Journal of Molecular and Cellular Cardiology 48 (2010) 152–160

Contents lists available at ScienceDirect

Journal of Molecular and Cellular Cardiology

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

Original article

Differential K_{ATP} channel pharmacology in intact mouse heart

Alexey V. Glukhov [a], Thomas P. Flagg [b,1], Vadim V. Fedorov [a], Igor R. Efimov [a], Colin G. Nichols [b,*]

[a] Department of Biomedical Engineering, Washington University, St. Louis, MO 63130, USA
[b] Department of Cell Biology and Physiology, Box 8228, Washington University School of Medicine, 660 South Euclid Avenue, St. Louis, MO 63110, USA

ARTICLE INFO

Article history:
Received 1 July 2009
Received in revised form 13 August 2009
Accepted 29 August 2009
Available online 8 September 2009

Keywords:
SUR1
ATP
Channel
Potassium channel opener
APD

ABSTRACT

Classically, cardiac sarcolemmal K_{ATP} channels have been thought to be composed of Kir6.2 (KCNJ11) and SUR2A (ABCC9) subunits. However, the evidence is strong that SUR1 (sulfonylurea receptor type 1, ABCC8) subunits are also expressed in the heart and that they play a significant functional role in the atria. To examine this further, we have assessed the effects of isotype-specific potassium channel-opening drugs, diazoxide (specific to SUR1>SUR2A) and pinacidil (SUR2A>SUR1), in intact hearts from wild-type mice (WT, $n=6$), $SUR1^{-/-}$ ($n=6$), and $Kir6.2^{-/-}$ mice ($n=5$). Action potential durations (APDs) in both atria and ventricles were estimated by optical mapping of the posterior surface of Langendorff-perfused hearts. To confirm the atrial effect of both openers, isolated atrial preparations were mapped in both WT ($n=4$) and $SUR1^{-/-}$ ($n=3$) mice. The glass microelectrode technique was also used to validate optical action potentials. In WT hearts, diazoxide (300 μM) decreased APD in atria (from 33.8±1.9 ms to 24.2±1.1 ms, $p<0.001$) but was without effect in ventricles (APD 60.0±7.6 ms vs. 60.8±7.5 ms, respectively, NS), consistent with an atrial-specific role for SUR1. The absence of SUR1 resulted in loss of efficacy of diazoxide in $SUR1^{-/-}$ atria (APD 36.8±1.9 ms vs. 36.8±2.8 ms, respectively, NS). In contrast, pinacidil (300 μM) significantly decreased ventricular APD in both WT and $SUR1^{-/-}$ hearts (from 60.0±7.6 ms to 29.8±3.5 ms in WT, $p<0.001$, and from 63.5±2.1 ms to 24.8±3.8 ms in $SUR1^{-/-}$, $p<0.001$), but did not decrease atrial APD in either WT or $SUR1^{-/-}$ hearts. Glibenclamide (10 μM) reversed the effect of pinacidil in ventricles and restored APD to control values. The absence of Kir6.2 subunits in $Kir6.2^{-/-}$ hearts resulted in loss of efficacy of both openers (APD 47.2±2.2 ms vs. 47.6±2.1 ms and 50.8±2.4 ms, and 90.6±5.7 ms vs. 93.2±6.5 ms and 117.3±6.4 ms, for atria and ventricle in control versus diazoxide and pinacidil, respectively). Collectively, these results indicate that in the same mouse heart, significant differential K_{ATP} pharmacology in atria and ventricles, resulting from SUR1 predominance in forming the atrial channel, leads to differential effects of potassium channel openers on APD in the two chambers.

1. Introduction

Sarcolemmal ATP-sensitive potassium (K_{ATP}) channels are prominently expressed throughout the heart [1,2]. In pathophysiological conditions, clear roles for K_{ATP} channels have been identified, including their participation arrhythmogenesis [3,4], and protection from the contractile impairment following ischemia-reperfusion [5–8]. At the molecular level, K_{ATP} channels are understood to be multisubunit protein complexes, and classically, cardiac sarcolemmal K_{ATP} channels have been thought to be primarily composed of Kir6.2 (inward-rectifier potassium channel 6.2, KCNJ11) and SUR2A (sulfonylurea receptor type 2A, ABCC9) subunits [9–11], together with other regulatory proteins (creatine kinase, GAPDH, and others) [12–15]. However, the evidence is strong that SUR1 (sulfonylurea receptor type 1, ABCC8) subunits are also expressed in the heart and that they play a significant functional role, especially in the atria [16–18]. Recently, we demonstrated that the SUR1 subunit is strongly expressed in the mouse atrium and that atrial sarcolemmal K_{ATP} requires SUR1 for functional channel expression [18].

The SUR subunit determines the specificity and selectivity of K_{ATP} agonists and antagonists [19]. Assessment of the functional effects of K_{ATP} agonists has been routinely exploited to infer the physiological outcome of K_{ATP} activation. A critical assumption in these experiments is that diazoxide does not affect sarcolemmal K_{ATP} channels. Because channel structure is regionally distinct, sarcolemmal K_{ATP} channels in the intact mouse heart are likely to exhibit chamber-specific pharmacology. In isolated cells, it has been shown that atrial K_{ATP} is more sensitive to diazoxide (specific to SUR1>SUR2A) than pinacidil (SUR2A>SUR1)[19], whereas ventricular K_{ATP} has the opposite specificity to the potassium channel opening drugs [18]. How and whether this differential pharmacology in isolated cells is manifest in the intact heart is unclear, and to examine this, we have simultaneously

* Corresponding author. Tel.: +1 314 362 6630; fax: +1 314 362 7463.
E-mail address: cnichols@wustl.edu (C.G. Nichols).
[1] Present and permanent address: Department of Anatomy, Physiology and Genetics, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Rd., C-2114, Bethesda, MD 20814, USA.

0022-2828/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.yjmcc.2009.08.026

monitored the effects of diazoxide and pinacidil on action potential duration (APD) in atria and ventricles of intact hearts from wild-type (WT) mice and mice with deletions of SUR1 ($SUR1^{-/-}$) and Kir6.2 ($Kir6.2^{-/-}$). Diazoxide shortened the action potential in atria, but not in ventricles, of WT hearts, but was without effect in both atria and ventricles in $SUR1^{-/-}$ hearts. Conversely, pinacidil significantly decreased ventricular APD in both WT and $SUR1^{-/-}$, but was without effect on atrial APD in both lines of mice. Knockout of Kir6.2 resulted in loss of efficacy of both openers, in both atria and ventricles.

2. Materials and methods

2.1. Generation and care of genetically modified mice

All procedures complied with the standards for the care and use of animal subjects as stated in the *Guide of the Care and Use of Laboratory Animals* (NIH publication No. 85-23, revised 1996) and protocols were approved by the Animal Studies Committee at Washington University School of Medicine. Data were obtained from adult (aged 11–20 weeks) wild-type mice (WT), sulfonylurea receptor type 1 knockout mice ($SUR1^{-/-}$) and Kir6.2 knockout mice ($Kir6.2^{-/-}$). The generation of $SUR1^{-/-}$ and $Kir6.2^{-/-}$ mice has been described elsewhere [20,21]. Separate groups of mice were studied as (1) whole heart preparations (WT, $n=6$; $SUR1^{-/-}$, $n=6$, and $Kir6.2^{-/-}$, $n=5$) and (2) isolated atrial preparations (WT, $n=4$, and $SUR1^{-/-}$, $n=3$).

2.2. Isolated heart preparations

The isolated heart preparation was performed as described previously [22]. Briefly, a Langendorff perfusion protocol modified for murine heart was used. Mice were anesthetized using a mixture of ketamine and xalazine, with 100 U of heparin. After mid-sternal incision, the heart was removed and placed in oxygenated (95% O_2, 5% CO_2) constant-temperature (37 ± 1 °C), modified Tyrode solution of the following composition: 128.2 mM NaCl, 4.7 mM KCl, 1.19 NaH_2PO_4, 1.05 mM $MgCl_2$, 1.3 mM $CaCl_2$, 20.0 mM $NaHCO_3$, and 11.1 mM glucose ($pH=7.35\pm0.05$). While bathed in the same solution, lung, thymus, and fat tissue were dissected and removed. A short section of aorta was attached to a custom made 21-gauge cannula. After cannulation, the heart was superfused and retrogradely perfused with Tyrode solution passed through the 5-μm filter (Millipore, Billerica) and warmed (37 °C) using a water jacket and circulator (ThermoNESLAB EX7, Newtown). Perfusion was performed using a peristaltic pump (Peri-Star, WPI, Sarasota) under constant aortic pressure of 60–80 mm Hg measured by pressure-amplifier (TBM4M, WPI, Sarasota). Cannulation was followed by (1) preparation of the intact heart or (2) isolated atria for image analysis.

A photograph of the intact mouse heart preparation is shown on Fig. 1A. The isolated heart was pinned at the apex to the Sylgard bottom of the chamber to prevent stream-induced movement. The right and left atrial appendages were stretched and pinned to flatten them and allow optical measurements from maximal surface of the atria. A small silicon tube, fixed by silk to the nearby connective tissue, was inserted into the left ventricle through the pulmonary vein, left atria, and tricuspid valve to prevent solution congestion and subsequent ischemia after suppression of ventricular contractions. This also prevented acidification of the perfusion solution and development of ischemia in the left ventricle, which otherwise developed without this precaution.

For the isolated atrial preparation (Fig. 5A), the heart was cannulated as described above, ventricles were dissected away, and the atria were stretched and then pinned to the bottom of a Sylgard-coated chamber and superfused with Tyrode solution at a constant rate of ~8 ml/min. Both left and right atria (LA and RA, correspondingly) as well as the atrioventricular junction (AVJ) were accessible in this preparation. A rim of ventricular tissue was preserved, to pin the preparation and prevent damage to the atria. The medial limb of the crista terminalis was cut to open the RA appendage, and the pacing electrode was located on the edge of the RA appendage. The interatrial septal tissue was partially removed to reduce the scattering from out of focus tissue. These procedures did not result in any irregularities in spontaneous rhythm.

The excitation–contraction uncoupler blebbistatin (10 μM, Tocris Bioscience) was used to prevent the effects of motion artifacts on the APD estimation. We showed in our previous studies in different species (dog, rabbit, rat, and human) that blebbistatin does not change action potential morphology or conduction properties [23]. In addition, we used microelectrode recordings to validate the optical signal. In this case, the application of blebbistatin induced a transient (5–10 min) increase of cycle length of spontaneous rhythm (data not shown).

2.3. Imaging system

Coronary perfused hearts were stained by perfusion with voltage-sensitive dye (RH-237, 5 μL of 1 mg/ml dimethyl sulfoxide, DMSO, in Tyrode solution) for 5–7 min. Isolated atrial preparations were stained by direct application of the dye dissolved in the bathing solution. Staining by RH-237 did not induce a significant change of cycle length, as typically observed for di-4-ANNEPS.

Excitation light (530/540 nm) was generated by a 250-W xenon arc lamp with a constant-current, low-noise, power supply (Oriel Instruments, Stratford, CT). The light was passed through a heat filter, a shutter, and excitation filter (530/40 nm). A flexible light guide directed the band-pass-filtered light onto the preparation, and a shutter was used to ensure that the preparation was exposed to light only during image acquisition. The fluorescent light emitted from the preparation was long-pass (>650 nm) filtered using an edge pass filter (Thorlabs, NJ) before reaching the camera. Emitted light was directed towards a MiCAM Ultima-L CMOS camera (SciMedia, CA) with high spatial (100×100 pixels, 230 ± 20 μm per pixel) and temporal (1000–3000 frames/s) resolution. The acquired fluorescent signal was digitized, amplified, and visualized using custom software (SciMedia, CA).

2.4. Experimental protocols

After isolation and cannulation, motion suppression, and dye staining, preparations were equilibrated for 5–10 min before imaging. Then, control measurements during both spontaneous rhythm and ventricular pacing were made. To estimate the effects of stimulation of different types of sulfonylurea receptors, we tested two potassium channel-opening drugs: diazoxide (specificity for SUR1>SUR2A) and pinacidil (specificity for SUR2A>SUR1)[19]. Diazoxide or pinacidil (300 μM) was delivered through both perfusion and superfusion solutions, and was applied for 20–25 min to reach steady-state effect. After optical measurements, hearts were washed out for 30 min before delivery of a second test compound. After washout, additional staining as well as additional injection of blebbistatin were performed if needed based on the quality of the optical signal. Following application of diazoxide and/or pinacidil, a selective blocker of KATP channels, glibenclamide (10 μM), was added to the solution to reverse the effect of channel openers. This protocol was applied for both intact heart and isolated atrial preparations.

2.5. Data processing

A customized Matlab-based computer program [24] was used to analyze optical signals. First, the signals were filtered using a low-pass Butterworth filter at 200 Hz. All optical action potentials were then averaged and normalized to a range from −85 to 15 mV. Finally, action potential duration at 90% of repolarization (APD90) and maximum upstroke derivative (dV/dt_{max}) were calculated for each action potential using the normalized optical signal and its derivatives.