Journal of Pharmacological Sciences 133 (2017) 195–202

Contents lists available at ScienceDirect

Journal of Pharmacological Sciences

journal homepage: www.elsevier.com/locate/jphs

Full paper

Permissive role of reduced inwardly-rectifying potassium current density in the automaticity of the guinea pig pulmonary vein myocardium

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ARTICLE INFO

Article history:
Received 12 September 2016
Received in revised form
12 December 2016
Accepted 18 December 2016
Available online 28 March 2017

Keywords:
Pulmonary vein myocardium
Potassium channel
Automaticity
Resting membrane potential
Intracellular Ca^{2+}

ABSTRACT

The electrophysiological properties underlying the automaticity of the guinea pig pulmonary vein myocardium were studied. About 30% of the isolated pulmonary vein tissue preparations showed spontaneous electrical activity, as shown by glass microelectrode recordings from their myocardial layer. The remaining quiescent preparations had a resting membrane potential less negative than that in the left atria. Blockade of the acetylcholine activated potassium current ($I_{K\text{-}ACh}$) by tertiapin induced a depolarizing shift of the resting membrane potential and automatic electrical activity in the pulmonary vein, but not in the atria. The tertiapin-induced electrical activity, as well as the spontaneous activity, was inhibited by the application of carbachol or by chelation of intracellular Ca^{2+} by BAPTA. The isolated pulmonary vein cardiomyocytes had an $I_{K\text{-}ACh}$ density similar to that of the atrial cardiomyocytes, but a lower density of the inwardly-rectifying potassium current (I_{K1}). Spontaneous Ca^{2+} transients were observed in about 30% of the isolated pulmonary vein cardiomyocytes, but not in atrial cardiomyocytes. The Ca^{2+} transients in the pulmonary vein cardiomyocytes were induced by tertiapin and inhibited by carbachol. These results indicate that the pulmonary vein cardiomyocytes have a reduced density of the inwardly-rectifying potassium current, which plays a permissive role in their intracellular Ca^{2+}-dependent automaticity.

1. Introduction

The pulmonary vein wall contains a myocardial layer connected to the left atrial myocardium, which generates spontaneous or triggered action potentials.[1] Although its contribution to the regulation of pulmonary circulation has been postulated,[2] the physiological role of the automaticity of the pulmonary vein myocardium remains to be clarified. On the other hand, clinical reports that paroxysmal atrial fibrillation is initiated by trains of rapid discharges from the pulmonary veins[3,4] attracted much attention. Since then the electrical activity of the pulmonary vein myocardium has been considered to play a central role in the generation and maintenance of atrial fibrillation, the most common type of arrhythmia in clinical practice. Thus, investigation of the mechanisms of automaticity in the pulmonary vein myocardium is physiological and clinical importance.

Automaticity of the pulmonary vein myocardium has been studied in various animal species. The pulmonary vein myocardium in general has a lower (less negative) resting membrane potential when compared to atrial myocardium[5–7] and its automaticity appears to be dependent on mechanisms different from that in the sinus node, although the mechanisms may vary among experimental animal species.[8] Concerning the mechanisms of the

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Peer review under responsibility of Japanese Pharmacological Society.

http://dx.doi.org/10.1016/j.jphs.2016.12.006

automaticity of the guinea pig pulmonary vein, microelectrode recording of the spontaneous action potential of the myocardial layer was performed by several researchers including ourselves[7,9,10] and the action of antiarrhythmic agents has been examined.[11–14] The spontaneous electrical activity of the guinea pig pulmonary vein myocardium was inhibited by ryanodine, which interferes with Ca^{2+} release from the sarcoplasmic reticulum, and SEA0400, a selective inhibitor of the plasmalemmal Na^{+}-Ca^{2+} exchanger.[10] These results indicated that the spontaneous firing of the pulmonary vein myocardium was dependent on Ca^{2+} released from the sarcoplasmic reticulum and the activity of the plasmalemmal Na^{+}-Ca^{2+} exchanger. Involvement of intracellular Ca^{2+} and the plasmalemmal Na^{+}-Ca^{2+} exchanger have also been reported in the rabbit[15] and rat[6,16] pulmonary vein myocardium. Concerning the mechanisms for the manifestation of electrical activity in the guinea pig pulmonary vein myocardium, the less negative resting membrane potential[7,9,10] implies that the reduced stability of membrane potential due to the reduced inwardly-rectifying potassium current allows repetitive firing.[8,10] However, the electrophysiological properties of the guinea pig pulmonary vein myocardium responsible for its automaticity has not yet been thoroughly examined. The present study was undertaken to clarify whether the automaticity of the guinea pig pulmonary vein myocardium could be attributed to the reduced inwardly-rectifying potassium current density. We compared the electrophysiological properties of the isolated pulmonary vein cardiomyocytes with that of atrial and examined the effect of pharmacological agents affecting the inwardly-rectifying potassium current.

2. Materials and methods

2.1. Action potential measurements

All experiments were performed in accordance with the Guiding Principles for the Care and Use of Laboratory Animals approved by The Japanese Pharmacological Society. Standard glass microelectrode experiments were performed with isolated guinea pig pulmonary vein and left atrial preparations as in our previous study.[10] The pulmonary veins of Hartley strain guinea pigs (weight, 300–500 g) were separated from the atrium at the left atrium-pulmonary vein junction, and separated from the lungs at the ending of the pulmonary vein myocardial sleeves. Tubular pulmonary veins were cut open and pinned down luminal side up on a silicon block placed at the bottom of a 20 ml recording chamber containing physiological salt solution of the following composition (mM): NaCl 118.4, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 24.9, glucose 11.1 (pH 7.4); it was gassed with 95% O_2–5% CO_2 and maintained at 36 ± 0.5 °C. The glass microelectrodes filled with 3 M KCl had resistances of 20–30 MΩ. The output of a microelectrode amplifier (MEZ8201; Nihon Kohden, Tokyo) was recorded and analyzed by Chart 7 software (AD Instruments, Dunedin, New Zealand). The action potential parameters: resting membrane potential (RMP); maximum diastolic potential (MDP); overshoot (OS); action potential duration at 20%, 50%, and 90% repolarization (APD_{20}, APD_{50}, APD_{90}, respectively); maximum rate of phase 0 depolarization ($\dot{V}_{max}$) and maximum rate of phase 4 depolarization (slope) were measured either under spontaneous firing or under field electrical stimulation at 1 Hz.

2.2. Current measurements

Langendorff-perfused hearts with the pulmonary veins attached were treated with 0.5 mg/mL collagenase (YK-102; Yakult Honsha Co, Ltd., Tokyo) and 0.1 mg/mL protease (type XIV; Sigma–Aldrich, St. Louis, Mo, U.S.A.) for about 15 min, after which cardiomyocytes were isolated from all four pulmonary veins and stored at 15–16 °C. The solution for cell storage contained (mM): glutamic acid 70, taurine 15, KCl 30, KH_2PO_4 10, $MgCl_2 \cdot 6\ H_2O$ 0.5, glucose 11, HEPES 10, EGTA 0.5 (pH 7.3/KOH).

The extracellular solution for current measurement and confocal microscopy contained (mM): NaCl 143.0, KCl 5.4, $MgCl_2$ 1.0, $CaCl_2$ 1.8, NaH_2PO_4 0.33, glucose 5.5, HEPES 5.0 (pH 7.4); it was gassed with 100% O_2 and maintained at 32 ± 0.5 °C. $CdCl_2$ (2 mM) and 4-aminopyridine (3 mM) were added to suppress the Ca^{2+} current and transient outward current, respectively. The borosilicate glass electrodes had tip resistances between 1.5 and 3 MΩ. The internal solution contained (mM): aspartic acid 70.0, KCl 40.0, KOH 100.0, EGTA 10.0, HEPES 10.0, ATP-Mg 5.0, Tris-GTP 0.3, Na_2-creatine phosphate 5.0 (pH 7.2/KOH). Inwardly-rectifying potassium currents were elicited by 300 ms hyperpolarizing voltage clamp pulses stepping from −40 mV to voltages between −120 mV and 0 mV with 0.1 Hz. The total inwardly-rectifying potassium current was recorded as 1 mM $BaCl_2$-sensitive current, and $I_{K\text{-}ACh}$ was recorded as 0.3 μM tertiapin-sensitive current, and I_{K1} was recorded as 0.3 μM tertiapin-insensitive and 1 mM $BaCl_2$-sensitive current.

2.3. Confocal microscopy

Confocal microscopic analyses of the Ca^{2+} dynamics in isolated pulmonary vein cardiomyocytes were performed with LSM 510 (Carl Zeiss, Jena, Germany) with procedures basically the same as previously described.[10] For the observation of intracellular Ca^{2+} movement, the isolated pulmonary vein cardiomyocytes were treated with fluo 4-AM (Dojindo Laboratories; Kumamoto), and superfused with the extracellular solution mentioned above. The cells were line scanned at a speed of line/960 μs. The excitation wavelength was 488 nm and fluorescence with wavelength above 505 nm was detected and analyzed.

2.4. Data analysis and statistics

Data were presented as mean ± standard error of the mean (S.E.M.). Statistical significance between means was evaluated by the paired *t*-test, the Student's *t*-test, Welch's *t*-test or one-way analysis of variance followed by the Tukey's test for multiple comparisons. *p* Values less than 0.05 were considered significant.

2.5. Drugs and chemicals

$BaCl_2$, carbachol, glibenclamide, $CdCl_2$, $NiCl_2$ and 4-aminopyridine were purchased from Wako Pure Chemical Industries, Ltd. (Osaka), tertiapin was purchased from Peptide Institute, Inc. (Osaka), 1,2-Bis (2-aminophenoxy) ethane-N,N,N′,N′-tetraacetic acid acetoxymethyl ester (BAPTA-AM) was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo) and E-4031 and chromanol293B were purchased from Sigma–Aldrich (St. Louis, MO, U.S.A.). SEA0400 was synthesized according to the reported methods.[17] Glibenclamide, BAPTA-AM, SEA0400 and chromanol293B were dissolved in DMSO and small aliquots were applied to the extracellular solution; the final concentration of DMSO was 0.05%. The other chemicals were dissolved in distilled water.

3. Results

3.1. Electrical activity of the pulmonary vein and atrial myocardium

To study the automaticity of the pulmonary vein myocardium, the membrane potential was recorded with microelectrode penetrations into the myocardial layer of isolated pulmonary vein tissue preparations (Fig. 1A, Table 1). Among the 78 isolated guinea pig