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Increased Ca^{2+} sparks and sarcoplasmic reticulum Ca^{2+} stores potentially determine the spontaneous activity of pulmonary vein cardiomyocytes

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ABSTRACT

Pulmonary veins (PVs) contain cardiomyocytes with spontaneous activity that may be responsible for PV arrhythmia. Abnormal Ca^{2+} regulation is known to contribute to PV arrhythmogenesis. The purpose of this study was to investigate whether PV cardiomyocytes with spontaneous activity have different intracellular Ca^{2+} ($[Ca^{2+}]_i$) transients, Ca^{2+} sparks and responses to isoproterenol and ryanodine receptor modulators (magnesium and FK506) than do PV cardiomyocytes without spontaneous activity and left atrial (LA) cardiomyocytes. Through fluorescence and confocal microscopy, we evaluated the $[Ca^{2+}]_i$ transients and Ca^{2+} sparks in isolated rabbit PV and LA cardiomyocytes. PV cardiomyocytes with spontaneous activity had larger $[Ca^{2+}]_i$ transients and sarcoplasmic reticulum (SR) Ca^{2+} stores than PV cardiomyocytes without spontaneous activity or LA cardiomyocytes. PV cardiomyocytes with spontaneous activity also had a higher incidence and frequency of Ca^{2+} sparks, and had Ca^{2+} sparks with larger amplitudes than other cardiomyocytes. Magnesium (5.4 mM) reduced the $[Ca^{2+}]_i$ transient amplitude and beating rate in PV cardiomyocytes with spontaneous activity. However, in contrast with other cardiomyocytes, low doses (1.8 mM) of magnesium did not reduce the $[Ca^{2+}]_i$ transients amplitude in PV cardiomyocytes with spontaneous activity. FK506 (1 μM) diminished the SR Ca^{2+} stores in PV cardiomyocytes with spontaneous activity to a lesser extent than that in other cardiomyocytes. Isoproterenol (10 nM) increased the $[Ca^{2+}]_i$ transient amplitude to a lesser extent in LA cardiomyocytes than in PV cardiomyocytes with or without spontaneous activity. In conclusion, our results suggest that enhanced $[Ca^{2+}]_i$ transients, increased Ca^{2+} sparks and SR Ca^{2+} stores may contribute to the spontaneous activity of PV cardiomyocytes.

Introduction

Atrial fibrillation (AF) is the most commonly sustained arrhythmia in clinical practice and may induce stroke and heart failure. Pulmonary veins (PVs) are common sources of ectopic beats initiating and maintaining atrial fibrillation (Pappone et al., 2000). Previous studies have indicated that abnormal Ca^{2+} regulation with the genesis of early afterdepolarization and delayed afterdepolarization (DAD) contribute to high arrhythmogenic activity in the PV cardiomyocytes (Honjo et al., 2003; Patterson et al., 2006; Chen et al., 2001, 2004; Chou et al., 2005; Wongcharoen et al., 2006; Huser et al., 2000). Honjo et al. found that even a low concentration of ryanodine induced PV firing, and suggested that enhancement of the Ca^{2+} leak may play an important role in PV electrical activity (Honjo et al., 2003). Patterson et al. indicated that an increase in the intracellular Ca^{2+} ($[Ca^{2+}]_i$) transients and Na^+–Ca^{2+} exchange may induce EADs and result in PV arrhythmogenesis (Patterson et al., 2006). Our previous study also found that PV electrical activity can be reduced by a Na^+–Ca^{2+} exchanger (NCX) inhibitor (KB-R7943), which reduced the $[Ca^{2+}]_i$ transients amplitude and sarcoplasmic reticulum (SR) Ca^{2+} stores (Wongcharoen et al., 2006). The above findings suggest that the regulation of $[Ca^{2+}]_i$ transients in PV cardiomyocytes plays an important role in PV arrhythmogenesis.

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Most $[Ca^{2+}]_i$ transients are induced by a Ca^{2+} influx triggering a large Ca^{2+} release from the SR. The major triggers were proposed to be inward NCX, L-type and T-type Ca^{2+} currents. There was no significant difference in the first two currents among the PV cardiomyocytes. However, the T-type Ca^{2+} current was more abundant in the PV cardiomyocytes with spontaneous activity than in either those without spontaneous activity or left atrial (LA) cardiomyocytes, suggesting that T-type Ca^{2+} current is responsible for PV arrhythmia (Chen et al., 2004). The $[Ca^{2+}]_i$ transients were also regulated by the SR Ca^{2+} store. As the SR Ca^{2+} content was increased, the SR released Ca^{2+} spontaneously and promoted the arrhythmia onset (Lukyanenko et al., 2001). Spontaneous non-propagating Ca^{2+} releases (Ca^{2+} sparks), are also mediated by the ryanodine receptor (RyR)/Ca^{2+} release channels in the SR. The cardiomyocytes from patients with AF have been shown to have more Ca^{2+} sparks than those without AF (Hove-Madsen et al., 2004). These findings indicate the importance of the Ca^{2+} sparks in the pathophysiology of AF. RyR modulators could also affect the PV arrhythmogenesis. FK506, an inhibitor of the FK506 binding protein on the RyR, can increase the diastolic Ca^{2+} release from the SR through the RyR, and therefore induce DAD and trigger cardiac arrhythmias (Wongcharoen et al., 2007). Magnesium, an important antagonist of Ca^{2+}, inhibits the Ca^{2+} release from the SR. It reduces the PV arrhythmogenesis and prevents postoperative atrial fibrillation (Miller et al., 2005; Chen et al., 2000). In addition, adrenergic stimulation was also demonstrated to enhance the PV spontaneous activity (Lo et al., 2007, Tai et al., 2000). Coutu et al. found that there was no significant difference in the SR Ca^{2+} stores or in the response to isoproterenol between the atrial and PV cardiomyocytes (Coutu et al., 2006). However, in their study, no spontaneous activity was detected in the PV cardiomyocytes. Thus, there is limited understanding of the determinants of $[Ca^{2+}]_i$ transients and SR Ca^{2+} stores in the PV cardiomyocytes with spontaneous activity and their responses to RyR modulators and isoproterenol.

Here, we studied the cellular mechanisms of Ca^{2+} handling and evaluated the response to RyR modulators in rabbit PV and LA cardiomyocytes. We demonstrate that there are larger $[Ca^{2+}]_i$ transients, more Ca^{2+} sparks and greater SR Ca^{2+} stores in the PV cardiomyocytes with spontaneous activity than in either the PV cardiomyocytes without spontaneous activity or LA cardiomyocytes, but that FK506 and magnesium do not reduce $[Ca^{2+}]_i$ transients as much in PV cardiomyocytes with spontaneous activity as in the other cardiomyocytes.

Materials and methods

Isolation of PV and atrial cardiomyocytes

The guidelines in the "Principles of laboratory animal care" (NIH publication No. 86-23, revised 1985) were followed and all procedures were approved by institutional committees. Using a procedure described previously (Chen et al., 2001; Wongcharoen et al., 2006), the rabbit PV and LA cardiomyocytes were enzymatically dissociated in normal Tyrode's solution (NaCl 137, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 0.5, HEPES 10 and glucose 11 mM; the pH was adjusted to 7.4 by titration with 1 N NaOH). Only cardiomyocytes that had clear striations and that could be electrically stimulated stably were used. The PV cardiomyocytes with spontaneous activity were identified by the presence of constantly beating.

Measurement of the intracellular calcium

The $[Ca^{2+}]_i$ were measured as described previously (Wongcharoen et al., 2006). In brief, cardiomyocytes were loaded with 25 μM of indo-1/AM (Sigma) at room temperature for 20 to 30 min and then perfused with a normal bath solution at 35±1 °C for at least 20 min to wash out the extracellular indicator and to allow for intracellular deesterification of the indo-1. Thereafter, the cells were excited at 360 nm with a xenon UV arclamp, which was controlled by a microfluorometry system (OSP100-CA, Olympus). The ratios of the indo-1 fluorescence at 410 and 485 nm ($R_{410/485}$) were used as the index of the $[Ca^{2+}]_i$ concentrations. During the fluorescence experiments at 35±1 °C, right atria pacemaker cells had a beating rate (1.4±0.1 Hz, n=6) similar to that (1.3±0.2 Hz) of PV cardiomyocytes with spontaneous activity (n=12). The cells were stimulated with a 1.5 Hz field-stimulation with 10-ms twice-threshold strength square-wave pulses. The time constant of the $[Ca^{2+}]_i$ transient decay (τ_{Ca}) was determined by a monoexponential least-squares fit. The SR Ca^{2+} content was stabilized after electrical stimulation at 1.5 Hz for at least 30 s and was estimated by adding 20 mM caffeine (Sigma). To evaluate the effects of magnesium and FK506 on Ca^{2+} handling in the cardiomyocytes, the $[Ca^{2+}]_i$ transients were measured in the normal Tyrode's solution and magnesium (1.8 or 5.4 mM) or FK506 (1 μM, Sigma) solutions. The effects of isoproterenol (10 nM, Sigma), calcium channel activator (Bay K 8644, 10 μM, Tocris) and $BaCl_2$ (1 mM, Sigma) on the spontaneous activity of PV cardiomyocytes were also evaluated.

Ca^{2+} sparks imaging with confocal microscope

PV and LA cardiomyocytes were loaded with a fluorescent Ca^{2+} (10 μM) fluo-3/AM for 30 min at room temperature. The excess extracellular dye was removed by changing the bathing solution and allowing the intracellular hydrolysis of the fluo-3/AM after 30 min. Fluorescence imaging was performed with a laser scanning confocal microscope (Zeiss LSM 510) and an inverted microscope (Axiovert 100). The Ca^{2+} sparks were detected through the line scan mode along a line parallel to the longitudinal axis of single cardiomyocytes, avoiding nuclei. The Fluo-3 fluorescence was excited with a 488-nm line of an argon ion laser. The emission was recorded at >515 nm. Cells were repetitively scanned at 3-ms intervals for a total duration of 6 s. Each line was composed of 512 pixels. The Ca^{2+} sparks were detected as an increase in the signal mass (less than 6 μm). The amplitudes of the Ca^{2+} sparks were presented as background-subtracted normalized fluorescence (F/F_0) where F was the fluorescence intensity and F_0 the resting fluorescence recorded under steady-state conditions. The Ca^{2+} spark frequencies were expressed as the number of observed sparks (per second and per mm of scanned distance) and the incidences as the percentage of cells exhibiting Ca^{2+} sparks in the resting stage of the LA cardiomyocytes and PV cardiomyocytes without spontaneous activity or in the diastolic phase of the PV cardiomyocytes with spontaneous activity.

Statistical analysis

All quantitative data are expressed as the mean±SEM. Differences between the PV and LA cardiomyocytes were compared by a one-way ANOVA with a post hoc analysis using the Tukey test. A paired t-test was used to compare the differences before and after drug administration. Nominal variables were compared by a Chi-square analysis with Yates correction or Fisher's exact test. A value of $P<0.05$ was considered statistically significant.

Results

$[Ca^{2+}]_i$ transients and Ca^{2+} stores in the PV and atrial cardiomyocytes

Fig. 1A shows the tracings of $[Ca^{2+}]_i$ transients in the PV and LA cardiomyocytes. There was no significant difference in the $[Ca^{2+}]_i$ transients between the LA cardiomyocytes and PV cardiomyocytes without spontaneous activity. However, the PV cardiomyocytes with spontaneous activity (n=15) had a higher $[Ca^{2+}]_i$ transient amplitude ($R_{410/485}$, 0.36±0.04, 0.25±0.02, 0.24±0.02, $P<0.05$), and a higher peak systolic ($R_{410/485}$, 1.16±0.05, 0.92±0.03, 0.96±0.02, $P<0.01$) and a