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Exercise-induced expression of cardiac ATP-sensitive potassium channels promotes action potential shortening and energy conservation

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Abstract

Physical activity is one of the most important determinants of cardiac function. The ability of the heart to increase delivery of oxygen and metabolic fuels relies on an array of adaptive responses necessary to match bodily demand while avoiding exhaustion of cardiac resources. The ATP-sensitive potassium (K_{ATP}) channel has the unique ability to adjust cardiac membrane excitability in accordance with ATP and ADP levels, and up-regulation of its expression that occurs in response to exercise could represent a critical element of this adaption. However, the mechanism by which K_{ATP} channel expression changes result in a beneficial effect on cardiac excitability and function remains to be established. Here, we demonstrate that an exercise-induced rise in K_{ATP} channel expression enhanced the rate and magnitude of action potential shortening in response to heart rate acceleration. This adaptation in membrane excitability promoted significant reduction in cardiac energy consumption under escalating workloads. Genetic disruption of normal K_{ATP} channel pore function abolished the exercise-related changes in action potential duration adjustment and caused increased cardiac energy consumption. Thus, an expression-driven enhancement in the K_{ATP} channel-dependent membrane response to alterations in cardiac workload represents a previously unrecognized mechanism for adaptation to physical activity and a potential target for cardioprotection.

Keywords

K_{ATP} ; K-ATP; remodeling; oxygen consumption; heart rate; exercise

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1. Introduction

Physical activity is one of the most important determinants of cardiac function. The ability of the heart to increase delivery of oxygen and metabolic fuels relies on an array of adaptive responses necessary to match bodily demand while avoiding exhaustion of cardiac energetic and functional resources [1]. Yet, the molecular mechanisms of these adaptive changes are not fully understood. The ATP-sensitive potassium (K_{ATP}) channel, one of the most abundant cardiac membrane protein complexes, has the unique ability to adjust membrane excitability in response to changes in ATP and ADP levels [2-6]. The channel complex is formed through physical association of the pore-forming inwardly rectifying potassium channel, Kir6.x, with the regulatory sulfonylurea receptor, SUR [2,5,7-12]. K_{ATP} channels in ventricular myocytes are composed primarily of Kir6.2 and SUR2A subunits, while in atria the combination of Kir6.2 or 6.1 with SUR1 and SUR2A has been reported [2,5-10]. The metabolic sensing of K_{ATP} channels occurs through modulation of the ATP sensitivity of the pore-forming subunit by the regulatory sulfonylurea receptor, closely integrated with cellular energetic networks [6,13-24]. When activated by reduced [ATP] to [ADP] ratio that occurs with increased cellular metabolic demand, K_{ATP} channel-dependent potassium efflux shortens cardiac action potentials [3,5,25,26]. This potassium efflux limits sodium and calcium entry into the cell and thus reduces energy requirements for ion homeostasis and contraction, as well as prolongs the diastolic interval that supports myocardial relaxation and replenishment of resources [4,5,21,25-41]. In this way, cardiac K_{ATP} channels serve a critical role in myocardial protection in response to vigorous exercise and heart rate acceleration [25,26], mandatory for optimal exercise capacity [25,29]. Furthermore, K_{ATP} channel expression up-regulation has been shown to be part of exercise-induced cardiac remodeling and has been linked to enhanced myocardial resistance to ischemic injury [42-45]. However, since the opening of only 1% of sarcolemmal K_{ATP} channels is sufficient to cause significant cardiac action potential shortening [5,16,19,46], it remains to be established how an increase in the expression of the already dense population of K_{ATP} channels could have a sufficient impact on cardiac excitability and energy balance.

Here, we demonstrate that an exercise-induced rise in K_{ATP} channel expression enhances the rate and magnitude of action potential shortening that occurs in response to heart rate acceleration, and that this adaptation in membrane excitability is critical to optimize cardiac energy consumption under conditions of increased demand. Thus, this study defines increased K_{ATP} channel membrane expression as part of the phenomenon of exercise-induced cardiac conditioning, and determines how this dynamic expression change is translated into the maintenance of cardiac energy homeostasis.

2. Materials and methods

2.1 Generation of genetically modified mice

Transgenic Tg[CX1-eGFP-Kir6.1AAA] mice (**Fig. 2**) were created on the FVB/N mouse strain using a non-conducting Kir6.1AAA pore mutant where a Gly-Phe-Gly motif critical for K^+ permeation was replaced by Ala-Ala-Ala [26,29,47]. Kir6.1AAA was subcloned downstream of the *loxP* site-flanked eGFP coding region and a stop codon, in such a way that dominant negative suppression of Kir6.2/SUR2A potassium efflux could be initiated by expression of Cre recombinase. Accordingly, cardiac-specific reduction of K_{ATP} channel activity was achieved by crossing Tg[CX1-eGFP-Kir6.1AAA] and Tg[α MHC-Cre] mice that express Cre recombinase under control of the α MHC promoter [48]. The resulting offspring are notated Tg[α MHC-Kir6.1AAA] to indicate cardiac specific expression of the dominant negative, non-functioning, pore-forming K_{ATP} channel subunit. Male WT, Tg[CX1-eGFP-Kir6.1AAA], and Tg[α MHC-Kir6.1AAA] littermate mice, aged 8-12 weeks, with and without exposure to 5 days treadmill exercise, were used for all experiments.

Protocols were approved by the University of Iowa Institutional Animal Care and Use Committee.

2.2 Isolated heart studies

Isolated hearts were retrogradely perfused at 90 mmHg with Krebs–Henseleit buffer bubbled with 95% O₂/5% CO₂, at 37°C and pH 7.4 [25,26,40]. The AV node was mechanically dissociated and hearts were paced (Bloom Electrophysiology, Fischer Imaging Corp., Denver, CO) using a bipolar platinum pacing catheter positioned in the right ventricular apex (NuMed; Hopkinton, NY).

A monophasic action potential (MAP) probe (EP Technologies; Sunnyvale, CA) was maintained at a single stable position on the LV epicardium, and amplified signals (IsoDam; World Precision Instruments; Sarasota, FL) were acquired at 2 kHz [25,26,40]. Using custom software, (MATLAB, Mathworks, Natick, MA) monophasic action potential recordings were analyzed for duration at 90% repolarization (APD₉₀). Changes in APD₉₀ (Δ APD₉₀) were calculated as the absolute change in milliseconds of the APD₉₀ using the duration of the last action potential at the previous pacing cycle length as a reference. The pacing protocol for Δ APD₉₀ calculations was 12 minutes of continuous pacing consisting of 3 minutes at each of the following sequential cycle lengths: 150, 130, 100 and 80 msec. Monophasic action potentials were only analyzed from tracings in which the MAP probe remained in a stable position throughout the entire pacing protocol, and in which pacing capture was maintained throughout the protocol without interruption. All calculations were manually reviewed to exclude artifacts.

Cardiac oxygen consumption was calculated based on Henry's Law as the difference between the oxygen partial tension measured in the perfusate prior (PO_{2pre}) and after (PO_{2post}) heart passage (Model 210, Instech Laboratories, Plymouth Meeting, PA). The rate of oxygen consumption ($\dot{V}O_2$) was calculated as: $\dot{V}O_2 = (PO_{2\text{entr}} - PO_2) \cdot \alpha \cdot v / m_h$, where v is rate of coronary flow (T402, Transonic System Inc., Ithaca, NY); $\alpha = 0.024$ ml O₂ per ml H₂O/760 mmHg denotes the Bunsen's solubility coefficient [26,49]; and m_h is heart wet weight. Measurements were taken at pacing cycle lengths of 150, 100 and 80 msec.

2.3 Patch-clamp studies

Single ventricular cardiomyocytes were enzymatically isolated as previously described [20,23]. Briefly, hearts were cannulated *in situ* then rapidly excised and retrogradely perfused at 90 mmHg for 5 min with Hepes buffer (Medium 199, Sigma), 1 min with a “low-calcium” medium (in mM): NaCl 100, KCl 10, KH₂PO₄ 1.2, MgSO₄ 5, glucose 20, taurine 50, HEPES 10, supplemented with (in mM) CaCl₂ 0.13, EGTA 2.1, then 13 min with “low-calcium” medium supplemented with 1% bovine serum albumin, CaCl₂ 0.2 mM, collagenase (type IV, 22 U/ml, Worthington) and pronase (100 µg/ml, Serva). Ventricles were dissected away, cut into pieces (~3×3 mm), and incubated at 37°C for 15 min in the enzyme solution with gentle stirring until dissociated cardiomyocytes could be collected.

Patch clamp studies were performed using an Axopatch 200B (Molecular Devices, Sunnyvale, CA) amplifier integrated with a Nikon TE2000-U microscope. Experiments were performed at 33–35°C using a temperature controller TC2r (Cell MicroControls, Norfolk, VA).

For whole-cell recording, pipettes (3–4 M Ω) were filled with internal solution (in mM): KCl 140, MgCl₂ 1, EGTA 5, ATP 5, HEPES-KOH 5 (pH 7.3). Cardiomyocytes were superfused with Tyrode solution (in mM): NaCl 136.5, KCl 5.4, CaCl₂ 1.8, MgCl₂ 0.53, glucose 5.5, HEPES-NaOH 5.5 (pH 7.4). Whole-cell current traces were obtained in response to rectangular pulses from a holding potential of –50 mV to test potentials from –100 to +40

mV. For quantification, whole cell K_{ATP} channel current was measured as the difference between baseline and stimulated current recorded just before the end of a 1-second applied voltage step from -50 to +40mV. For cell attached and inside-out single channel recording, myocytes were bathed in internal solution (mM) KCl 140, MgCl₂ 1, EGTA 5, HEPES-KOH 5 (pH 7.3), supplemented with glucose (1 g/liter). Pipettes (4-5 M Ω) were filled with "Electrode Solution" (in mM): KCl 140, CaCl₂ 1, MgCl₂ 1, HEPES-KOH 5 (pH 7.3). Pipette potential was +40 mV. All recordings were monitored, sampled and analyzed using pCLAMP software (Molecular Devices, Sunnyvale, CA).

2.4 Exercise protocol

Three days before exercise, mice were acclimated daily for 45 min on a nonmoving treadmill (Columbus Instruments, Columbus, OH) followed by 15 min at a velocity of 3.5 m/min. After this training period mice were exercised daily for 5 consecutive days at a speed of 12 m/min and inclination of 15° for 45 minutes. Mice were sacrificed for further studies on the last day of the exercise protocol.

2.5 Experimental Protocols

Quantitative real-time PCR—Total RNA was isolated from liquid N₂ freeze-clamped left ventricles using a commercial kit (RNeasy, Mini Kit, Qiagen) according to the manufacturer's instructions. Strand cDNA was synthesized with random hexanucleotides from 1 μ g of total RNA using a reverse transcription system kit (Superscript III First Strand Synthesis, Invitrogen). Quantitative PCR was done using Biorad's iQ SYBR Green Supermix (iCycler, Biorad). The primer sets were as follows: Kir6.2 Forward: GAA-GCC-TGT-ACC-GGG-TTA-TT, Reverse: GCT-TTA-GAG-GCC-CTG-AAC-CT, 118bp product; SUR2A Forward: GAG-TGT-CAG-ACC-TGC-GCT-TCT, Reverse: GCT-GCT-CAG-CAG-GAT-TGG-TCT-C, 251bp product; HPRT Forward: GAA-CCT-CTC-GAA-GTG-TTG-GAT-AC, Reverse: GCT-CAT-CTT-AGG-CTT-TGT-ATT-TGG-CT, 165bp product. The quality of the RT-qPCR product was routinely checked by the thermal denaturation curve following RT-qPCR reactions. The expression of RNA encoding the channel subunits was normalized to Hypoxanthine-Guanine Phosphoribosyltransferase (HPRT) and quantification of relative mRNA levels was performed by ΔC_T method.

Western blotting—Protein extracts were prepared by homogenizing ventricular tissue in NaCl 150 mM, Tris-HCl 50 mM (pH 7.8), supplemented with 1% Triton X-100, protease and phosphatase inhibitors (Roche). Electrophoresis was performed on 3-8% gradient Nu-Page Tris-Acetate gel and transferred to 0.2 μ m Sequi-Blot PVDF membranes (Bio-Rad). Rabbit polyclonal antibodies against SUR2, goat polyclonal antibodies against Kir6.2, and secondary anti-rabbit and anti-goat HP-conjugated antibodies were used (Santa Cruz Biotechnology). Densitometric analysis of the Western blot was performed using Adobe Photoshop (Adobe Systems, San Jose, CA).

2.6 Statistical analysis

Results are expressed as mean $\pm$ SEM. Comparisons between two groups were made using the 2-sided Student's t-test and between more than two groups using analysis of variance (ANOVA). A *p* value <0.05 was considered statistically significant.

3. Results

3.1. Exercise increases the expression of functional K_{ATP} channels

Exercise causes significant cardiac remodeling [50]. We used short-term exposure to exercise training to evaluate if increased cardiac K_{ATP} channel expression is part of activity-

induced cardiac remodeling. A densitometric analysis of western blots (**Fig. 1A, B**) revealed a significant increase in the expression of Kir6.2 and SUR2A subunits in protein extracts from ventricles of mice with exposure to 5 days exercise compared to sedentary controls ($45\pm 5\%$ increase for Kir6.2 and $32\pm 7\%$ increase for SUR2A, **Fig. 1B**). These changes in protein expression were accompanied by a gain in the number of functional K_{ATP} channels at the sarcolemma and an increase in the K_{ATP} channel current stimulated by application of the K_{ATP} channel specific opener pinacidil, and mitochondrial uncoupler 2,4-dinitrophenol (DNP). Specifically, membrane patches on ventricular cardiomyocytes from exercised animals had on average 8.59 ± 1.07 channels vs. 4.66 ± 0.41 channels in sedentary animals ($p<.05$, **Fig. 1C, D**). Similarly, whole cell K_{ATP} channel current in myocytes isolated from hearts of mice after exposure to exercise training was 25.19 ± 2.09 pA/pF vs. 17.75 ± 1.34 pA/pF in sedentary controls ($p<.05$, **Fig. 1E, F**). No changes in sensitivity to ATP, single channel conductance or open channel probability were detected in response to exercise (**Fig. 1G, H**). The $[IC_{50}]$ for ATP in the exercise group was $26\pm 2.1\mu M$ vs. $31\pm 1.1\mu M$ for the sedentary group ($p=NS$). The Hill coefficient was $1.0\pm 0.3\mu M$ for exercise vs. $1.1\pm 0.2\mu M$ for sedentary ($p=NS$). Single channel conductance was 70.9 ± 5 pS ($n=5$ patches) for exercise vs. 71.4 ± 6 ($n=4$ patches) for sedentary conditions ($p=NS$). Open probability was $.84\pm .05$ ($n=5$ patches) for exercise vs. $.82\pm .03$ ($n=4$ patches) for sedentary conditions ($p=NS$). Thus, short-term exposure to exercise caused a significant increase in the expression of functional K_{ATP} channels without altering their ATP-dependent gating properties or conductance.

3.2. Exercise-induced increase in K_{ATP} channel current capacity is eliminated by cardiac-specific expression of a non-functional K_{ATP} channel pore-forming subunit

Cardiac remodeling may be beneficial independently from changes in K_{ATP} channel expression. In order to determine if changes in K_{ATP} channel expression after exposure to exercise effect cardiac function and energetics, we explored a model of cardiac-specific disruption of K_{ATP} channel function as a control. Tg[CX1-eGFP-Kir6.1AAA] mice, which express eGFP in all somatic cells (**Fig. 2A**, upper panel) but are otherwise phenotypically normal [26,47], were crossed with αMHC -Cre transgenic animals [48]. The resulting Tg[αMHC -Kir6.1AAA] progeny displayed cardiac-specific potassium impermeable Kir6.1AAA expression tracked by selective cardiac elimination of eGFP fluorescence (**Fig. 2A**, bottom). The eGFP fluorescence was not eliminated in any other tissue, including brain (**Fig. 2A**, bottom, left panel), skeletal muscle (**Fig. 2A**, bottom, middle panel), vasculature, or pancreas (not shown), consistent with previous reports [47,48], and supporting the expression of the dominant negative Kir6.1AAA construct only in cardiomyocytes.

There was no difference in the number of K_{ATP} channels/patch (20.88 ± 1.18 pA/pF, $n=6$, $p=NS$) or whole cell K_{ATP} channel current in cardiomyocytes from sedentary Tg[CX1-eGFP-Kir6.1AAA] ($4.5\pm .64$, $n=10$, $p=NS$, tip size $4.74\pm .21$ m Ω) compared to sedentary WT littermates (data in section 3.1). However, cardiac expression of the mutant Kir6.1-AAA subunit significantly reduced the number of functional K_{ATP} channels per patch (**Fig. 2B, C**) and whole cell K_{ATP} channel current (**Fig. 2D, E**). Specifically, when sedentary Tg[αMHC -Kir6.1AAA] mice were compared to their sedentary WT or Tg[CX1-eGFP-Kir6.1AAA] littermates, the number of functional K_{ATP} channels/patch was reduced by 76% (**Fig. 1C, D** vs. **2B, C**; $p<.05$) and the whole cell K_{ATP} channel current by 86% (**Fig. 1E, F** vs. **2D, E**).

Formation of K_{ATP} channels requires 4 pore-forming subunits, and since inclusion of only one mutant Kir6.1AAA subunit is sufficient to eliminate K^+ conductance, an increase in Kir6.2 expression is not expected to easily overcome the dominant-negative effect of the Kir6.1AAA transgene. As such, expression of the non-functional pore-forming subunit not only reduced the K_{ATP} channel current capacity in sedentary animals, but also prevented an exercise-induced increase in the number of functional K_{ATP} channels and K_{ATP} channel current capacity. Specifically, patch clamp assays performed on ventricular myocytes

isolated from Tg[α MHC-Kir6.1AAA] with and without exposure to exercise revealed $.94 \pm .18$ and $1.1 \pm .23$ channels per patch and whole cell current densities of $2.3 \pm .5$ and $2.5 \pm .6$ pA/pF, respectively (**Fig. 2C-E**, $p=NS$). Thus, cardiac-specific transgenic expression of the nonfunctional Kir6.1AAA subunit significantly reduced K_{ATP} channel current capacity and prevented its increase by exercise.

3.3. Increased K_{ATP} channel expression promotes action potential shortening in response to heart rate acceleration

During exercise, bodily energy consumption increases 20-100 times over the basal level [51]. The ability of the cardiovascular system to deliver required oxygen and other metabolic fuels is critical for such physical performance, but is limited by the capacity to achieve and maintain sufficient heart rate and stroke volume [1]. Acceleration of heart rate requires shortening of the action potential duration to adjust myocyte refractoriness and to balance augmented cardiac contractile function with relaxation and resource replenishment [4,5,21,25-32]. We have previously shown that this action potential shortening in response to an increase in heart rate has a prominent K_{ATP} channel-dependent component [26]. Therefore, to determine if the witnessed exercise-induced increase in functional K_{ATP} channel expression is sufficient to have a measurable and physiologically relevant impact on the regulation of cardiac excitability, we evaluated action potential duration changes in response to heart rate acceleration. Specifically, we measured monophasic action potential duration in retrogradely perfused hearts isolated from exercised and sedentary mice, paced over a range of heart rates (450-720 beats/min corresponding to cycle lengths of 150-80 msec). When the change in APD_{90} (ΔAPD_{90}) was tracked as a function of time following heart rate acceleration, we found that ΔAPD_{90} follows a complex function with an immediate decrease of several milliseconds, followed by a rapid rebound back towards baseline, and then a slower (1-2 min) decline to a steady state. Wild type hearts exposed to exercise, associated with an increase in K_{ATP} channel expression, demonstrated enhanced APD_{90} shortening at higher heart rates (**Fig. 3**). Specifically, for the transitions 130-to-100, and 100-to-80 msec cycle length (**Fig. 3A** middle and left panels), there was a significantly faster and greater extent of APD_{90} shortening in hearts with increased K_{ATP} channel expression. There was no significant effect of exercise on the extent of APD_{90} shortening at the slower cycle length transition, 150-to-130 msec, which apparently did not increase energy consumption to a degree sufficient to promote K_{ATP} channel opening (**Fig. 3A** first panel). To quantify the magnitude and rate of the ΔAPD_{90} response to heart rate transitions, we examined the area above the ΔAPD_{90} -time curve or “integrated APD_{90} shortening” as a parameter which reflects both the magnitude and the rate of change. We also calculated the half-time of the ΔAPD_{90} response in order to assess rate alone (**Fig. 3C, D**). The 130-to-100 and 100-to-80 msec cycle length transitions resulted in a consistently greater integrated APD_{90} shortening and shorter response half times for hearts from exercised compared with sedentary mice (**Fig. 3C, D**, $p<.05$). APD_{90} reduction in response to heart rate increase was significantly blunted in both exercise and sedentary group hearts perfused with the specific K_{ATP} channel blocker glyburide (**Fig. 3A**) at dose of $10\mu M$, sufficient to completely block cardiac K_{ATP} channel current [52].

Furthermore, to establish that the differential effect of heart rate acceleration on action potential shortening between exercise and sedentary groups is indeed a result of augmented K_{ATP} expression, and not due to other beneficial effects of exercise, we examined ΔAPD_{90} in the hearts of Tg[α MHC-Kir6.1AAA], in which expression of the dominant negative Kir6.1AAA pore-forming subunit prevented an increase in K_{ATP} channel current capacity after exposure to exercise (**Fig. 2, 3**). These experiments revealed a significantly decreased integrated ΔAPD_{90} shortening and half time of ΔAPD_{90} for the 130-to-100 and 100-to-80 msec cycle length transitions in the transgenic hearts compared to the hearts of WT with or

without exposure to exercise (**Fig. 3A, C**). The total shortening of APD₉₀ at steady state was similar in Tg[α MHC-Kir6.1AAA] and sedentary WT hearts after the 130-to-100 msec transition, indicating that the reduced K_{ATP} channel current capacity in the Tg[α MHC-Kir6.1AAA] decelerated but did not eliminate APD₉₀ adjustment at this level of energy demand (**Fig. 3A**). There was no difference in the APD₉₀ shortening between Tg[α MHC-Kir6.1AAA] hearts with and without exercise training (**Fig. 3A, C**). Thus, an exercise-induced increase in cardiac K_{ATP} channel expression enhanced the rate and magnitude of action potential shortening in response to heart rate acceleration.

3.4. Exercise-induced increase in K_{ATP} channel expression limits escalation of cardiac oxygen consumption during heart rate acceleration

K_{ATP} channels are critical regulators of myocyte energy consumption. To investigate whether the increase in K_{ATP} channel expression and consequent enhanced action potential shortening in response to acceleration of heart rate is sufficient to impact cardiac energetic status, we compared the cardiac oxygen consumption of isolated hearts extracted from exercised and sedentary WT and Tg[α MHC-Kir6.1AAA] littermates (**Fig. 4**). For WT animals, oxygen consumption, normalized to heart weight and coronary flow, was comparable when hearts were paced at a cycle length of 150 msec. Shortening the pacing cycle length from 150 to 100 msec, or 100 to 80 msec, produced an expected increase in oxygen consumption but significantly less so for the exercise group compared to the sedentary group (**Fig. 4B**). Specifically, the oxygen consumption of sedentary WT mice was $.17 \pm .01$, $.23 \pm .01$, and $.29 \pm .02$ ml/min/g at cycle lengths of 150, 100, and 80 msec, respectively, while oxygen consumption for exercised mice was $.14 \pm .02$, $.16 \pm .003$, and $.22 \pm .01$ at the same cycle lengths ($p < .05$ for 100 and 80 msec cycle lengths). By comparison, hearts from sedentary Tg[α MHC-Kir6.1AAA] animals had significantly greater oxygen consumption at every cycle length ($.22 \pm .01$, $.30 \pm .03$, and $.37 \pm .03$, $p < .05$ for each cycle length) and exercised Tg[α MHC-Kir6.1AAA] animals did not demonstrate a reduction in oxygen consumption ($.23 \pm .02$, $.31 \pm .03$, and $.37 \pm .02$, $p = \text{NS}$ compared to sedentary). These data confirm that normal K_{ATP} channel current capacity is necessary to properly regulate cardiac energy use, and that augmentation of K_{ATP} channel expression increases cardiac energetic efficiency at higher heart rates.

3.5. Exercise increases transcription of ABCC9 encoding the SUR2A regulatory subunit

Exercise is a well-established inducer of cardioprotective gene reprogramming [50]. To investigate whether exercise-activated transcription of *ABCC9* and *KCNJ11* genes encoding K_{ATP} channel subunits underlies changes in the number of plasmalemmal K_{ATP} channels, Kir6.2 and SUR2A mRNA were measured using RT-PCR. Exposure to exercise caused an approximately 1.5-fold increase in the level of SUR2A mRNA, while Kir6.2 mRNA did not change (**Fig. 5A**). This is in agreement with the previously established role of transcription of the *ABCC9* gene, encoding the SUR2A regulatory subunit, as a rate limiting factor in the assembly of functional K_{ATP} channels [53-55].

The transcription of *ABCC9* has been shown to be increased by the activator protein-1 (AP-1) transcription factor complex [53]. The activity of this complex is upregulated by exposure to short-term exercise [56-58] and depends on the presence of phosphorylated cJUN kinase [59]. In our model, activation of *ABCC9* transcription in hearts exposed to exercise training was paralleled by an increased myocardial content of phosphorylated cJUN (P-cJUN), while the level of total cJUN was not significantly changed (**Fig. 5B, C**). Specifically, densitometry of western blots revealed a relative P-cJUN density of $.59 \pm .09$ for exercise and $.32 \pm .02$ for sedentary groups ($p < .05$), and a P-cJUN/cJUN ratio of $.76 \pm .06$ and $.56 \pm .05$, respectively ($p < .05$). Thus increased K_{ATP} channel expression after exposure to exercise was associated with increased transcription of the SUR2A encoding gene.

4. Discussion

K_{ATP} channels are unique membrane protein complexes critical for preservation of myocardial energy balance under normal and disease conditions [4,5,7,21,25,26,40]. Such a basic physiologic role in cardiac adaptation to increased workload is consistent with the dense expression of these channels in cardiomyocytes as well as their evolutionary conservation across a broad range of species [4-7,19,21,25,26]. Here we demonstrated the significance of physiologic regulation of K_{ATP} channel expression for cardiac energy homeostasis. Short-term exposure to exercise caused significant up-regulation of functional K_{ATP} channels at the cardiomyocyte sarcolemma. This increase in K_{ATP} channel expression enhanced APD₉₀ shortening and reduced cardiac energy consumption in response to workload imposed by heart rate acceleration. Disruption of exercise-induced K_{ATP} channel current up-regulation in Tg[α MHC-Kir6.1AAA] transgenic mice eliminated this adaptive energy saving response.

Several earlier studies associated exposure to exercise with augmented K_{ATP} channel expression and reduced susceptibility to ischemic injury [42-45]. Our data corroborate these findings and provide a mechanistic explanation for this phenomenon. Here, enhanced expression of K_{ATP} channels increased the magnitude and rate of action potential shortening in response to workload intensification. Metabolic sensing by the channel occurs through modulation of the K⁺ pore ATP-sensitivity by the SUR subunit, which is also required for channel activation by MgADP and potassium channel openers as well as inhibition by sulfonylurea drugs [2-24,60]. Nucleotide exchange between the SUR2A ATPase and intracellular metabolic pathways provides a mechanism coupling K_{ATP} channel function with cellular energetics and allows precise modulation of membrane potential-dependent cellular functions in response to changes in cellular energetics [13-15,20,23,61]. However, since the bulk cellular ATP/ADP ratio remains unchanged under all but the most severe metabolic insults, the concept of local energetic signaling has been proposed [26,62]. Specifically, compartmentalization of the intracellular milieu provides conditions such that membrane ATPases may reduce the local ATP concentration, and elevate the local ADP concentration, significantly, without bulk ATP/ADP changes. Thus, K_{ATP} channels could be differentially activated according to their proximity to these ATPases [26,62,63]. We hypothesize that the findings described in the current study may be explained by the compartmentalized cellular environment in which increased expression of K_{ATP} channels could result in a greater number of channels under the influence of ATP consuming sites, thus accounting for an increased magnitude of response. Furthermore, in the presence of a larger number of channels, the probability is increased that K_{ATP} channels would be located in a closer proximity to such sites, which may explain the increased rate of action potential shortening. A faster response in the potassium efflux-driven repolarization, following an increase in heart rate, would accelerate action potential shortening and thus improve the energy efficiency of myocyte contraction and preservation of ion homeostasis [26]. This change in K_{ATP} channel-dependent membrane response, and the consequent beneficial effect on myocardial oxygen consumption, may also provide a mechanistic explanation for the previously reported increased resistance to ischemic injury of hearts from rats with exercise-induced enhancement of K_{ATP} channel expression [42-45].

Action potential shortening in response to heart rate acceleration is critical to reduce refractory period and preserve diastolic filling time [30-32]. This response includes an immediate change (first 100 milliseconds) and a longer-term (1-2 minutes) phase [30,64]. The molecular mechanisms of this response are not yet fully understood, but likely embrace complex changes in the balance between inward and outward ion currents [30-32]. The phenomenon of immediate change in action potential duration in response to a sudden increase in stimulation frequency has been attributed to incomplete recovery of ion currents

from the prior cardiac cycle, such as ongoing inactivation of L-type Ca^{++} channels and continuing decay of outward K^{+} fluxes [30-32]. The next, more prolonged phase of action potential shortening, has been linked to activation of the $\text{Na}^{+}/\text{Ca}^{++}$ exchanger and the $\text{Na}^{+}/\text{K}^{+}$ ATPase as a result of changes in intracellular sodium and calcium content, as well as extracellular potassium accumulation [30-32]. Recently, we reported in a murine model that action potential adjustment in response to heart rate acceleration also depends on opening of the K_{ATP} channel [26]. The current study confirms these previous findings and also reveals the time course of these changes in the APD. An immediate APD shortening occurred with the first beat after heart rate acceleration and was similar in all experimental groups without relation to exercise or disruption of the K_{ATP} channel current. This was followed by a rapid rebound of the APD, possibly associated with activation of calcium-dependent calmodulin kinase (CaMKII) and increased calcium influx [65], which was counterbalanced by a prolonged and more prominent APD shortening phase that depended substantially on K_{ATP} channel current. The observed K_{ATP} channel-dependent APD shortening took approximately 1-2 minutes before reaching steady state. As expected, the effect was more prominent at high heart rates, likely due to an increase in Ca^{++} and Na^{+} turnover and activation of $\text{Na}^{+}/\text{K}^{+}$ and Ca^{++} ATPases [26,63]. We interpret these changes as confirmation that K_{ATP} channels are critical for myocyte excitability adjustment under normal physiologic dynamics of cardiac workload and that increased K_{ATP} channel expression accelerates and augments this response as part of the physiologic adaptation to exercise training.

Beneficial effects of exercise involve a broad variety of molecular targets and mechanisms [1,50]. To dissect the K_{ATP} channel-dependent mechanisms we used a transgenic model, Tg[α -MHC-Kir6.1AAA], with cardiac-specific functional disruption of sarcolemmal K_{ATP} channel potassium conductance. Expression of the dominant negative Kir6.1AAA mutant subunit has been successfully used to disrupt normal potassium conductance of sarcolemmal K_{ATP} channels in the heart, skeletal muscles and endothelium [26,29,47]. Of note, no expression of this transgene has been found in the mitochondria [29], and therefore disruption of mitochondrial K_{ATP} channels in the Tg[α -MHC-Kir6.1AAA] mice can be excluded. In this study, we use transgenic mice with mutant Kir6.1AAA expression sufficient to reduce K_{ATP} channel current density by 80%, but not to completely eliminate it. Accordingly, this reduction in K_{ATP} channel current capacity does not eliminate K_{ATP} channel-dependent APD shortening (compare to glyburide-treated hearts), but rather significantly attenuates it – an effect achieved in part by slowing the K_{ATP} channel-dependent response rate. Furthermore, exposure to exercise did not significantly change the cardiac K_{ATP} channel current capacity, APD₉₀ response, or oxygen consumption with heart rate acceleration in Tg[α -MHC-Kir6.1AAA] mice. In fact, the oxygen consumption of Tg[α -MHC-Kir6.1AAA] hearts was greater than WT at all cycle lengths, even following the 150-to-130 msec transition when significant differences in ΔAPD_{90} were not found. This is in agreement with our previously published findings [26], and may reflect a difference in the resolution of the MAP vs. oxygen consumption techniques. Indeed, monophasic action potential recordings in murine hearts may correlate with, but not exactly reproduce, the absolute transmembrane action potential duration [66]. Here, we used each MAP position as its own control, and examined changes, rather than absolute duration, in order to eliminate this potential source of error. However, when true transmembrane action potentials were measured in isolated ventricular cardiomyocytes expressing Kir6.1AAA, the action potentials were found to be significantly longer than in WT, even during resting conditions, and this was corroborated by longer ventricular refractory periods during pacing studies in isolated hearts [29]. Therefore, an overall increase in APD could account for the differences in oxygen consumption seen at slower heart rates. Also, similar to previously published transmembrane action potentials in ventricular myocytes from Tg[α -MHC-Kir6.1AAA] mice [29], we see a more prominent action potential plateau. This suggests increased calcium entry, increased calcium release, and thus increased SERCA2A activity to restore diastolic

calcium concentrations. This, possibly coupled with increased contraction, would be expected to increase ATP hydrolysis and oxygen consumption. Thus, the data reported in the current study support a previously unrecognized homeostatic feedback loop for myocardial energy conservation: exercise-related increase in cardiac energy demand triggers an increase in K_{ATP} channel expression which, in turn, enhances APD shortening and thus limits workload-driven energy consumption.

Defining this mechanism further expands our understanding of exercise-induced cardiovascular conditioning leading to improved cardiovascular performance [1]. Indeed, exposure to exercise causes a broad spectrum of beneficial changes which promote cardiac and general stress resistance [1,50]. One of the reported consequences of exercise-related gene reprogramming associated with short-term exposure to exercise (up to 5 days) is activation of the c-Jun/NH2-terminal kinase signaling cascade [56,58], critical for activation of SUR2A encoding gene transcription [53]. Indeed, our data indicate that the exercise-induced increase in phosphorylated cJUN was paralleled by a 50% increase in the presence of SUR2A encoding mRNA, comparable with the change in functional K_{ATP} channel expression. No change was evident in the level of Kir6.2 encoding mRNA in agreement with previously reported data which indicate that Kir6.2 mRNA is present in relative abundance to SUR encoding mRNA, and does not correlate with K_{ATP} channel complex expression [53]. Although detailed definition of the mechanism underlying up-regulation of K_{ATP} channels by exercise will require future study, we interpret the current findings as an indication that elevated transcription of *ABCC9* promotes production of SUR2A, driving the increased expression of functional K_{ATP} channels in response to short-term exposure to exercise.

A limitation of this study is that we did not measure energy consumption by membrane ATPases or sub-membrane ATP/ADP levels. These future experiments would provide a more comprehensive understanding of K_{ATP} channel responses under physiologic or pathophysiologic conditions. It will also be critical to investigate the role of K_{ATP} channels in APD adaptation to heart rate acceleration in other animal models to exclude this as a species-specific phenomenon related to the unique composition of ion channel currents comprising the murine action potential.

In this study we examined K_{ATP} channel-dependent changes associated with short-term exposure to exercise. It has previously been reported that long-term exposure to exercise also augments K_{ATP} channel expression [42]. It is possible that these changes in K_{ATP} channel expression are driven by different mechanisms and not by activation of SUR2A transcription, as AP1 activity is known to be reduced in chronically trained hearts [56,58]. It would be important to evaluate K_{ATP} channel subunit stability, localization, trafficking, endocytosis, and degradation, as well as AP1 independent transcription activation. It will also be important to define the duration of K_{ATP} channel expression and cardiac energy consumption changes in response to exercise.

5. Conclusions

This study demonstrates that dynamic regulation of cardiac K_{ATP} channel expression modulates the speed and magnitude of the membrane electrical response to changes in the metabolic state of the cell. This mechanism allows the cardiomyocyte to adapt energy consumption to available resources more efficiently, and thereby maintain cardiac well-being under a range of physiologic stresses. Further understanding of the mechanisms responsible for K_{ATP} channel regulation provides novel insights into endogenous cardioprotection, and the development of strategies for the management and prevention of cardiovascular injury and diseases.

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Figure 1. Short-term exercise increases cardiac expression of functional K_{ATP} channels

A. Representative Western blots of K_{ATP} channel subunits Kir6.2 and SUR2A in protein extracts from ventricles of mice under sedentary and exercise conditions. **B.** Summary statistics indicating increased expression of Kir6.2 and SUR2A subunits over baseline in response to exercise (both $n=10$ and $*p<.05$ vs. baseline). **C.** Representative cell-attached K_{ATP} channel recordings in ventricular myocytes isolated from hearts of sedentary and exercised mice. Channel opening is stimulated by 2,4-dinitrophenol (DNP) 200 μ M and pinacidil 100 μ M in the perfusate. **D.** Summary statistics indicating the number of K_{ATP} channels/patch detected in cardiomyocytes isolated from exercised ($n=22$ patches, 5 mice) and sedentary ($n=29$ patches, 6 mice) animals ($*p<.001$). The patch pipette tip size was not significantly different between groups ($4.71\pm.31$ vs. $4.65\pm.22$ M Ω , respectively, $p=NS$). **E.** Representative whole cell K_{ATP} current recordings in cardiomyocytes isolated from hearts of exercised and sedentary mice in response to DNP 50 μ M and pinacidil 100 μ M in the perfusate. Inset: stimulation pattern. Current is normalized to membrane capacitance (pA/pF). **F.** Summary statistics of whole cell K_{ATP} channel current normalized to cell capacitance (pA/pF) in response to DNP 50 μ M and pinacidil 100 μ M for cardiomyocytes isolated from hearts of exercised ($n=19$ patches, 5 mice) vs. sedentary ($n=10$ patches, 6 mice) animals ($*p<.05$). **G.** Representative inside-out patch recordings of K_{ATP} channel activity in isolated cardiomyocytes from hearts of exercised and sedentary mice in response to escalating concentrations of ATP in the perfusate. **H.** K_{ATP} channel activity in membrane patches (inside-out configuration), calculated relative to activity in the absence of ATP, and fitted by the Hill equation ($y=[IC_{50}]/([IC_{50}]^h + [ATP]^h)$, where h is the Hill coefficient, $[ATP]$ is the ATP concentration, and $[IC_{50}]$ is the half-maximal inhibitory ATP concentration ($n=10$ exercise and 9 sedentary).

Figure 2. Expression of a dominant negative K_{ATP} channel pore-forming subunit results in significant reduction of functional cardiac channels and interference with exercise-induced K_{ATP} current up-regulation

A. Representative images of tissues from transgenic mice under UV light. Tg[$\text{CX1-eGFP-Kir6.1AAA}$] mice express eGFP in all cells (top panel). Crossing Tg[$\text{CX1-eGFP-Kir6.1AAA}$] with Tg[$\alpha\text{MHC-Cre}$] generated Tg[$\alpha\text{MHC-Kir6.1AAA}$] progeny with cardiomyocyte specific Kir6.1AAA transgene expression tracked by selective elimination of eGFP fluorescence in the heart only (bottom panel). **B.** Representative cell-attached K_{ATP} channel recordings in response to DNP 200 μM and pinacidil 100 μM in the perfusate. **C.** Summary statistics indicating the number of K_{ATP} channels/patch in cell-attached

recordings following exposure to DNP 200 μ M and pinacidil 100 μ M performed in cardiomyocytes isolated from exercised (n=5 mice and 17 patches) *vs.* sedentary (n=5 mice, 10 patches) Tg[α MHC-Kir6.1AAA] animals (p=NS). The patch pipette tip size was not significantly different between groups ($4.62 \pm .09$ *vs.* $4.44 \pm .07$ M Ω , respectively, p=NS). **D.** Representative whole cell K_{ATP} channel current recordings in cardiomyocytes isolated from hearts of exercised and sedentary Tg[α MHC-Kir6.1AAA] mice in response to DNP 50 μ M and pinacidil 100 μ M in the perfusate. Inset shows stimulation pattern. Current is normalized to cell capacitance (pA/pF). **E.** Summary statistics of whole cell K_{ATP} channel current normalized to cell capacitance (pA/pF) in response to DNP 50 μ M and pinacidil 100 μ M for cardiomyocytes isolated from hearts of exercised (n= 3 mice and 11 patches) *vs.* sedentary (n= 3 mice and 11 patches) Tg[α MHC-Kir6.1AAA] animals (p=NS).

Figure 3. Exercise-induced increase in K_{ATP} channel expression translates to enhanced action potential shortening in response to heart rate acceleration

A. Summary plots of changes in monophasic action potential shortening (ΔAPD_{90}) as a function of time following each of three pacing cycle length transitions in isolated, perfused hearts from WT exercised ($n=7$), WT sedentary ($n=8$), TG exercised ($n=7$), and TG sedentary ($n=7$) mice ($*p<.05$ for WT exercise vs. WT sedentary). Also graphed are glyburide ($10\mu M$) treated hearts from WT exercise ($n=3$) and WT sedentary mice ($n=3$). Each graph indicates shortening as compared to the steady state APD_{90} measured for the previous pacing cycle length. There is no difference in steady state APD_{90} after 3 minutes of pacing at 150 msec. (59.3 ± 3.5 , 57.4 ± 3 , 59.6 ± 3.3 , 57.4 ± 4.1 , 60.3 ± 4.1 and 59.5 ± 3.8 msec, respectively, $p=NS$). The ΔAPD_{90} of the first action potential following the transition is graphed, followed by every 40th action potential thereafter for 2 minutes. **B.** Representative examples of monophasic action potentials recorded from the left ventricular epicardium of isolated, perfused hearts from exercised WT and Tg[αMHC -Kir6.1AAA] mice after 3 minutes of pacing at the displayed cycle length, in accordance with the 12 minute protocol of progressive cycle length shortening. Dotted lines indicate the 90% repolarization level. **C.** Summary statistics showing the integrated APD_{90} shortening, defined as the area above the curve of the ΔAPD_{90} time course (n = same as in Fig. 3A, $*p<.05$ compared to WT sedentary). **D.** Summary statistics showing the half time ($t_{1/2}$) of ΔAPD_{90} response (n = same as in Fig. 3A, $*p<.05$ compared to WT sedentary). WT = wild-type, TG = Tg[αMHC -Kir6.1AAA].

Figure 4. Rate of cardiac oxygen consumption in response to heart rate acceleration is reduced in animals with increased K_{ATP} channel expression following exercise

A. Representative tracing of raw oxygen consumption data (perfusate oxygen tension – effluent oxygen tension) in response to pacing cycle length transitions in an isolated perfused heart from a WT sedentary animal. **B.** Summary statistics indicating steady state oxygen consumption rate ($\dot{V}O_2$), normalized to heart weight and coronary flow (see Methods), at each of three pacing cycle lengths in isolated perfused hearts from WT exercise (n=7), WT sedentary (n=7), TG exercise (n=4) and TG sedentary (n=5) mice (*p<.05 compared to WT sedentary). WT = wild-type, TG = Tg[α MHC-Kir6.1AAA].

Figure 5. Increased cardiac K_{ATP} channel expression in response to exercise is associated with increased transcription of the SUR2A encoding gene

A. Summary of the relative levels of SUR2A (left bar graph) and Kir6.2 (right bar graph) mRNA in extracts from ventricles isolated from hearts of exercised and sedentary mice (n=9 for each group, *p<.05). **B.** Representative western blots of cJUN and P-cJUN compared to GAPDH in extracts from ventricles isolated from hearts of exercised and sedentary mice. **C.** Summary of cJUN and phosphorylated cJUN (P-cJUN) expression relative to GAPDH in extracts from ventricles of hearts from exercised (n=3) and sedentary (n=3) mice (*p<.05 for Pc-JUN and Pc-JUN/cJUN exercise vs. sedentary).