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Inhibition of the reverse mode of the Na^+/Ca^{2+} exchange by KB-R7943 augments arrhythmogenicity in the canine heart during rapid heart rates

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Abstract To test the hypothesis that the reverse mode of the Na^+/Ca^{2+} exchange augmented by a rapid heart rate has an antiarrhythmic effect by shortening the action potential duration, we examined the effects of KB-R7943 (2-[2-[4-(4-nitrobenzyloxy)phenyl]ethyl] isothiourea methanesulfonate), a selective inhibitor of the reverse mode of the Na^+/Ca^{2+} exchange, to attenuate this effect. We recorded the electrocardiogram, monophasic action potential (MAP), and left ventricular pressure in canine beating hearts. In comparison to the control, KB-R7943 significantly increased the QTc value and MAP duration. MAP alternans and left ventricular pressure alternans were observed after changing the cycle length to 300 milliseconds in the control studies. KB-R7943 magnified both types of alternans and produced spatially discordant alternans between right and left ventricles. Early after-depolarizations and nonsustained ventricular tachycardia occurred in the presence of KB-R7943. Our data suggest that the reverse mode of the Na^+/Ca^{2+} exchange may contribute to suppression of arrhythmias by abbreviating action potential duration under pathophysiological conditions. This conclusion is based on further confirmation by future studies of the specificity of KB-R7943 for block of the reverse mode of the Na^+/Ca^{2+} exchange.

Keywords: Reverse mode of Na^+/Ca^{2+} exchange; KB-R7943; Arrhythmia; Heart

1. Introduction

Contractile force, left ventricular pressure, and the amplitude of $[Ca^{2+}]_i$ transients increase when stimulation rate is increased (positive force-frequency relation) [1,2], and this frequency-dependent potentiation is related to the intracellular Ca^{2+} cycling that is linked to intracellular Na^+ homeostasis mediated by the Na^+/Ca^{2+} exchange of the hearts [2]. At potentials less than the reversal potential and normal intracellular Na concentration ($[Na^+]_i$), the Na^+/Ca^{2+} exchanges functions in the Na^+ influx–Ca^{2+} efflux (forward mode) and reduces the cytosolic Ca^{2+} concentration by extruding Ca^{2+}. However, rapid pacing of cardiac myocytes for a long period causes a larger percentage increase in $[Na^+]_i$ relative to $[Ca^{2+}]_i$ [2-6] and augments the driving force for the Na^+/Ca^{2+} exchange in the reverse mode. The reverse mode of the Na^+/Ca^{2+} exchange can promote Ca^{2+} entry and trigger Ca^{2+} release from the sarcoplasmic reticulum (SR), leading to an increased cytosolic Ca^{2+} concentration [3], and contribute to the rate-dependent increase in left ventricular pressure (LVP) [5,6]. Because of its 3 Na^+/1 Ca^{2+} stoichiometry, the reverse mode of the outward Na^+/Ca^{2+} exchange current shortens the action potential duration. The reverse mode of the Na^+/Ca^{2+} exchange capable of triggering Ca^{2+} release from the SR may shorten the cardiac action potential duration indirectly by modifying Ca^{2+}-sensitive currents including the L-type Ca^{2+} current (I_{Ca}), slowly activating delayed rectifier potassium current (I_{KS}) and the Ca^{2+}-sensitive transient outward current ($I_{to(Ca)}$) [3,7].

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Thus, we speculated that the reverse mode of the Na^+/Ca^{2+} exchange augmented by a rapid heart rate had the beneficial effect of suppressing ventricular arrhythmias by shortening the action potential duration. KB-R7943 (2-[2-[4-(4-nitrobenzyloxy)phenyl]ethyl] isothiourea methanesulfonate) is a novel isothiourea derivative that preferentially blocks the reverse mode of the Na^+/Ca^{2+} exchange rather than the Ca^{2+} efflux mode [3,8-10]. To test our hypothesis, we examined the electrophysiological effects of KB-R7943 on beating canine hearts and suggested a physiological role for the reverse mode of the Na^+/Ca^{2+} exchange to prevent ventricular arrhythmias during fast heart rates.

2. Methods

2.1. In vivo canine beating hearts

We anesthetized 10 mongrel dogs of either sex (weight 12-15 kg) with sodium pentobarbital 30 mg/kg intravenously (IV) and made a midsternal incision in each dog. The dogs were ventilated with a respirator (model SN-480, Shimano Inc, Tokyo, Japan), and each dog's heart was suspended in a pericardial cradle. We measured the arterial pH, P_{O_2}, P_{CO_2}, and serum K^+ level periodically and maintained them at normal levels by adjusting the ventilatory volume or frequency or by administering IV sodium bicarbonate or KCl. Electrical stimuli with a 2-millisecond pulse duration and at twice the diastolic threshold strength were delivered through a bipolar platinum wire electrode positioned in the right ventricular apex close to a suction electrode. We measured the surface temperature of the heart directly with a thermistor probe and kept it at 36°C with hot packs on both the cervical and femoral regions [11,12]. This study conformed to the Guidelines for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

2.2. Simultaneous recording of the ECG, monophasic action potential, and LVP

We used a suction electrode to record the monophasic action potential (MAP) by the technique described previously [11,12]. In this experiment, we kept the negative pressure of the suction electrodes between 20 and 30 mm Hg to minimize the myocardial cell damage from the combined effects of the negative pressure and resultant ischemia. We checked the MAP configurations to satisfy the MAP quality criteria described by Franz [13] during experiments. The LVP was measured with a tip-manometer angiocatheter. We simultaneously recorded the MAP, electrocardiogram (ECG) limb lead II, and LVP with a multichannel recorder (MP100 system; BIOPAC System, Inc, Gioleta, Calif). These signals were digitized at 1-millisecond interval with an online analog-to-digital converter, then displayed and stored on the hard drive of a computer. We measured the MAP durations (MAPDs) at 30% and 100% repolarization levels ($MAPD_{30}$ and $MAPD_{100}$, respectively), and the magnitudes of the MAP alternans and the LVP alternans were defined as the differences between the 30th and 31st $MAPD_{30}$ and between the 30th and 31st LVP, respectively, after the cycle length was changed [11,12].

ECG measurements including the RR interval, PR interval, QRS duration, and QT interval were determined manually using on-screen cursors. Five successive ECG complexes were analyzed, and the intervals were averaged for each experiment. The QT interval (QTc) was corrected for heart rate by using Bazett formula. We analyzed the hemodynamic and electrophysiological data during sinus rhythm and rapid ventricular pacing at a cycle length of 300 milliseconds. When we stimulated the canine hearts at a cycle length of 300 milliseconds, the coupling interval between the first stimulus and the last spontaneous sinus beat was fixed at 300 milliseconds. After the control studies, we infused KB-R7943 IV at a dose of 2 mg/kg at a constant rate of 1.0 $mg \cdot kg^{-1} \cdot min^{-1}$ because Miyata et al [3] reported that the IV infusion of 1 to 5 mg/kg at a constant rate of 1.0 $mg \cdot kg^{-1} \cdot min^{-1}$ preferentially inhibited the reverse mode of the Na^+/Ca^{2+} exchange. Electrophysiological measurements were performed 10 minutes after the administration. KB-R7943 was a gift from Nippon Organon KK and was dissolved in dimethyl sulphoxide (Sigma, St Louis, MO). The final concentration of dimethyl sulphoxide was expected to be less than 0.1%, and this amount of dimethyl sulphoxide had no significant effect on any of the parameters examined.

2.3. Statistical analysis

Data are presented as mean ± SEM, and the Mann-Whitney U test was used for statistical analysis. A P value of $<.05$ was considered statistically significant.

3. Results

3.1. Electrocardiographic and hemodynamic values during sinus rhythm

The baseline values for the heart rate, PR intervals, and QRS durations were 115.1 ± 7.1 beats per minute, 105.5 ± 5.3 milliseconds, and 73.2 ± 4.3 milliseconds, respectively. The administration of KB-R7943 did not affect these parameters, as shown in Table 1. However, the QTc value increased significantly from 413.8 ± 6.5 at baseline to 444.0 ± 10.6 after we administered KB-R 7943 ($n = 10$, $P < .01$). In comparison with the control, KB-R7943 significantly increased the right ventricle (RV) $MAPD_{100}$, left ventricle (LV) $MAPD_{30}$, and LV $MAPD_{100}$ from 250.8 ± 8.5, 165.1 ± 7.2, and 252.1 ± 8.0 milliseconds to 271.2 ± 7.5, 180.6 ± 9.4, and 269.3 ± 8.1 milliseconds, respectively ($n = 10$, $P < .01$). The LVP and the peak positive and negative dP/dt remained constant throughout these experiments, and there was no significant difference between the control hearts and the hearts treated with KB-R7943 (Table 1).

Fig. 1 shows typical results from 10 experiments to demonstrate the effects of KB-R7943 on the heart during