The Rho/Rho-kinase Systems Are Involved in Rapid Pacing-induced Changes of Atrial Refractory Period in a Canine Model

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Introduction: The Rho/Rho-kinase pathway has been related to various physiological responses of the cardiovascular system. Previous reports have suggested a significant effect of Rho signals on the electrophysiological characteristics of the heart. We hypothesized that the Rho/Rho-kinase system would contribute to the rapid pacing-related change of atrial effective refractory period (AERP).

Methods and Results: In 17 dogs, AERP was measured at the right atrial appendage (RAA) and posterior left atrium (LA) before, during, and after 6-hours rapid atrial pacing at 500 bpm. Saline control (n = 5), verapamil (n = 5), or fasudil (n = 7) were infused throughout the protocol. The shortening of AERP after rapid pacing was abrogated by the administration of verapamil, as reported in previous studies. Furthermore, fasudil (Rho/Rho-kinase inhibitor) influenced the change of AERP in a manner similar to the infusion of verapamil throughout the experiments.

Conclusions: Since the AERP was attenuated by fasudil, rapid pacing-related atrial electrophysiological changes might involve the Rho/Rho-kinase pathway.


Key words: Fasudil, Electrophysiology, Effective refractory period, Rho-kinase inhibitor

Rho-kinase mediated pathway was involved in various cardiovascular responses under pathophysiological conditions. Some reports have indicated the important contribution of Rho signaling in the regulation of AV conduction and sinus node func-
Furthermore, Wei et al. suggested the possibility of proarrhythmic effects of disruption of the Rho pathway. However, the relationships between Rho/Rho-kinase signaling and the electrophysiological characteristics of the atrium have not been well discussed. Therefore, we investigated the contribution of the Rho/Rho-kinase pathway on changes of the atrial electrical characteristics related to rapid pacing in a canine model.

Methods

Animal preparation

The protocol was approved by the ethical committees of the Kanazawa University School of Medicine and was performed in accordance with institutional guidelines. Animals used in this study received humane care in compliance with the "Principles of Laboratory Animal Care", formulated by the National Society for Medical Research, and the "Guide for the Care and Use of Laboratory Animals", prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication 85-23, revised 1996).

Seventeen adult mongrel dogs weighing 12 to 20 kg were utilized in these experiments. The animals were anesthetized with sodium pentobarbital (25 mg/kg i.v., followed by bolus doses of 4 mg/kg as needed). Artificial respiration was maintained via an endotracheal tube connected to a Harvard mechanical ventilator (Harvard Apparatus, Holliston MA). The chest was opened through the left fifth intercostal space. Two bipolar electrodes (interelectrode distance, 1.5 mm) were sutured to the right atrial appendage (RAA), and left atrium (LA) just beside the left pulmonary veins for electrophysiological study. Another electrode of the same type was sutured to the left atrial appendage (LAA) for rapid atrial pacing. A unipolar epicardial pacing lead (OSCAR MEDICAL, Tokyo, Japan) was sutured to the right ventricle, connected to a programmable VVI pacemaker (Pacesetter, St. Jude Medical, St. Paul, MN, USA) and was placed in a subcutaneous pocket. Complete atrioventricular block was created by radiofrequency ablation, and VVI pacing was initiated at 80 pulses per minute. Upon completion of surgery, the dogs were administered antibiotics and allowed to recover.

Experimental preparation

After 1 to 2 weeks from the operation and with conformation of the physical recovery of the dogs, re-anesthesia and ventilation were performed in the same manner. Arterial blood pressure (RM-7000, NIHON KODEN, Tokyo, Japan) and arterial blood oxygen concentration were continuously monitored (ABL-5, Radiometer, Copenhagen, Denmark) throughout the protocols. The respiratory parameters were adjusted to maintain arterial blood gases at physiological levels (SaO₂ > 92%; pH 7.35 to 7.45). An electric blanket was utilized to maintain the body temperature at 37 ± 1°C. Pharmacological autonomic blockade was achieved by i.v. bolus injections of atropine (0.04 mg/kg) and propranolol (0.2 mg/kg), followed by a continuous infusion throughout the experiment (0.007 and 0.04 mg/kg/h, respectively). Thirty minutes after the initial infusion of atropine and propranolol, and stability of the heart rate and the blood pressure were confirmed, infusion of saline or experimental drugs was started.

Electrophysiological study

A cardiac stimulator (BC-02A, Fukuda Denshi, Tokyo, Japan) was utilized to deliver square-wave impulses of 1 ms duration. The surface ECG (lead II) and intracardiac electrograms were continuously monitored and recorded (Mingograph 7, Siemens-Elema AB, Solna, Sweden). Thirty minutes after initiation of experimental drug infusion, the AERP of the RAA and LA were measured at a basic cycle length (BCL) of 350 and 250 ms as baseline data. Seven basic drive stimuli were followed by a single

Figure 1 Typical recordings of electrocardiograms (lead II) and intracardiac electrograms at baseline (left) and during atrial extrastimuli (right). At baseline, ventricular pacing at 80 bpm with complete atrio-ventricular block was shown. The interval between the atrial electrograms was 360 ms. Extrastimuli at the RAA with basic cycle length 250 ms and S1–S2 interval 190 ms were followed by the excitation. RAA indicates right atrial appendage, and LAA left atrial appendage.
continuous infusion of 15 mg/kg for the first 3 minutes, followed by a protocol. Fasudil was administered at a dose of 5 mg/kg and the AERP was considered to indicate the differences between groups. A two-sided probability level of $p < 0.05$ was considered to indicate statistical significance.

**Results**

**Control condition**

The absolute value of AERP at the LA was shorter compared with that at the RAA ($115 \pm 13$ ms versus $126 \pm 20$ ms at a BCL of 350 ms, $p = 0.01$; $110 \pm 13$ ms versus $119 \pm 17$ ms at a BCL of 250 ms, $p = 0.05$) even after administration of autonomic blockade (Table 1). The shortening of the AERP began in the first hour and remained consistent throughout the subsequent five hours of rapid pacing. The AERPs at the RAA after 6 hours of pacing were $26 \pm 7$ ms.

**Infusion of drugs**

Infusion of the Rho/Rho-kinase inhibitor fasudil (n = 7), verapamil (n = 5), or saline as control (n = 5) was initiated 30 minutes before baseline measurements and maintained until the completion of the protocol. Fasudil was administered at a dose of 1 mg/kg for the first 3 minutes, followed by a continuous infusion of $15 \mu g/kg/min$. This was similar to that used in previous reports, and did not alter the hemodynamic parameters.6,7) Verapamil administration was initiated with a loading dose of 0.1 mg/kg for the first 2 minutes, followed by a continuous infusion of $5 \mu g/kg/min$. The drug and control experiments were performed in a randomized manner.

**Statistical analysis**

All values were expressed as the mean ± SD. Continuous values were compared with paired t test. ANOVA with Dunnett’s test was utilized to evaluate the differences between groups. A two-sided probability level of $p < 0.05$ was considered to indicate statistical significance.

**Table 1** Changes in mean AERPs before, during, and after rapid atrial pacing.

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<td></td>
<td>0</td>
<td>60</td>
<td>180</td>
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<td>104 ± 13*</td>
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<tr>
<td>Control</td>
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Values are given as mean ± SD. †P < 0.01; *P < 0.05 compared with baseline (time 0). SR (= sinus rhythm) indicates time after offset of rapid pacing. AERP = atrial effective refractory period; BCL = basic cycle length; RAA = right atrial appendage; LA = left atrium.
Figure 2  Time course of atrial effective refractory period (AERP) changes produced by rapid atrial pacing at the right atrial appendage (RAA) and left atrium (LA) in the control group (n = 5). The baseline AERP at the LA tended to be shorter than that at the RAA at basic cycle lengths (BCLs) of 350 and 250 ms. The AERP was attenuated during rapid pacing at both BCLs, and the AERP differences from BCLs gradually tended to be smaller during rapid pacing. After cessation of rapid pacing, the AERP at the RAA was restored to the baseline within 30 minutes, but the recovery of the AERP was slower at the LA at both BCLs. SR indicates the time after offset of rapid pacing.

Figure 3  Time course of atrial effective refractory period (AERP) changes produced by atrial pacing during infusion of fasudil, verapamil, or saline at the right atrial appendage (RAA) and left atrium (LA) at the BCL of 350 ms. During infusion of verapamil, the AERP did not shorten during rapid atrial pacing at the RAA and the LA. During infusion of fasudil, the AERP was slightly attenuated at the RAA but not at the LA. The AERP shortening at the RAA by the high-frequency pacing was significantly reduced in the fasudil group compared to the control. SR indicates the time after offset of rapid pacing.
involve the Rho/Rho-kinase systems. These findings are the first to demonstrate that rapid pacing, in a manner similar to the infusion of verapamil, abrogated by continuous administration of the Rho/Rho-kinase pathway responsible for the changes in the AERP.

**Administration of verapamil or fasudil**

The AERP remained unchanged during rapid atrial pacing after the infusion of verapamil (Table 1). During infusion of fasudil, the AERP at the LA also remained unchanged throughout rapid atrial pacing. Furthermore, the shortening of AERP at the RAA tended to be smaller after administration of fasudil compared with control conditions (−15 ± 14 and −10 ± 10 ms versus baseline at BCLs of 350 (p < 0.05) and 250 ms (p < 0.05), respectively) (Figure 2).

Only LA extra-stimuli induced short duration atrial fibrillation in several dogs. Furthermore, fasudil and verapamil did not interfere with this induction ability. None of the dogs exhibited induction of atrial fibrillation by RA extra-stimuli (data not shown).

In response to the initial bolus intravenous injection of verapamil or fasudil, the arterial pressure tended to be slightly attenuated, but this change was not statistically significant (from 154 ± 44/80 ± 21 to 152 ± 42/81 ± 16 mmHg with verapamil, and from 163 ± 46/87 ± 18 to 137 ± 29/67 ± 9 mmHg with fasudil, respectively). There were no significant changes during continuous infusion throughout the experiments.

**Discussion**

The major findings of this study are as follows: (1) the AERP was significantly shortened during rapid atrial pacing in this closed-chest dog model at the left atrium and right atrial appendage, and (2) the change of the AERP during rapid pacing was abrogated by continuous administration of the Rho/Rho-kinase inhibitor, fasudil, especially in the LA, in a manner similar to the infusion of verapamil. Thus, these findings are the first to demonstrate that rapid pacing-induced atrial electrophysiological changes involve the Rho/Rho-kinase systems.

Experimental and clinical studies have demonstrated a progressive shortening of atrial refractoriness in response to rapid atrial pacing, and this phenomenon is defined as “atrial electrical remodeling.” The shortening of the AERP induced by short-term rapid atrial pacing has been shown to be blocked by the L-type calcium channel blocker, verapamil, and is accentuated by hypercalcemia. The autonomic responses, atrial mechanical stretch and depletion of high-energy phosphates have been reported to exert very limited influence on AERP shortening. Therefore, calcium loading might be responsible for this type of atrial electrophysiological change. Our data, under control conditions demonstrated AERP shortening by an average of 18 to 26 ms from the baseline after 6 hours of rapid pacing. This electrophysiological change was abrogated by verapamil, which was quite similar to previous reports. However, the intracellular signaling pathway responsible for the changes in the AERP after the calcium influx into the cell remains to be elucidated.

Several studies have demonstrated regional difference of AERP. We observed 9 to 30 ms differences of the AERP between the RAA and LA at the baselines. Furthermore, in our study the recovery of the AERP at the LA after cessation of the rapid pacing was significantly slower, compared with that at the RAA, similar to the findings of previous studies.

The low-molecular-weight G protein, Rho, is a member of the Rho family of small GTPases, which also includes Rac and Cdc42. These GTPases act as molecular switches to regulate cellular function. The role of the Rho/Rho-kinase pathway has been discussed mainly in smooth muscle, regulating myosin light chain (MLC2) phosphorylation, and has been reported to be an important factor especially in hypertension or coronary vasospasm. Furthermore, Rho-kinase has been recognized to promote hypertrophic changes and fibrosis in cardiac cells. By contrast, the interaction between the Rho/Rho-kinase signal and atrial muscle contractility has been demonstrated in rats and humans. The Rho/Rho-kinase pathway has also been demonstrated to play an important role in regulating the development of the cardiac conduction system. Wei et al. reported that inhibition of Rho family protein activities stimulated atrioventricular conduction abnormalities with prolonged atrial refractory periods and suggested the possible contribution of Rho signals towards atrial arrhythmias.

According to the current findings with fasudil, the shorting of the AERP after rapid atrial pacing was...
partially but significantly reduced by inhibition of the Rho/Rho-kinase pathway. The direct interaction of the Rho/Rho-kinase system and calcium kinetics has only been demonstrated in vitro. In general, the effect(s) of the Rho/Rho-kinase pathway on smooth muscle contraction has been considered to occur via a calcium-independent pathway. However, Rho-kinase inhibitors have been shown to reduce the noradrenaline-evoked stimulation of calcium influx, distinct from voltage-operated calcium channels or thapsigargin-activated store-operated channels in vascular smooth muscle. In addition, the species-specific cross-interaction of Rho-kinase with L-type calcium channel activation has recently been suggested. In brief, the Rho-kinase inhibitor directly inhibited smooth muscle contraction through interference with L-type calcium channel inactivation in rat ureter smooth muscle. Furthermore, tracheal smooth muscle contraction was mediated through the Rho-kinase pathway via increased calcium influx and augmented calcium sensitization. Thus, the interactions between calcium kinetics and the Rho/Rho-kinase pathway may be different between organs and under various pathophysiological conditions. In this study, the effects of fasudil and verapamil showed some tendency to differ between the atria. A certain signaling pathway accompanied with these drugs affecting the AERP changes by rapid atrial pacing might be different between the atria. The changes of the AERP throughout rapid pacing after administration of verapamil and fasudil were similar throughout this investigation, at least in the LA. These electrophysiological characteristics might be influenced by mechanical stress, in addition to electrical stress. However, we only performed provocation for 6 hours and administered artificial atrio-ventricular block to exclude the effect of documented hemodynamic changes, such as cardiac dysfunction. None of the dogs exhibited any significant changes of systemic blood pressure, nor showed apparent tachy- or bradycardia throughout this investigation. We carefully conducted this experiment to avoid with the effects of physiological and other types of stress.

In summary, the atrial electrical remodeling evoked by 6 hours of rapid atrial pacing was initiated by intracellular calcium overload. The Rho-kinase inhibitor, fasudil, reduced the changes in AERP throughout rapid atrial pacing. Based on these findings, the Rho/Rho-kinase system is significantly involved in the pacing-induced shortening of the atrial effective refractory period in a canine model.

References


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