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Effects of Amiodarone on the Electrophysiological Characters of Rabbit Atrial Myocytes

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Abstract: Amiodarone is a potent antiarrhythmic agent in the treatment of the atrial fibrillation and flutter. However, underlying electrophysiological mechanism are not clarified yet. In the present study, we investigated the acute effects of amiodarone on the action potentials in rabbit atrial myocytes. Amiodarone (10 μM) prolonged the action potential durations (APDs) at lower stimulation frequencies (e.g. 38 ± 8 ms control vs. 45 ± 6 ms amiodarone at 0.1 Hz, n=3, P<0.05). In contrast, amiodarone shortened the APDs at higher frequencies (e.g. 81 ± 11 ms control vs. 25 ± 10 ms amiodarone at 3.0 Hz, n=3, P<0.05). These results suggest that the antiarrhythmic action of amiodarone on atrial arrhythmias exhibits frequency-dependence.

Key words: amiodarone, rabbit, atrial, action potential, frequency dependency

Amiodarone has been shown to be an effective Class III antiarrhythmic drug, especially in the treatment of life-threatening atrial fibrillation and flutter (Chun SH, et al. 1995). Amiodarone has a multifaced pharmacological profile including prolongation of cardiac repolarization and refractory period (Kodama I, et al. 1997) through inhibition of various K^+ channels, Na^+ channels (Honjo H, et al. 1991), L-type Ca^{2+} channels (Nishimura M, et al. 1989), and anti-adrenergic activity (Polster P, et al. 1976).

Effects of amiodarone on action potential duration (APD) have been controversial. In atrial muscle, APD was prolonged (Yabek SM, 1986; Northover BJ, 1984), shortened (Sun W, et al. 2002), and observed no significant changes (Kadoya M, et al. 1985) by perfusion of amiodarone. These conflicting results may be explained by differences in animals, preparations and experiment conditions such as stimulation frequency.

The purpose of the current study was to investigate the frequency-dependent effects of amiodarone on the action potentials in single rabbit atrial myocyte.

Materials and Methods

Three Japanese male white rabbits weighing from 1.5 to 2.0kg were anesthetized with thiamylal sodium after being heparinized. The hearts were quickly excised and mounted on a Langendorff perfusion apparatus. They were initially perfused via the aorta with normal Tyrode's solution composed of (mM) 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). This solution was gassed with 100% O_2 at 37°C. When the hearts became clear of blood, perfusion was continued with calcium-free Tyrode's solution until the heart stopped beating. The heart was then perfused with the same solution containing collagenase (0.20 mg/ml, Yakult, Japan) for 20 min. After washing the heart with high-potassium storage solution (KB solution) for 5 min, atria of the heart were cut into small pieces. KB solution was composed of (mM) KOH 82, 1-glutamic acid 50, KCl 40, taurine 20, KH_2PO_4 20, glucose 10, HEPES 10, EGTA 0.5 and $MgCl_2$ 3 (pH=7.35 with KOH). Cells were gently agitated, filtered, and washed. Isolated myocytes were stored at 4°C in KB solution. An aliquot of the cell suspension was placed in the recording chamber on the stage of an inverted microscope (Diaphoto; Nikon Co., Tokyo, Japan) with normal Tyrode's solution. The bath temperature in all experiments was 35°C. A single-pipette whole-cell clamp method was employed to record the action potential. The resistance of the glass pipette was 3~5 MΩ after filling with an internal pipette solution composed of (μM) 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, pCa=8.0). The action potential was elicited by applying a 5-ms depolarizing pulse through the pipette and was recorded at various stimulation frequencies. Amiodarone was dissolved in dimethyl sulfoxide (DMSO) to make a 50-mM stock solution and was subsequently diluted by the superfusate to achieve a final concentration (10 μM). Amiodarone was perfused after recording the control action potential. Data were stored and analyzed on an IBM-PC computer by using PCLAMP software (version 6.0, Axon Instruments, U.S.A.). Data were expressed as mean ± SEM. Paired t-test was applied to evaluate statistical significance,

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and differences were considered significant at $P<0.05$.

Results

Action potentials were recorded at varying stimulation frequencies from 0.1 Hz to 3.0 Hz. The APD was measured at 90% repolarization (APD_{90}). During the stimulation frequency was increased from 0.1 Hz to 3.0 Hz, APD increased when the stimulation frequency more than 1.0 Hz. And the notches observed at the phase 1 of action potentials stimulated at lower frequencies less than 1.0 Hz disappeared at higher stimulation frequencies (Fig. 1A). After amiodarone (10 μM) was perfused for 5 min, APD was prolonged significantly by amiodarone at lower stimulation frequencies from 0.1 Hz to 0.3 Hz (e.g. 38 ± 8 ms control vs. 45 ± 6 ms amiodarone at 0.1 Hz, n=3, $P<0.05$). In contrast, APD was shortened significantly by amiodarone at higher frequencies from 2.0 Hz to 3.0 Hz (e.g. 81 ± 11 ms control vs. 25 ± 10 ms amiodarone at 3.0 Hz, n=3, $P<0.05$) (Fig. 1B).

Discussion

In our present study using rabbit atrial myocytes, amiodarone caused biphasic changes in APD depending on the stimulation frequencies. Amiodarone prolonged the APD at stimulation frequencies lower than 0.5 Hz. In contrast, amiodarone shortened the APD at frequencies higher than 1.0 Hz. The APD shortening at higher stimulation frequencies are consistent with that previously reported in rabbit isolated atrial muscle (Sun et al. 2002).

The dual effects of amiodarone on APD in response to stimulation frequency observed in our study may be attributed to the balance of inhibitory actions on inward Na^+ current and outward K^+ current. Amiodarone blocked Na^+ current in a use-dependent manner (Follmer, et al. 1987). At lower stimulation frequencies, amiodarone did not cause sufficient Na^+ current block to shorten APD. Instead, the rapid component of the outward delayed rectifier K^+ current (I_{Kr}) was blocked (Kamiya et al. 1995) to prolong the APD. At higher stimulation frequencies, however, amiodarone blocked Na^+ current use-dependently and lead to APD shortening. The APD prolonging effect of I_{Kr} blockade might be canceled out.

APD is determined not only by Na^+ current and I_{Kr} but also other currents such as calcium current (I_{Ca}) and transient outward current (I_{to}). The unique frequency response on APD caused by amiodarone might be also resulted from effects on I_{Ca} and I_{to}. I_{Ca} has been reported to be blocked by amiodarone at higher concentrations (Kodama et al. 1996), but I_{to} was unaffected by 10 μM amiodarone in rabbit ventricular myocytes (Kamiya et al. 1995). Further experiments are required to clarify these points.

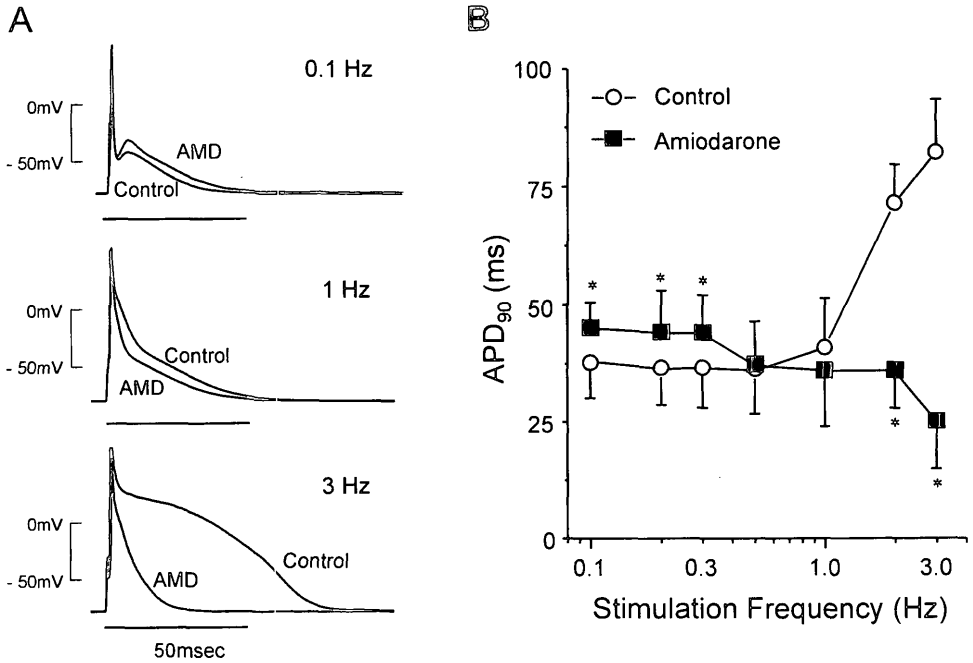

Fig. 1 Effects of amiodarone (10 μM) on action potentials in rabbit atrial myocytes.
A, representative action potentials recorded at 0.1 Hz, 1.0 Hz and 3.0 Hz under control conditions (Control) and in the presence of amiodarone (AMD). Action potentials were recorded at 35°C. B, quantitative data of frequency-dependence of APD to 90% repolarization (APD_{90}) under control conditions (○) and in the presence of amiodarone (■). Data are expressed means ± SEM. * $P < 0.05$ for difference between drug effect versus control value at each frequency. n=3.

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