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Regional variation of the inwardly rectifying potassium current in the canine heart and the contributions to differences in action potential repolarization

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ABSTRACT

The inward rectifier potassium current, I_{K1}, contributes to the terminal phase of repolarization of the action potential (AP), as well as the value and stability of the resting membrane potential. Regional variation in I_{K1} has been noted in the canine heart, but the biophysical properties have not been directly compared. We examined the properties and functional contribution of I_{K1} in isolated myocytes from ventricular, atrial and Purkinje tissue. APs were recorded from canine left ventricular midmyocardium, left atrial and Purkinje tissue. The terminal rate of repolarization of the AP in ventricle, but not in Purkinje, depended on changes in external K^+ ($[K^+]_o$). Isolated ventricular myocytes had the greatest density of I_{K1} while atrial myocytes had the lowest. Furthermore, the outward component of I_{K1} in ventricular cells exhibited a prominent outward component and steep negative slope conductance, which was also enhanced in 10 mM $[K^+]_o$. In contrast, both Purkinje and atrial cells exhibited little outward I_{K1}, even in the presence of 10 mM $[K^+]_o$, and both cell types showed more persistent current at positive potentials. Expression of Kir2.1 in the ventricle was 76.9-fold higher than that of atria and 5.8-fold higher than that of Purkinje, whereas the expression of Kir2.2 and Kir2.3 subunits was more evenly distributed in Purkinje and atria. Finally, AP clamp data showed distinct contributions of I_{K1} for each cell type. I_{K1} and Kir2 subunit expression varies dramatically in regions of the canine heart and these regional differences in Kir2 expression likely underlie regional distinctions in I_{K1} characteristics, contributing to variations in repolarization in response to in $[K^+]_o$ changes.

1. Introduction

Repolarization of the cardiac action potential (AP) is controlled by several voltage-dependent K^+ currents. In the canine ventricle, four K^+ currents play important roles in controlling the cardiac action potential duration [1–5]: (i) a Ca^{2+}-independent transient outward K^+ current (I_{to1}) (in contrast to the Ca^{2+}-dependent current, I_{to2} [6]); (ii) a rapid and slow delayed rectifier K^+ current (I_{Kr} and I_{Ks}, respectively) and (iii) an inwardly rectifying K^+ current (I_{K1}). I_{K1} has been identified in the myocardium of most mammalian species (for review see [7,8]), but the density of I_{K1} among different species as well as among different cardiac tissue types is highly variable [9–11]. Previous studies have shown that the I_{K1} density is large in the ventricle, small in atria and largely absent in sinoatrial node [12–14].

The salient features of I_{K1}, such as its negative slope conductance and the crossover of the I–V relation upon increases in external K^+ have long been identified [15], and their physiological significance has been extensively studied [16,17] (see Ref. [18]). The strong rectification of I_{K1} is the result of voltage-dependent block by intracellular Mg^{2+} [19] and polyamines [20], primarily spermine and spermidine [21]. The channels that carry cardiac I_{K1} belong to the Kir2.x subfamily, namely Kir2.1 through Kir2.3 [8,22]. Each Kir2 channel is composed of four subunits which form monomeric channels, although native I_{K1} channels likely exist as heteromultimers [23–25]. When expressed in mammalian cells, all Kir2.x channels exhibit properties that are similar to native I_{K1} [10], although each Kir2.x channel has a different current profile [10], reflecting the differences in polyamine induced rectification [26]. For example, Kir2.1 and Kir2.2 channels show a prominent negative slope conductance and rectify 'completely' above −20 mV, while

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Kir2.3 channels pass significant current at these potentials [10]. The distinct properties of different Kir2.x channels have been implicated in regional and species differences in I_{K1}, as observed in sheep heart where the incomplete rectification of I_{K1} in atria is attributed to the predominant expression of Kir2.3 [10]. Furthermore, individual reports [27–29] indicate the I_{K1} in canine heart appears to differ among ventricular, Purkinje and atrial myocytes. However, I_{K1} has never been directly compared between these three tissue types.

Since the canine heart is used as both a model for safety pharmacology and to stimulate cardiac electrophysiological diseases, such as atrial fibrillation [30], Long QT [31,32] and Brugada Syndromes [33], the present study compares the electrophysiological and molecular constituents of I_{K1} in single myocytes isolated from the canine heart. Here we show that the maximal rate of repolarization of the AP strongly depends on external K^+ ($[K^+]_o$) in ventricle, but not in Purkinje. We also show that the biophysical and molecular properties of I_{K1} differ between ventricular, Purkinje and atrial cells. While Kir2.1 is highly expressed in the ventricle, the other putative Kir2 subunits, Kir2.2 and Kir2.3, were more equally distributed in the three cardiac tissue types. Taken together, our data suggest that prominent differences in the expression Kir2 subunits and the characteristics of I_{K1} contribute to the differences of AP repolarization and its response to $[K^+]_o$ among atrial, ventricular and Purkinje tissues.

2. Materials and methods

2.1. Isolated tissue action potential recordings

This investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (Eighth Edition, 2011), and all protocols were approved by the Animal Care and Use Committee at the Masonic Medical Research Laboratory, and hearts from 39 total canines were used in the study. The mongrel canines of either sex were anti-coagulated with heparin and anesthetized with pentobarbital (30–35 mg/kg, i.v.). The chest was opened via left thoracotomy, the heart excised, placed in a cardioplegic solution (4 °C-Tyrode's solution with 12 mM $[K^+]_o$). Left ventricle midmyocardial (Mid-LV) (approximately $1.0 \times 0.5 \times 0.1$ cm), left atrial tissue and Purkinje tissue were isolated from hearts. The preparations of ventricle consisted of dermatome shavings (Davol Simon Dermatome Power Handle 3293 with cutting head 3295, Cranston, R.I.) obtained from the left ventricular free wall. The tissues were superfused with oxygenated (95% O_2/5% CO_2) Tyrode's solution maintained at 36–37 °C. The composition of the Tyrode's solution was (in mM): NaCl 129, KCl 5.4, NaH_2PO_4 0.9, $NaHCO_3$ 20, $CaCl_2$ 1.8, $MgSO_4$ 0.5, and D-glucose 5.5; pH = 7.4. All preparations were allowed to equilibrate until the action potentials achieved steady-state (usually 3–4 h). The tissues were stimulated at basic cycle lengths (BCL) ranging from 1000 and 2000 ms using rectangular stimuli (2-5 ms duration, 2–2.5 times diastolic threshold intensity) delivered through thin silver bipolar electrodes. Transmembrane action potentials were recorded from the two tissues simultaneously using glass microelectrodes filled with 2.7 M KCl (10–30 MΩ: DC resistance) connected to a high input-impedance amplification system (Electro 705 Electrometer, World Precision Instruments). The signals were digitized (model 1401 AD/DA system, Cambridge Electronic Designs [C.E.D.]) and analyzed (Spike 2 acquisition and analysis module, C.E.D.).

2.2. Isolation of canine cardiomyocytes

Left ventricular or left atrial preparations were dissected and cells were dissociated as described previously [3]. For isolation of mid-left ventricular cells (Mid-LV), thin slices of tissue from the Epi and Endo were shaved from the wedge using a dermatome to expose the midmyocardium, and the midmyocardium was subsequently shaved and removed for further digestion. For atrial cells, the pectinate and appendage muscle were isolated. The tissue was then agitated in the enzyme solution at 37 °C for cell dissociation. Canine Purkinje cells were isolated using techniques previously described [34]. Briefly, Purkinje fibers from both ventricles were dissected out and placed in a small dish. Fibers were then subjected to enzyme digestion with the nominally Ca^{2+}-free solution supplemented with 1.0 mg/ml collagenase (Type II, Worthington) and 30 mM 2,3-butanedione monoxime (Sigma-Aldrich) at 36 °C. Dissociation of cells from the fibers was aided by agitation of the enzyme solution with a small stir bar. Periodically, enzyme solution containing Purkinje cells in suspension was removed and added to a modified storage solution. Fresh enzyme solution was added to the undigested Purkinje fibers to maintain a volume of about 2 ml. Digestion of the Purkinje fibers into individual myocytes typically required 15–35 min. The cells were kept in 0.1 mM Ca^{2+} solution at room temperature until use.

2.3. Single cell electrophysiology

Voltage-clamp recordings were made using a MultiClamp 700A amplifier and MultiClamp Commander (Molecular Devices, Foster City, CA). Patch pipettes were fabricated from borosilicate glass capillaries (1.5 mm O.D., Fisher Scientific, Pittsburgh, PA). The pipettes were pulled using a gravity puller (Narishige, Tokyo, Japan) and the pipette resistance ranged from 1 to 3 MΩ. The cardiomyocytes were superfused with a HEPES buffer of the following composition (mM): NaCl 135, KCl 5.4, $MgCl_2$ 1.0, $CaCl_2$ 1.8, HEPES 10, NaH_2PO_4 0.33 and D-glucose 10, pH adjusted to 7.4 with NaOH. The patch pipette solution (K_{INT}) had the following composition (mM): K-aspartate 125, KCl 10, $MgCl_2$ 1.0, EGTA 5, MgATP 5, HEPES 10, NaCl 10, pH = 7.2 with KOH. All experiments were performed at 37 °C. After a whole-cell patch was established cell capacitance was measured by applying −5 mV voltage steps. Series resistance was compensated to 60–70%. All analog signals were acquired at 10–50 kHz, filtered at 4–6 kHz and digitized with a Digidata 1322 converter (Molecular Devices). I_{K1} currents were elicited by step increments of 10 mV between −120 and 0 mV from a holding potential of −80 mV and currents were evaluated at the end of the 500-ms step pulse, in the absence and presence of 100 μM $BaCl_2$, where I_{K1} was considered to be the Ba^{2+}-sensitive current. For analysis of kinetics of I_{K1} activation, hyperpolarizing steps down to −110 mV were applied to the cells in 10 mV decrements from a holding potential of −30 mV. The P/5 method was used to subtract the capacitance transient from fast activating I_{K1} currents, or K^+-free HEPES buffer was rapidly eliminate I_{K1} in order to isolate it from the capacitance currents, as similarly performed in Panama et al. [11]. For action potential clamp experiments, pre-recorded action potentials from ventricular, Purkinje and atrial myocytes served as voltage waveforms. Action potentials of single cells were recorded using whole cell patch pipettes. The resistance of the electrodes was 1–3 MΩ when filled with the K_{INT} pipette solution. Action potentials were elicited using a 3 ms current pulse at 120% threshold amplitude.

2.4. Electronic expression of I_{K1}

The electronic expression of I_{K1} was performed in the whole cell configuration, similarly as previously described [35] using a Cybercyte system (Cytocybernetics Inc., Pendleton, NY). I_{K1} I–V relations for Mid-LV and Purkinje were fit with the following function:

$$I_{K1} = G \times \left(g_{K1} \times \frac{V_m - V_{rev}}{1 + \exp\left(k^{-1} \times \left(V_m - V_{\frac{1}{2}}\right)\right)} + g_{linear} \times (V_m - V_{rev}) \right).$$

Cells were bathed in Tyrode solution and experiments were conducted at room temperature for increased patch stability. Model parameters were fit by adjusting g, g_{K1}, g_{linear} V_{rev}, $V_{1/2}$, and k to experimental data manually using a visual goodness of fit procedure, comparisons of