Journal of Molecular and Cellular Cardiology 64 (2013) 79–89

Contents lists available at ScienceDirect

Journal of Molecular and Cellular Cardiology

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

Original article

Developmental changes in expression and biophysics of ion channels in the canine ventricle

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ARTICLE INFO

Article history:
Received 2 May 2013
Received in revised form 12 August 2013
Accepted 2 September 2013
Available online 10 September 2013

Keywords:
K^+ current
Ventricular arrhythmias
Sudden death
Developmental electrophysiology

ABSTRACT

Background: Developmental changes in the electrical characteristics of the ventricular myocardium are not well defined. This study examines the contribution of inwardly rectifying K^+ current (I_{K1}), transient outward K^+ current (I_{to}), delayed rectifier K^+ currents (I_{Kr} and I_{Ks}) and sodium channel current (I_{Na}) to repolarization in the canine neonate myocardium. *Methods*: Single myocytes isolated from the left ventricle of 2–3 week old canine neonate hearts were studied using patch-clamp techniques. *Results*: Neonate cells were ~6-fold smaller than those of adults (28.8 ± 8.8 vs. 176 ± 6.7 pF). I_{K1} was larger in neonate myocytes and displayed a substantial inward component and an outward component with negative slope conductance, peaking at −60 mV (4.13 pA/pF). I_{Kr} tail currents (at −40 mV), were small (<20 pA). I_{Ks} could not be detected, even after exposure to isoproterenol (100 nM). I_{to} was also absent in the neonate, consistent with the absence of a phase 1 in the action potential. Peak I_{Na}, late I_{Na} and I_{Ca} were smaller in the neonate compared with adults. *KCND3*, *KCNIP2* and *KCNQ1* mRNA expression was half, while *KCNH2* was equal and *KCNJ2* was greater in the neonate when compared with adults. *Conclusions*: Two major repolarizing K^+ currents (I_{Ks} and I_{to}) present in adult ventricular cells are absent in the 2 week old neonate. Peak and late I_{Na} are significantly smaller in the neonate. Our results suggest that the absence of these two currents in the neonate heart may increase the susceptibility to arrhythmias under certain long QT conditions.

1. Introduction

It is well established that prolongation of the QT interval can increase the risk for developing cardiac arrhythmias. In the neonate heart, prolongation of the QT interval may be due to defects in cardiac ion channels which may be responsible for some reported cases of sudden infant death syndrome (SIDS) [1]. Approximately 95% of all SIDS cases occur before the age of 6 months. Recent studies suggest that approximately 1 out of 5 SIDS case may be due ion channel variations [2]. These ion channel defects would be expected to reduce the repolarization reserve of the ventricular myocardium, leading to the development of Torsade de Pointes (TdP) arrhythmias. The extent to which the neonatal canine and human heart is compromised and made vulnerable by mutations and drugs that reduce sodium and/or potassium channel currents is not well understood, because the ionic basis for repolarization in neonate cells are not well defined.

In the adult mammalian heart, marked differences in the shape and duration of the cardiac action potential have been well described in different regions of ventricle [3–5]. These differences are due to differences in a number of time- and voltage-dependent K^+ currents which control repolarization. In adult canine heart at least four K^+ currents play important roles in regulating the cardiac action potential duration: an inwardly rectifying K^+ current, I_{K1}; a Ca^{2+}-independent transient outward K^+ current, I_{to}; and rapid and slow components of the delayed rectifier K^+ currents, I_{Kr} and I_{Ks} [6,7].

In contrast, the neonatal heart exhibits prominent differences in action potential waveform compared to the adult heart. For example, in neonatal ventricular tissue isolated from dog [8,9] and rat [10] the very rapid phase 1 (normally present in adult tissue) is small or absent, suggesting that I_{to}, is absent. In addition, other experimental studies demonstrated that class III anti-arrhythmic agents produce a greater prolongation on action potential duration in the neonatal heart [11], suggesting that the rapid and slow delayed rectifier currents are reduced in the young heart. These results suggest that the underlying ionic currents are different at different stages of development. However, the complement of K^+ currents is not well understood at the early stages of development in the canine heart.

The present study was designed to examine the contribution of sodium channel current (I_{Na}), I_{Ca}, I_{K1}, I_{to}, I_{Kr} and I_{Ks} in the 2 week old canine

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http://dx.doi.org/10.1016/j.yjmcc.2013.09.001

neonate ventricular myocardium. Our data indicate that two of the major repolarizing K^+ currents (I_{Ks} and I_{to}) present in adult ventricular cells are absent in 2 week old neonate ventricular cells. In addition, both peak and late I_{Na} are also reduced in the neonate. Since I_{Ks} is absent in the neonate heart, repolarization is mainly dependent on I_{Kr}. Preliminary results have been presented in abstract form [12,13].

2. Methods

2.1. Isolation of neonate cells

Myocytes were prepared from 2 week old canine hearts using techniques previously described with minor modifications [14]. Briefly, male and female mongrel dogs were anesthetized with sodium pentobarbital (35 mg/kg i.v.), their hearts were rapidly removed and placed in nominally Ca^{2+}-free Tyrode's solution. The heart was then cannulated through the aorta and perfused with nominally Ca^{2+}-free Tyrode's solution containing 0.1% BSA for about 5 min. The heart was then subjected to enzymatic digestion with the nominally Ca^{2+}-free solution supplemented with 0.5 mg/ml collagenase (Type II, Worthington) and 1 mg/ml BSA for 7–9 min. After perfusion, the left ventricle was removed, minced and incubated in fresh buffer containing 0.5 mg/ml collagenase, 1 mg/ml BSA and agitated. The supernatant was filtered, centrifuged and the pellet containing the myocytes was stored in KB solution at room temperature. All animal procedures were in accordance with previously established guidelines (NRC publication, Guide for care and use of laboratory animals. Eighth Edition. 2011).

2.2. Solutions

All solutions were made with Milli-Q grade water. Nominally Ca^{2+}-free buffer had the following composition (mM): NaCl 129, KCl 5.4, $MgSO_4$ 2.0, NaH_2PO_4 0.9, glucose 5.5, and $NaHCO_3$ 20. This solution was bubbled with 95% O_2/5% CO_2. The modified KB storage solution [15] had the following composition (mM): potassium glutamate 100, potassium aspartate 10, KCl 25, KH_2PO_4 10, $MgSO_4$ 2, taurine 20, creatine 5, EGTA 0.5, glucose 20, HEPES 5, and BSA 0.2%, pH adjusted to 7.2 with KOH. Myocytes were superfused with Tyrode's solution of the following composition (mM): NaCl 140, KCl 4, $MgCl_2$ 1.0, $CaCl_2$ 2.0, HEPES 10, and glucose 10. pH was adjusted to 7.4 with NaOH.

2.3. Action potential and voltage clamp recordings of K^+ currents

Voltage and current clamp recordings were made using both whole cell and amphotericin perforated patch techniques [7,16]. Patch pipettes were fabricated from glass capillaries (1.5 mm o.d., Fisher Scientific, Pittsburg, PA). Patch pipettes were pulled from glass capillaries (1.5 mm o.d. and 1.1 mm i.d.) on a Model PP-830 vertical puller (Narashige Instruments, Japan) and filled with pipette solution of the following composition (mM): potassium aspartate 120, KCl 10, HEPES 10.0, $MgCl_2$ 1.0, EGTA 5.0, NaCl 10. pH was adjusted to 7.2 with KOH. In some experiments, amphotericin B (240 μg/ml) was included in the pipette solution. The pipette resistance ranged from 2 to 4 MΩ when filled with the internal solution. Action potentials were elicited using a 3 ms current pulse at 120% threshold amplitude and cells were paced at a cycle length of 1 Hz. Current and voltage signals were recorded using a MultiClamp 700A amplifier (Molecular Devices Inc., Foster City, CA) and series resistance errors were electronically compensated by 70–80%. All signals were acquired at 5–50 kHz (Digidata 1322, Molecular Devices) and analyzed with pClamp 9 software (Molecular Devices). All K^+ current experiments were performed at 36 °C.

2.4. Voltage clamp recordings of peak I_{Na}

Early sodium current, I_{Na}, was measured as previously described with minor modifications [17,18]. Patch pipettes were pulled from glass capillaries and the electrode resistance was 0.9–2.0 MΩ when filled with the internal solution (see below). The membrane was ruptured by applying negative pressure and series resistance compensated by 70–80%. Whole cell current data was acquired at 20–50 kHz and filtered at 5 kHz. Currents were normalized to cell capacitance.

External solution contained (in mM): Choline Cl 120, NaCl 10, Na^+ acetate 2.8, $CaCl_2$ 0.5, KCl 4, $MgCl_2$ 1.5, $CoCl_2$ 1, glucose 10, HEPES 10, NaOH 5, and $BaCl_2$ 0.1, pH adjusted to 7.4 with NaOH/HCl. The pipette solution contained (mM): NaCl 15, CsF 120, $MgCl_2$ 1, KCl 5, HEPES 10, Na_2ATP 4, and EGTA 10, pH adjusted to 7.2 with CsOH. Peak sodium current was dramatically reduced in the low extracellular sodium to ensure adequate voltage control, as gauged by the slope of a Boltzmann fit to the steady state activation curve [19] and peak I_{Na} was recorded at room temperature. When measuring sodium channel kinetics and density the holding potential was −120 mV to recruit all available sodium channels. In addition, recordings of I_{Na} were made at least 5 min after rupture to minimize the effects of time-dependent negative shift of steady-state inactivation that occurs in conventional voltage clamp experiments. Whole cell currents were analyzed using the *Clampfit* analysis program from pClamp 9 (Axon Instruments).

2.5. Voltage clamp recordings of late I_{Na}

Late I_{Na} density was a measured in full external Na^+ at 37 °C as previously described [20,21]. The external solution contained (in mM): NaCl 140, $CaCl_2$ 2.0, $MgCl_2$ 1, glucose 10, and HEPES 10, pH adjusted to 7.4 with NaOH. Pipette solution contained (mM): NaCl 10, aspartate 130, $MgCl_2$ 1, CsCl 10, HEPES 10, MgATP 5, and EGTA 10, pH adjusted to 7.2 with CsOH.

Late I_{Na} density was recorded in cells that were held at −80 mV. To remove steady-state inactivation and recruit all Na^+ channels, a pulse to −120 mV was applied before a 500-ms pulse to −40 mV. The protocol was repeated following rapid application of 10 μM TTX. Late I_{Na}, characterized as the TTX-sensitive difference current, was measured as total charge movement during application of the 500 ms step to −40 mV.

2.6. Voltage clamp recordings of I_{Ca}

I_{Ca} was recorded as previously described with minor modifications [22]. The external solution contained (in mM): NaCl 140, $CaCl_2$ 2.0, $MgCl_2$ 1, glucose 10, and HEPES 10, pH adjusted to 7.4 with NaOH. Pipette solution contained (mM): CsCl 120, $MgCl_2$ 1.0, EGTA 10, MgATP 5, HEPES 10, and $CaCl_2$ 5, pH = 7.2 with CsOH. Ca^{2+} currents were recorded during a 300 ms step depolarization from a holding potential of −40 mV [23].

2.7. Isolated ventricular preparations

Left ventricular tissues from epicardial, midmyocardial and endocardial regions (approximately 1.0 × 0.5 × 0.1 cm) were isolated from hearts removed from anesthetized (sodium pentobarbital, 35 mg/kg) 2 week old mongrel dogs. The preparations 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 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 studied in the same bath and allowed to equilibrate until the action potentials achieved steady-state (usually 4–5 h). The tissues were stimulated at basic cycle lengths (BCL) ranging from 300 to 2000 ms using rectangular stimuli (2–5 ms