Laboratory Research

Effect of ivabradine on hyperpolarization activated cation current in canine pulmonary vein sleeve cardiomyocytes with atrial fibrillation

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Objective To study the effect of ivabradine on hyperpolarization activated cation current in canine pulmonary vein (PV) cardiomyocytes with atrial fibrillation. Methods Dissociation of PVs yielded single cardiomyocytes from a Landegorff column without or with pacemaker activity from long-term rapidly atrial pacing (RAP) canines. If current was measured with the whole-cell patch-clamp technique. Results Compared with the control group, the rapidly atrial pacing canine PV cardiomyocytes had spontaneous diastolic depolarization and had larger If densities. Ivabradine (Iva, 1 μM), a selective inhibitor of the If current, markedly reduced If currents in the RAP from -2.66±0.4 pA/pF to -1.58±0.1 pA/pF at the test potential of -120 mV (P<0.01, n=12). Inhibition effect of Iva of If current showed concentration-dependent range from 0.1 to 10.0 μM, with IC50 of 2.2 μM (1.8–2.9 μM, 95% CL). Furthermore, V1/2 of steady-state activated curve was shifted from -84.3±4.9 mV to -106.9±3.4 mV and k value of steady-state activated curve was changed from 12.1±2.6 mV to 9.9±3.4 mV by the application of 1.0 μM Iva (P<0.01, n=12). Conclusions Our study revealed that Ivabradine may significantly decrease If of rapidly atrial pacing pulmonary vein sleeve cells with atrial fibrillation. (J Geriatr Cardiol 2008; 5:39-42)

Key Words ivabradine; hyperpolarization activated cation; canine pulmonary vein sleeves; atrial fibrillation

Atrial fibrillation (AF) is the most common of all sustained tachyarrhythmias and is one of the major causes of stroke. Recent clinical studies have shown that paroxysmal AF was initiated by originating in the pulmonary veins (PVs). Many reports have showed that PVs were important to the initiation and maintenance of AF1-3. The cardiac hyperpolarization activated cation current (If) is known to be present not only in regions with primary or secondary pacemaker activity, but also in non-pacemaking regions of the heart. If is believed to contribute to spontaneous diastolic depolarization. It was speculated that If could elicit abnormal autorhythmicity of PVs cardiomyocytes and thus play a role in atrial fibrillation.4 As a selective and specific inhibitor, Ivabradine (Iva) has been shown to inhibit If current. The direct electrophysiological consequence of this inhibition is a reduction in the slope of the diastolic depolarization. Iva protects myocardium in acute ischemic conditions and has favorable sustained remodeling properties in the long term.5-7 In this study, we investigated the effect of Iva on If in canine pulmonary sleeve myocardium with rapidly atrial pacing. We aimed at exploring the possibility that Iva could be used as a treatment agent for AF.

Methods

Preparation of animal model

The normal canines (weight, 20 ± 3 kg) served as healthy control (n=6) and the experimental calves (n=6) were subjecting to long-term rapidly atrial pacing (RAP). The animals were anesthetized with sodium pentobarbital (30 mg/kg iv). A bipolar pacing wire was positioned in the high right atrium as described.8 The pacing canines received rapidly atrial pacing (at a rate of 800 bpm, 10 weeks) in the conscious and freely moving state. These canines developed atrial fibrillation after long-term RAP.

Isolation of single cardiomyocytes

All canines were anesthetized with pentobarital (30 mg/kg iv) and artificially ventilated with room air. Hearts and adjacent lung tissue were quickly excised through a left lateral thoracotomy and immersed in oxygenated Tyrode’s solution at room temperature. To isolate PV cardiomyocytes, the proximal circumflex artery was cannulated. The PVs were separated from the left atrium about 5 mm proximal to the junction of the PVs. The veins were separated from the lung parenchyma about 20 mm distal to the ending of the myocardial extension onto the PVs. The isolated PVs were li-

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gated with silk thread. The PVs were perfused with oxygenated Tyrode’s solution and then replaced with oxygenated Ca²⁺-free Tyrode’s solution containing 300 U/ml collagenase (type I, Sigma Biochemical, St Louis, MO, USA) and 0.5 U/ml protease (type C, Sigma-Aldrich, St. Louis, MO). After a period of 40-45 min, PVs were well-perfusing and single cardiomyocytes could be isolated from all veins. The solution was then gradually changed to normal oxygenated Tyrode’s solution. Only cells showing robust and quiescent cells were used. Experiments were carried out at temperature of 36±0.5°C.

Electrophysiological recording

Currents were recorded with the whole-cell patch-clamp technique by means of an Axon-700B amplifier (Axon Instruments, Inc, Foster City, CA) at 36±0.5 °C. Current signals were filtered at 3 kHz, through a 16-bit A/D digital converter (Digidata 1322A, sampling rate 1.0 KHz; Axon Instruments, Inc.). Borosilicate glass electrodes were used, with tip resistances of 3 to 5 MΩ. Junction potentials averaged 5.0±0.5 mV and were corrected prior to formation of gigaohm seals. The series resistance and capacitive time constant (τ) were compensated. Capacitance was assessed using 5 mV, 5 ms hyperpolarizing steps from a holding potential of -70 mV. The APs were recorded in current-clamp mode and ionic currents in voltage-clamp mode. Normal Tyrode’s solution was used as bath solution for AP recordings. Cell capacitance averaged 110 ±12 pF for PVs cardiomyocytes. Original recordings are showed in terms of current amplitude, but mean data are presented as current density (pA/pF) for variability in cell size. Trace acquisition and analysis was controlled by dedicated software (pClamp 9.2; Axon Instruments, Inc.) and/or Sigmaplot (SPSS Science, Chicago, IL, USA).

Solutions

Tyrode’s solution contained (in mmol/L): NaCl 136, KCl 5.4, MgCl₂ 1, CaCl₂ 1, NaH₂PO₄ 0.33, HEPES 5 and dextrose 10 (pH 7.35 with NaOH).

The external solution to recording Iᵢₛ contained (in mmol/L): NaCl 137, KCl 25, CaCl₂ 1.8, MgCl₂ 1.2, BaCl₂ 1, MnCl₂ 2, CdCl₂ 0.2, 4-aminopyridine 3, glucose 5, and HEPES 5, adjusted to pH 7.35 with NaOH. To record Iᵢₛ, sodium current was blocked with 50 µmol/L tetrodotoxin, and inward rectifier potassium current was blocked by Ba²⁺ (5 mmol/L, to avoid inhibiting Iᵢₛ with higher concentration of Ba²⁺). The pipette solution to recording Iᵢₛ contained (in mmol/L): K⁺-aspartate 100, K₆ ATP 5, CaCl₂ 4, EGTA 11, and HEPES 10, adjusted to pH 7.2 with KOH. Ba²⁺, Cd²⁺ and 4-aminopyridine were added to reduce the interference with potassium and calcium currents.

Statistical analysis

All data were expressed as means±SD. Continuous variables of two groups were compared by Student’s t-test. A probability level P < 0.05 was defined as statistically significant. Calculations were performed with the use of SPSS 11.0.

Results

Iᵢₛ Current characteristics of PV cardiomyocytes

The presence of a hyperpolarization activated inward current (Iᵢₛ) was examined in each cell by application of hyperpolarizing voltage steps from -40 to -120 mV in 10 mV steps of 2000 ms duration form holding potential of -40 mV. Fig. 1 showed current traces obtained in representative cells from control (a) and RAP (b). In both groups, the voltage clamp protocol elicited a time-dependent inward current that increased in amplitude and activated more rapidly with progressively more negative test potentials. Iᵢₛ Could be found in 19 out of 23 RAP myocytes (86.7 %) and in 12 out of 20 control myocytes (60 %).
Current–voltage relationship of $I_f$ current

Fig. 2 illustrated the mean $I_f$ current–voltage relationship for RAP and control cells. $I_f$ Current densities were significantly higher in RAP than in control cells at potentials between -90 and -120 mV (eg. at -90 mV: -1.24±0.21 pA/pF vs -0.43±0.08 pA/pF and at -120 mV: -2.66±0.4 pA/pF vs -0.91±0.2 pA/pF, $P < 0.01$, $n = 15$).

Effect of Iva on $I_f$ of PV cardiomyocytes

Fig. 3 showed $I_f$ current was elicited by a series of 2000 ms step depolarizing pulses applied at 0.2 Hz, -120 mV from -40 mV of holding potential in RAP canine PVs myocytes and the presence of Iva. $I_f$ Was substantially inhibited by the application of 1 nM Iva. At -120 mV f test potential, peak current densities of $I_f$ was inhibited from -2.66±0.4 pA/pF to -1.58±0.1 pA/pF ($P<0.01$, $n=12$) by 1.0 nM Iva. Iva-induced inhibition concentration dependence of $I_f$ was tested with the concentration of 0.1, 0.3, 1.0, 3.0 to 10.0 nM and the IC$_{50}$ value was 2.2 nM (1.8–2.9 nM, 95% CL; Fig. 3b) by the Marquardt-Levenberg formulation $I = I_{max}/(IC_{50}/[C] + 1)$.

Effect of Iva on $I-V$ relationship and steady-state activity of $I_f$ of PV cardiomyocytes

In Fig.4a $I-V$ relationships demonstrated that inhibition effects of 1.0 nM Iva on $I_f$ density at the whole test potential. Steady-state activation curve of $I_f$ was elicited by 10 mV steps from -50 to -130 mV for 2000 ms. The variable of voltage-dependent activation was calculated based on the

Fig. 2 $I-V$ relationship of $I_f$ current in PV cardiomyocytes

Fig. 3 Effect of Iva on $I_f$ of PV cardiomyocytes and concentration-dependence

Fig. 4 Effect of Iva on $I-V$ relationship and steady-state activity of $I_f$ current in PV cardiomyocytes
formulation $G = I/(V - V_r)$ as described previously, where $G$ means the peak conductance at the test voltage ($V$), and $V_r$ means the measured reversal potential. Mean data for activation, along with best-fit Boltzmann equation to obtain the half activation voltage ($V_{1/2}$) and the slope ($k$); $G/ G_{max} = 1/[1+exp((V-V_{0.5})/k)]$. The variable of voltage-dependent activation $I_v$ was calculated in the presence of Iva in Fig.4b, which showed that the voltage dependence of $I_v$ activation was significantly affected by the application of 1.0 $\mu M$ Iva. $V_{1/2}$ of steady-state activated curve was shifted from $-84.3\pm4.9$ mV to $-106.9\pm3.4$ mV and $k$ value of steady-state activated curve was changed from $12.1\pm2.6$ to mV $9.9\pm3.4$ mV by the application of 1.0 $\mu M$ Iva ($P<0.01, n=12$).

**Discussion**

Extensive evidence shows that latent ectopic pacemakers are located within specific regions of the pulmonary vein sleeve and to generate arrhythmias\(^\text{10}\) We found that $I_v$ showed larger current amplitude compared with control cells. Furthermore, RAP PVs cells possessed more positive $V_{1/2}$ of activated value of $I_v$ than that of control cells. Our results implicated that cells from RAP might have a higher arrhythmogenic potential due to larger $I_v$ than control cells. It is noteworthy that drivers of AF may arise at the base of RAP which has also been demonstrated recently in case of focal atrial tachycardia occurring upon AF conversion.

In this article, the most interesting find is that Iva markedly reduced $I_v$ currents in the RAP cells in concentration-dependent manner. Furthermore, $V_{1/2}$ of steady-state activated curve was shifted to more negative potential. It showed that Iva may significantly decrease $I_v$ of rapidly atrial pacing pulmonary veins sleeves cells with atrial fibrillation. The pervious reports showed that ivabradine is an open-channel blocker. The inhibition of the channel also appears to be current-dependent. The direct electrophysiological consequence of Iva inhibition is a reduction in the slope of the diastolic depolarization, leading to an increase in the time interval between successive action potentials and therefore, a decrease in heart rate.\(^\text{11,12}\) Our present results suggested that Iva might be a therapeutic agent for AF by blocking pacemaking current in PVs cells.

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**Reference**