr with collagenase (2 mg/ml) in modified Barth’s medium and then defolliculated manually with fine forceps. The oocytes were injected with Kv1.2 or Kv1.4 transcript (40–50 nl per oocyte). Injected oocytes were incubated for 2–5 days at 19°C in modified Barth’s medium before the electrophysiological experiments. During the electrophysiological experiments, the oocytes were bathed in ND96 solution. The standard two-microelectrode voltage-clamp method was used for recording whole-cell current with 3-M KCl-filled electrodes. All experiments were carried out at 20–23°C.

The composition of ND96 solution was as follows: 96 mM NaCl, 2 mM KCl, 1.8 mM CaCl2, 1 mM MgCl2 and 5 mM HEPES (pH 7.5). Drugs and chemicals were obtained from the following sources: d-sotalol (Bristol-Myers Squibb Company, Wallingford, CT, U.S.A.); E-4031 (Eisai, Tokyo); MS-551 (Mitsui Pharmaceuticals, Inc., Tokyo); tetraethylammonium chloride (TEA), 4-aminopyridine (4-AP) and quinidine sulfate dihydrate (Wako, Osaka); and HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid, Dojin, Kumamoto). All drugs were dissolved in ND96 solution, and the pH of the solution was adjusted to 7.5. Drugs were applied in a cumulative manner, and measurements of IKvl.2 and IKvl.4 in the presence of drugs were carried out about every 1 min after increasing drug concentrations. Peak IKvl.2 and IKvl.4 used for constructing current-voltage relationships in the presence of drugs and concentration-response curves were obtained about 5 min after changing drug concentrations.

In one of the experiments, the current was measured during the 400-msec test pulse to potentials between −80 and +20 mV from a holding potential of −80 mV at a 60-sec pulse interval. Figure 1A shows typical recordings of IKvl.2 and IKvl.4 ob-
Fig. 1. Reduction of $I_{Kv1.2}$ and $I_{Kv1.4}$ by 4-AP and quinidine. A. Typical recordings of $I_{Kv1.2}$ and $I_{Kv1.4}$ in the control and the preparations with drugs (10 pM, 100 nM, 1 mM). The currents were elicited by test pulses to +20 mV from a holding potential of -80 mV. B. Peak current-voltage relationships in the control and the preparations with drugs (100 pM, 1 mM). C. Concentration-response curves of peaks of $I_{Kv1.2}$ and $I_{Kv1.4}$ for 4-AP and quinidine ($n = 4-5$).

Fig. 2. Absence of effects of d-sotalol, E-4031 and MS-551 on $I_{Kv1.2}$ and $I_{Kv1.4}$. A, B. Typical recordings of $I_{Kv1.2}$ and $I_{Kv1.4}$ in the control and the preparations with drugs (1 pM, 10 pM, 100 pM, 1 mM). The currents were elicited by test pulses to +20 mV from a holding potential of -80 mV. C, D. Peak current-voltage relationships in the control and the preparations with drugs (100 pM).
tained with 4-AP (1 μM – 1 mM) and quinidine (1 μM – 1 mM). Both drugs blocked the currents in a dose-dependent manner (Fig. 1, B and C). However, class III antiarrhythmic agents, d-sotalol (1 μM – 1 mM), E-4031 (1 μM – 1 mM) and MS-551 (1 μM – 1 mM), had little effect on I_{Kv1.2} and I_{Kv1.4} (Fig. 2, typical results). TEA (10 mM), a non-specific K channel blocker, also had no effect (n = 5, data not shown). As class III agents have a reverse use-dependency characteristic (4), we changed interpulse intervals in the order of 30, 60, 120 and 500 sec. However, no marked changes occurred (n = 4, data not shown).

Recent electrophysiological studies in single myocytes by the patch-clamp technique indicate that d-sotalol and MS-551 block I_K, I_to and I_K1 and that E-4031 blocks I_K (4, 5). It is also known that on low-frequency stimulation, quinidine, a class Ia antiarrhythmic agent, has little or no effect on the Na channel, but markedly increases APD by nonselectively blocking I_{Ks}, I_{to} and I_{K1}, acting like a "pure" class III antiarrhythmic agent (4). The current through our cloned K channel, I_{Kv1.4}, seems similar to the transient outward current (I_{to}) in terms of the kinetics and the sensitivities to 4-AP and TEA (6). I_{Kv1.4} was blocked by quinidine, but, unexpectedly, not affected by d-sotalol and MS-551. The magnitude of native K currents recorded from native cardiac tissue by the patch-clamp method is in the order of nA, whereas those recorded from K channels expressed in oocytes is in the order of μA; the latter is one thousand times larger than the former. However, the concentrations of 4-AP and quinidine required to inhibit I_{to} in the Xenopus oocyte expression system is nearly equal to those in the native cell membrane (6, 7), so that it is unlikely that the concentrations of d-sotalol and MS-551 studied were too low.

The Na and Ca channels each consist of more than two subunits. In both channels, it has been reported that coexpression of the main pore forming subunits with the other subunits results in normalization of current kinetics (8, 9). It is possible that our cloned K channels lack some structural proteins, such as subunits in the case of the Na or the Ca channel, which are necessary for the inhibitory action of d-sotalol and MS-551.

The properties of I_{Kv1.2} are distinct from those of the commonly observed cardiac delayed rectifier current but practically identical to those of a novel type of K channel current in rat atrium (10). Although at present no information is available about whether class III antiarrhythmic agents have effects on the novel type K channel, this is the first report demonstrating that these agents have little effect on I_{Kv1.2}.

In conclusion, both I_{Kv1.2} and I_{Kv1.4} were sensitive to 4-AP and quinidine but insensitive to d-sotalol, E-4031 and MS-551. A further study is necessary to elucidate the mechanism responsible for the insensitivity to class III agents.

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