Journal of Molecular and Cellular Cardiology 108 (2017) 34–41

Contents lists available at ScienceDirect

Journal of Molecular and Cellular Cardiology

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

Original article

The mechano-sensitivity of cardiac ATP-sensitive potassium channels is mediated by intrinsic MgATPase activity

Mohammad Fatehi, Christian C. Carter, Nermeen Youssef, Peter E. Light *

Department of Pharmacology, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, Alberta T6G 2E1, Canada

ARTICLE INFO

Article history:
Received 5 April 2017
Received in revised form 3 May 2017
Accepted 4 May 2017
Available online 5 May 2017

Keywords:
ATP-sensitive potassium channels
K_{ATP} channels
Kir6.2
SUR2A
SUR1
Stretch
Mechanosensitivity
MgATPase activity

ABSTRACT

Cardiac ATP-sensitive K^+ (K_{ATP}) channel activity plays an important cardio-protective role in regulating excitability in response to metabolic stress. Evidence suggests that these channels are also mechano-sensitive and therefore may couple K_{ATP} channel activity to increased cardiac workloads. However, the molecular mechanism that couples membrane stretch to channel activity is not currently known. We hypothesized that membrane stretch may alter the intrinsic MgATPase activity of the cardiac K_{ATP} channel resulting in increased channel activation. The inside-out patch-clamp technique was used to record single-channel and macroscopic recombinant K_{ATP} channel activity in response to membrane stretch elicited by negative pipette pressure. We found that stretch activation requires the presence of the SUR subunit and that inhibition of MgATPase activity with either the non-hydrolysable ATP analog AMP-PNP or the ATPase inhibitor BeFx significantly reduced the stimulatory effect of stretch. We employed a point mutagenic approach to determine that a single residue (K1337) in the hairpin loop proximal to the major MgATPase catalytic site in the SUR2A subunit is responsible for the difference in mechano-sensitivity between SUR2A and SUR1 containing K_{ATP} channels. Moreover, using a double cysteine mutant substitution in the hairpin loop region revealed the importance of a key residue-residue interaction in this region that transduces membrane mechanical forces into K_{ATP} channel stimulation via increases in channel MgATPase activity. With respect to K_{ATP} channel pharmacology, glibenclamide, but not glicalizide or repaglinide, was able to completely inhibit K_{ATP} channel mechano-sensitivity. In summary, our results provide a highly plausible molecular mechanism by which mechanical membrane forces are rapidly converted in changes in K_{ATP} channel activity that have implications for our understanding of cardiac K_{ATP} channels in physiological or pathophysiological settings that involve increased workload.

© 2017 Elsevier Ltd. All rights reserved.

1. Introduction

Since the discovery of plasma-membrane ATP-sensitive potassium (K_{ATP}) channels in cardiac myocytes, activation of these channels has been shown to be cardio-protective during ischemic episodes and periods of increased workload such as exercise, via control of cardiac excitability, action potential duration and calcium homeostasis [1–8]. Furthermore, genetic ablation of K_{ATP} channels results in the development of heart failure [9,10]. K_{ATP} channels have also been implicated in the powerful cardio-protective phenomenon of ischemic preconditioning [4,11].

K_{ATP} channels function as molecular transducers by adjusting cellular electrical excitability in response to alterations in cellular metabolism. However, as the IC_{50} for ATP inhibition in isolated channels is in the low micromolar range, cardiac K_{ATP} channels display a marked insensitivity to normal intracellular ATP levels in the 1–5 millimolar range, therefore other pathways and mechanisms likely play a major role in the dynamic regulation of K_{ATP} channel activity under physiological conditions in the intact myocardium [3]. In this regard, several studies have shown that cardiac K_{ATP} channels are mechano-sensitive providing a potentially important mechanism by which alterations in cardiac workload may be rapidly coupled to K_{ATP} channel activity via membrane stretch [12,13]. Despite the important roles that K_{ATP} channels play in cardiac physiology and pathophysiology, the precise molecular mechanisms underlying their mechano-sensitivity have still not been fully determined. Recent studies have shown K_{ATP} channel mediated action potential shortening is linked to left ventricular pressure and the actin cytoskeleton plays a key role in transducing membrane stretch to K_{ATP} channel mechano-sensitivity [13,14]. However, the intrinsic molecular mechanism within the channel itself is not known. In this regard, our group and others have characterized another important property of K_{ATP} channels as enzymes capable of converting ATP to ADP via intrinsic MgATPase activity that may also be sensitive to mechanical forces within the localized membrane environment of the channel [15–17]. Furthermore, it has previously been shown that the MgATPase activity of

* Corresponding author at: Department of Pharmacology, Alberta Diabetes Institute, 1-005 Li Ka Shing Centre for Health Research Innovation, University of Alberta, Edmonton, Alberta T6G 2E1, Canada.
E-mail address: plight@ualberta.ca (P.E. Light).

http://dx.doi.org/10.1016/j.yjmcc.2017.05.004
0022-2828/© 2017 Elsevier Ltd. All rights reserved.

cardiac K_{ATP} channels is a key mechanism for regulating channel activity and may also play an important role in regulating other cellular functions that are associated with opening of K_{ATP} channels [18].

Cardiac K_{ATP} channels are hetero-octameric trans-membrane protein complexes comprised of four pore-forming inward rectifying potassium Kir6.2 channel subunits coupled to four regulatory sulfonylurea receptor (SUR) subunits that bestow the intrinsic catalytic MgATPase activity of the channel complex.

The major site for the inhibitory action of ATP is located within the pore-forming Kir6.2 subunits. In contrast, ADP can release the inhibition by ATP, leading to increases in channel activity via molecular interactions within the SUR subunits. The SUR subunit contains two nucleotide binding domains (NBDs) with each NBD containing a Walker A and B motif that bestow intrinsic MgATPase catalytic activity to the K_{ATP} channel [19]. It is this MgATPase function that is considered important for regulating the appropriate K_{ATP} channel activity via ADP-induced K_{ATP} channel activation [15,20]. As recent evidence suggests that ventricular tissue expresses the SUR2A isoform whereas atrial tissue expresses SUR1, the overall aim of this study was to determine whether the intrinsic MgATPase activity of recombinant cardiac K_{ATP} channels containing SUR1 or SUR2A underlies the effects of mechanical stretch on channel activity [21].

2. Methods

2.1. Cell culture, transfection and electrophysiology

HEK293T cells were cultured in DMEM/FBS and then transfected with the human Kir6.2, SUR1 and SUR2A clones using the calcium phosphate precipitation technique. Transfected cells were identified using fluorescent optics in combination with co-expression of an enhanced green fluorescent protein plasmid. K_{ATP} channel recordings were then performed 48–72 h after transfection. The excised inside-out patch clamp technique was used to measure single channel and macroscopic recombinant K_{ATP} channel currents in transfected cells as described in detail previously [16]. Experiments were performed at room temperature (21 °C). Linear stepwise changes in pipette pressure were applied to the inside of the recording pipette using a high-speed pressure clamp system (HSPC-1, ALA Scientific, Farmingdale NY). Beryllium fluoride (BeF_2) was dissolved in 50 mM KF (33% w/v) to produce a sufficient amount of the ATP γ-phosphate mimetic BeF_x (BeF^{3-} and BeF_2^{4-}) [22]. In bath solutions used for experiments in the presence of BeF_x, 50 mM KCl was replaced with 50 mM KF.

2.2. Experimental Compounds

BeF_2, MgATP, AMP-PNP, diazoxide and the Na^+ salt of GTP were obtained from Sigma-Aldrich (Oakville, Canada). MgATP, AMP-PNP and GTP were prepared as 10 mM stock solutions in ddH_2O immediately prior to use.

2.3. Site-directed mutagenesis

The full-length human SUR1 and SUR2A DNA constructs were a generous gift from Dr. J. Bryan (Pacific Northwest Diabetes Research Institute, Seattle, WA). All mutants used in this study were generated using site-directed mutagenesis (QuickChange, Stratagene) and subsequently confirmed by sequence analysis.

2.4. In silico homology modeling

The homology model of the SUR1 NBD1 and NBD2 dimer (17), based on the prokaryotic ATP binding cassette (ABC) protein crystal structure of MJ0796 (PDB accession #1F30,) was generously provided by Dr. C.G. Nichols (Washington University, St. Louis, MO) and was used to visualize and predict the location and interactions of key regions and residues in the NBD2 region of SUR2A and SUR1 using Pymol software [23].

2.5. Cysteine cross-linking

Disulfide bond formation was induced by exposing excised patches expressing WT, single and double cysteine mutant SUR1 subunits to 0.3% H_2O_2. The effect of H_2O_2 on WT and mutant channels was assayed by measuring the amplitudes of currents elicited by 1 mM GTP and negative pipette pressure in the presence of 0.1 mM MgATP before and after H_2O_2 application. The reversibility of disulfide bond formation was examined by exposing patches to the reducing agent dithiothreitol (DTT, 10 mM) and measuring the macroscopic current amplitudes induced by 1 mM GTP and pipette pressure in the presence of 0.1 mM MgATP.

2.6. Statistical analysis

Macroscopic K_{ATP} channel currents were normalized and expressed as changes in test current relative to control current. Macroscopic current analysis and single-channel unitary current amplitude and open probability (Po) analysis was performed using pClamp 10.0 (Axon Instruments, Foster City, CA) and Origin 6.0 software. Statistical significance was assessed using the unpaired Student's *t*-test or one-way ANOVA with a Bonferroni post hoc test. P values < 0.05 were considered statistically significant. Data are expressed as the mean ± S.E.M.

3. Results

3.1. Sensitivity to membrane stretch is bestowed upon the K_{ATP} channel complex by the SUR subunit

It has previously been shown that mechanical membrane stretch increases native K_{ATP} channel activity from cardiac myocytes [12,13]. In order to allow us to probe the molecular mechanisms and subunit contributions, we studied heterologously expressed human recombinant K_{ATP} channels composed of the major cardiac SUR2A isoform and Kir6.2. We confirmed that the mechano-sensitivity is also preserved in recombinant cardiac K_{ATP} channels (Fig. 1A) and that the maximum membrane stretch effect was elicited by −15 mm Hg pipette pressure before patch seals became unstable at more negative pressures (Fig. 1E). Furthermore, we also confirmed that the stimulatory effects of membrane stretch are mediated by increases in single channel open probability rather than any changes in unitary single channel current amplitude (Fig. 1A, B). The use of a recombinant expression system allowed us to investigate which subunit is responsible for transducing membrane stretch in changes in channel open probability by expression of the Kir6.2C-terminal truncation mutation, Kir6.2ΔC26, that allows for surface expression of the pore forming Kir6.2 subunit in the absence of the SUR subunit [24,25]. The effects of membrane stretch on the activity of a population of Kir6.2ΔC26 K_{ATP} channels in a macroscopic patch are completely lost in the absence of the SUR2A subunit (Fig. 1C, D, E).

3.2. GTP enhances the stretch-induced increase in K_{ATP} channel open probability

Our group and others have shown that K_{ATP} channels possess intrinsic enzymatic MgATPase activity bestowed upon the channel by the SUR subunit [15–17]. As the stimulatory effect of membrane stretch is lost in the absence of the SUR2A subunit, we then investigated whether K_{ATP} channel MgATPase activity, residing in the SUR subunit, may be enhanced by membrane stretch and is responsible for the observed mechano-sensitivity of the channel. The inside-out patch technique was used to measure macroscopic K_{ATP} channel currents in response to application of GTP that acts as a substrate for MgATPase activity generating GDP that stimulates K_{ATP} channel activity. Therefore, an

Download English Version:

https://daneshyari.com/en/article/5533534

Download Persian Version:

https://daneshyari.com/article/5533534

Daneshyari.com