Neural control of the heart: developmental changes in ionic conductances in mammalian intrinsic cardiac neurons

D.J. Adams, A.A. Harper, R.C. Hogg

Department of Physiology and Pharmacology, School of Biomedical Sciences, University of Queensland, Brisbane, Queensland 4072, Australia

Abstract

The expression and properties of ionic channels were investigated in dissociated neurons from neonatal and adult rat intracardiac ganglia. Changes in the hyperpolarization-activated and ATP-sensitive K+ conductances during postnatal development and their role in neuronal excitability were examined. The hyperpolarization-activated nonselective cation current, Ih, was observed in all neurons studied and displayed slow time-dependent rectification. An inwardly rectifying K+ current, IK(IR), was present in a population of neurons from adult but not neonatal rats and was sensitive to block by extracellular Ba2+. Using the perforated-patch recording configuration, an ATP-sensitive K+ (KATP) conductance was identified in z50% of intracardiac neurons from adult rats. Levomakalim evoked membrane hyperpolarization, which was inhibited by the sulphonylurea drugs, glibenclamide and tolbutamide. Exposure to hypoxic conditions also activated a membrane current similar to that induced by levomakalim and was inhibited by glibenclamide. Changes in the complement of ion channels during postnatal development may underlie observed differences in the function of intracardiac ganglion neurons during maturation. Furthermore, activation of hyperpolarization-activated and KATP channels in mammalian intracardiac neurons may play a role in neural regulation of the mature heart and cardiac function during ischaemia–reperfusion.

Keywords: Development; Ih; IK(IR); KATP; Intracardiac neurons

Introduction

Autonomic control of heart rate changes during early postnatal development in the rat whereby the intrinsic heart rate and maximal parasympathetic control of heart rate in response to direct vagal nerve stimulation decreases with postnatal age (Quigley et al., 1996). In autonomic ganglion neurons from immature animals, the electrical properties have been reported to be different from those of mature, adult animals (e.g. Hirst and Anderson). The electrical properties of a neuron can change during development by up- or down-regulation of ion channel expression or as a result of modulation of the existing complement of ion channel subunits. In autonomic neurons, membrane hyperpolarization can activate two inwardly rectifying conductances: a nonselective cation current (Ih), with Na+ and K+ as the charge carriers, and a K+-selective current (IK(IR)).

ATP-sensitive K+ (KATP) channels have been reported in the canine intracardiac ganglia and have been proposed to be functionally important during oxidative challenge. Recordings from canine intracardiac ganglia in situ, using extracellular microelectrodes, demonstrate that administration of cromakalim in the local blood supply causes a decrease in neuronal activity (Thompson et al., 1998). Furthermore, transient coronary occlusion has been shown to alter the firing activity of intrinsic cardiac neurons (Huang et al., 1993). KATP channel activation may contribute to changes in neuronal excitability in response to ischaemia (hypoxia and/or metabolic inhibition) by inducing membrane hyperpolarization. The present study demonstrates that the expression of Ih, IK(IR) and IATP in rat intracardiac neurons are related to the stage of postnatal development.
Methods

The isolation of parasympathetic neurons from neonatal and adult rat intracardiac ganglia has been described in detail previously (Hogg et al., 2001) and were in accordance with the guidelines of the University of Queensland Animal Experimentation Ethics Committee. Current and voltage clamp recordings were made at 22 °C using the amphotericin perforated-patch whole-cell configuration. Membrane current and voltage were recorded using an Axopatch 200A patch clamp amplifier (Axon Instruments, Union City, CA), filtered at 1 kHz and digitized at 5 kHz (Digitdata 1200A interface, Axon Instruments). Data are presented as mean±S.E.M. The control external solution was physiological saline solution containing (in mM): 140 NaCl, 3 KCl, 2.5 CaCl₂, 1.2 MgCl₂, 7.7 glucose and 10 HEPES–NaOH, pH 7.2. The pipette solution for perforated-patch whole-cell recordings contained (in mM): 75 K₂SO₄, 55 KCl, 5 MgSO₄ and 10 HEPES–N-methyl-D-glucamine, pH 7.2. Extracellular K⁺ concentration was changed by equimolar substitution of KCl for NaCl. Symmetrical 140 mM K⁺ solutions contained 5 mM TEACl extracellularly. Hypoxic solutions were bubbled with 100% nitrogen for at least 2 h prior to the experiment. O₂ partial pressure (P O₂) was measured via a probe in the recording chamber attached to an oxygen meter (Strathkelvin Instruments, Glasgow, UK). Pharmacological agents were bath applied at the concentrations indicated. All chemicals used were of analytical grade. The following drugs were used: amphotericin B, glibenclamide, tolbutamide, tetraethylammonium chloride (Sigma), N-ethyl-1,6-dihydro-1,2-dimethyl-6-(methylamino)-N-phenyl-4-pyrimidinamine hydrochloride (ZD 7288: Tocris Cookson, Bristol, UK) and levcromakalim (BRL 38227) (SmithKline Beecham Pharmaceuticals, UK).

Results

Hyperpolarization-activated currents, Iₜₜ and Iₖ(IR)

The relationship between the stage of postnatal development and the properties of hyperpolarization-activated currents were investigated in rat intracardiac ganglion neurons from neonatal (2–5 days) and adult (5–6 weeks) rats. An inwardly rectifying nonselective cation current, Iₜₜ, has been described in neonatal (Cuevas et al., 1997) and adult (Xi-Moy and Dun, 1995) rat intracardiac neurons. Iₜₜ was observed in all intracardiac neurons studied, displayed slow time-dependent rectification and was isolated by blockade with 2 mM Cs+ externally. Current density of Iₜₜ was significantly greater in neurons from neonatal as compared to adult rats; however, the reversal potential and activation parameters were unchanged (see Table 1). Iₜₜ was sensitive to changes in external Na⁺ and K⁺ concentrations and was irreversibly blocked by 10–100 μM ZD 7288, a selective inhibitor of Iₜₜ (Bosmith et al., 1993). Fig. 1A and B shows hyperpolarization-activated currents and the corresponding current–voltage (I–V) relationships recorded in the absence and presence of ZD 7288 (100 μM) and the ZD 7288-sensitive current (Iₜₜ) obtained by subtraction.

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<th>Developmental changes in the passive electrical properties and characteristics of Iₜₜ, Iₖ(IR) and Iₖ(ATP) in intracardiac neurons from neonatal and adult rats</th>
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<td><strong>Passive electrical properties</strong></td>
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Values are mean ± S.E.M, number of neurons in parenthesis, nd, not detected. E m values calculated for −50 pA hyperpolarizing current steps. Current densities of Iₜₜ and Iₖ(IR) were determined at −130 mV and Iₖ(ATP) at −50 mV in physiological (3 mM) external K⁺ solutions. Temperature: 22 °C (Cuevas et al., 1997; Hogg et al., 2001; Hogg and Adams, 2001).
An additional inwardly rectifying K+ current, $I_{K(IR)}$, was present in neurons from adult but not neonatal rats. $I_{K(IR)}$ was present in approximately half the adult rat intracardiac neurons studied, with a current density of $-0.61\pm0.26$ pA/pF ($-130$ mV, $n=6$) and was blocked by externally applied Ba$^{2+}$ (10 μM). $I_{K(IR)}$ displayed rapid activation kinetics but did not exhibit time-dependent rectification and was sensitive to changes in external K+ but not Na+, whereby raising external K+ from 3 to 15 mM shifted the reversal potential by approximately $+36$ mV.

**ATP-sensitive K+ current**

Under current clamp conditions in physiological salt solutions, activation of $K_{ATP}$ channels by levcromakalim (3 μM) caused a 15.5±2.9 mV ($n=4$) hyperpolarization from the resting membrane potential (Fig. 1C). Bath application of either glibenclamide or tolbutamide depolarized adult intracardiac neurons by 3–5 mV, suggesting that a $K_{ATP}$ conductance contributes to the resting membrane potential. Under voltage clamp conditions in physiological solutions, bath application of levcromakalim (3 μM) shifted the $I-V$ relationship to more negative membrane potentials and was reversed in the presence of 10 μM glibenclamide (Fig. 1D). Under voltage clamp conditions in symmetrical (140 mM) K+ solutions, external application of the selective $K_{ATP}$ channel opener, levcromakalim (3 μM), activated an inward current at negative membrane potentials, with an EC$_{50}$ of 1.6 μM. The levcromakalim-induced response had a current density of $-8.1\pm2.6$ pA/pF at $-50$ mV, reversed close to 0 mV and was completely inhibited by 10 μM glibenclamide.

The $K_{ATP}$ conductance was also activated in hypoxic conditions ($P_{O2}$, 16 mm Hg), causing membrane hyperpolarization that was inhibited by 10 μM glibenclamide (not shown). In contrast, in intracardiac neurons isolated from neonatal rats, neither hypoxia nor levcromakalim (10 μM) activated a conductance change ($n=7$) and glibenclamide (10 μM) did not affect the resting membrane potential.

![Figure 1](https://example.com/figure1.png)

**Fig. 1.** Isolation of $I_{K}$ and levcromakalim-activated currents in adult rat intracardiac ganglion neurons. Currents were evoked by 2-s hyperpolarizing steps to test potentials between $-60$ and $-130$ mV from a holding potential of $-50$ mV. (A) $I_{K}$, recorded from an adult neuron in control conditions and in the presence of 100 μM ZD 7288. The ZD 7288-sensitive current was obtained by subtraction. (B) $I-V$ relation of the steady-state current recorded in the absence (■) and presence of 100 μM ZD 7288 (▲) and the ZD 7288-sensitive component (▲). (C) Under current clamp conditions, 3 μM levcromakalim evoked a 16-mV hyperpolarization, which was inhibited by 10 μM glibenclamide. (D) Under voltage clamp conditions in normal PSS, 3 μM levcromakalim shifted the reversal potential of the membrane current obtained in response to voltage ramps (from $-120$ to $+10$ mV) from $-53$ to $-87$ mV. Glibenclamide (10 μM) reversed the effect of levcromakalim (adapted from H siege et al., 2001; H siege and Adams, 2001).
Discussion

These results indicate that \( I_{\text{K(IR)}} \) density increases as a function of development in rat intracardiac neurons, which together with a relative decrease in \( I_h \) may contribute to changes in the control of neuronal excitability in adult versus neonatal intracardiac neurons. The appearance of \( I_{\text{K(IR)}} \) during postnatal development coincided with an approximate 50% decrease in \( I_h \) current density. \( I_h \) was irreversibly blocked by the bradycardic agent ZD 7288 (100 \( \mu \)M), leaving an inward hyperpolarization-activated \( K^+ \) current sensitive to blockade by 10 \( \mu \)M Ba\( ^{2+} \). The ion channel inhibitors, Cs\(^+ \) and Ba\(^{2+} \), have been shown to differentially affect the vagally induced pacemaker response in anesthetized dogs, whereby BaCl\(_2\) attenuated the vagally induced bradycardia without affecting other components of the response. In contrast, CsCl had no effect on the initial vagal slowing of atrial rate but abolished the acceleratory portion of the response (Wallick et al., 1997). Local arterial infusion of BaCl\(_2\) to the right atrial ganglionated plexus has also been shown to directly modulate the electrical activity of canine intracardiac neurons in situ (Thompson et al., 2000). The inhibition of \( I_h \) and \( I_{\text{K(IR)}} \) by Cs\(^+ \) and Ba\(^{2+} \), respectively, may contribute to the observed changes in neuronal excitability of mammalian intrinsic cardiac ganglia. Furthermore, the changes in the functional expression of \( I_h \) and \( I_{\text{K(IR)}} \) with postnatal development suggest that different ionic mechanisms may contribute to modulating neuronal excitability in neonatal and adult intracardiac ganglion neurons.

The present study demonstrates the presence of an ATP-sensitive \( K^+ \) conductance in adult but not neonatal rat intracardiac neurons. \( K_{\text{ATP}} \) channels have been suggested to be present in the canine intracardiac ganglia and to be functionally important during oxidative challenge. The location of the mammalian intracardiac ganglia in the atrial epicardium make them susceptible to the effects of myocardial ischemia and reperfusion associated with coronary heart disease (Armour, 1999). Recently, sensory neurons in the guinea pig enteric nervous system have been shown to sense changes in extracellular glucose levels via \( K_{\text{ATP}} \) channel activity and are sensitive to tolbutamide (Liu et al., 1999). The physiological and pathophysiological modulation of \( K_{\text{ATP}} \) channels in intrinsic cardiac ganglia may be important in neuroeffector transmission in the heart and regulation of heart rate. The presence of \( K_{\text{ATP}} \) channels in adult intracardiac neurons only may contribute to the differential effects of hypoxia on heart rate in neonates versus adults (see Gootman and Gootman, 2000).

References


