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Reduction in number of sarcolemmal K_{ATP} channels slows cardiac action potential duration shortening under hypoxia

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Abstract

The cardiovascular system operates under demands ranging from conditions of rest to extreme stress. One mechanism of cardiac stress tolerance is action potential duration shortening driven by ATP-sensitive potassium (K_{ATP}) channels. K_{ATP} channel expression has a significant physiologic impact on action potential duration shortening and myocardial energy consumption in response to physiologic heart rate acceleration. However, the effect of reduced channel expression on action potential duration shortening in response to severe metabolic stress is yet to be established. Here, transgenic mice with myocardium-specific expression of a dominant negative K_{ATP} channel subunit were compared with littermate controls. Evaluation of K_{ATP} channel whole cell current and channel number/patch was assessed by patch clamp in isolated ventricular cardiomyocytes. Monophasic action potentials were monitored in retrogradely perfused, isolated hearts during the transition to hypoxic perfusate. An 80-85% reduction in cardiac K_{ATP} channel current density results in a similar magnitude, but significantly slower rate, of shortening of the ventricular action potential duration in response to severe hypoxia, despite no significant difference in coronary flow. Therefore, the number of functional cardiac sarcolemmal K_{ATP} channels is a critical determinant of the rate of adaptation of myocardial membrane excitability, with implications for optimization of cardiac energy consumption and consequent cardioprotection under conditions of severe metabolic stress.

Keywords

ATP-sensitive potassium channel; K_{ATP} ; heart; glyburide; monophasic action potential

1. Introduction

The cardiovascular system operates under a wide span of demands, ranging from conditions of rest to extreme stress. Identification of mechanisms underlying cardiac stress resistance is

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an area of active investigation. One mechanism of cardiac stress tolerance is through action potential duration (APD) shortening driven by ATP-sensitive potassium (K_{ATP}) channels [1-14].

K_{ATP} channels are comprised of the pore-forming inwardly rectifying potassium channel, Kir6.x, with the regulatory sulfonylurea receptor, SUR [5,15-18]. The metabolic sensing of K_{ATP} channels occurs through modulation of the ATP sensitivity of Kir6.x by SUR which is closely integrated with cellular energetic networks [6,19-27]. When activated by a reduced ATP/ADP ratio, reflecting increased cellular metabolic demand, K_{ATP} channel-dependent potassium efflux shortens cardiac APD [1-3,5,28]. This potassium efflux limits sodium and calcium entry into the cell and thus reduces energy requirements for ion transport/exchange and contraction as well as prolongs the diastolic interval that supports myocardial relaxation and restoration of ion gradients and energetic resources [1-14].

Reflecting the importance of myocardial energy homeostasis, cardiac K_{ATP} channels are among the most densely expressed membrane protein complexes in cardiomyocytes [5,14,21,28]. Indeed, models based on data obtained in isolated cardiomyocytes, and indirect evidence in intact hearts, indicate that opening of <1% of sarcolemmal K_{ATP} channels is sufficient to dramatically shorten the APD [5,20,24,29-31]. By implication, K_{ATP} channel physiologic effects should thus be insensitive to K_{ATP} channel expression increases, and buffered against all but the most severe expression decreases. However, we have recently shown that changes in K_{ATP} channel expression have a significant physiologic impact with regard to both APD shortening and myocardial energy consumption in response to physiologic increases in heart rate [1]. Compared with control, a ~50% increase in K_{ATP} channel membrane expression results in greater and more rapid APD shortening associated with less myocardial oxygen consumption in response to heart rate acceleration within the physiologic range, while an ~80% reduction in functional K_{ATP} channels has the opposite effect [1]. These findings indicate the importance of the level of K_{ATP} channel expression in sensing slight changes in cardiomyocyte energetics coupled to physiologic increases in workload. Still, the effect of reduced channel expression on APD shortening in response to a severe metabolic stress that activates most or all available K_{ATP} channels, is yet to be established.

Here, we demonstrate that in hearts exposed to severe hypoxia, an 80-85% reduction in K_{ATP} channel current density results in a normal magnitude of APD shortening, but occurring at a significantly slower rate. Thus, this study provides direct evidence to support the previously proposed models linking dramatic shortening of the cardiac APD to activation of a small percentage of K_{ATP} channels. This study further indicates that the number of functional K_{ATP} channels is a critical determinant of the rate of adaptation of membrane excitability. These findings have implications for optimization of cardiac energy consumption and consequent cardioprotection, under conditions of severe metabolic stress.

2. Materials and Methods

2.1 Generation of genetically modified mice

Transgenic Tg[CX1-eGFP-Kir6.1AAA] mice (Fig. S1) were created on an FVB background using a non-conducting Kir6.1AAA pore mutant where a Gly-Phe-Gly motif critical for K^+ permeation was replaced by Ala-Ala-Ala [8]. Expression of the Kir6.1AAA transgene has been shown to dramatically reduce K_{ATP} channel current in tissues with channels comprised of either Kir6.2 or Kir6.1 isoforms [1,3,8,32]. Kir6.1AAA was subcloned downstream of a *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 [33]. The resulting offspring are notated Tg[α MHC-Kir6.1AAA]. Male WT, Tg[CX1-eGFP-Kir6.1AAA], and Tg[α MHC-Kir6.1AAA] littermate mice, aged 8-12 weeks, were used for experiments. Protocols conform to the Guide for the Care and Use of Laboratory Animals and were approved by the University of Iowa Institutional Animal Care and Use Committee.

2.2 Isolated heart studies

Hearts were extracted from anesthetized mice and retrogradely perfused at 90 mmHg with Krebs-Henseleit buffer bubbled with 95% O₂/5% CO₂, at 37°C and pH 7.4 [1-3,12]. The AV node was mechanically dissociated and hearts paced at 130 ms cycle length (Bloom Electrophysiology, Fischer Imaging Corp., Denver, CO) using a platinum pacing catheter positioned in the right ventricle (NuMed; Hopkinton, NY). For hypoxia, a separate reservoir of Krebs-Henseleit buffer was bubbled with 95% N₂/5% CO₂, at 37°C and pH 7.4 [12]. A valve followed by a small bubble chamber and an oxygen sensor immediately above the heart was used to quickly switch the perfusing solutions. Oxygen partial tension was measured in the perfusate prior to heart passage (Model 210, Instech Laboratories, Plymouth Meeting, PA). Coronary flow was measured in series with the aortic cannula (T402, Transonic Systems, Ithica, 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 [1-3,12]. MAP recordings were analyzed (MATLAB, Mathworks, Natick, MA) for duration at 90% repolarization (APD₉₀). Changes in APD₉₀ (Δ APD₉₀) were calculated using the duration of the MAP just prior to the initiation of hypoxia as a reference. MAPs were only analyzed from tracings in which pacing capture was maintained without interruption. All calculations were manually reviewed.

2.3 Patch-clamp studies

Single ventricular cardiomyocytes were enzymatically isolated [25,26] and patch clamp studies performed as previously described [1].

2.4 Imaging

Bright field and fluorescence images were captured using an Olympus SZX12 stereoscope with 1x and 40X objectives and a fiberoptic white light or 100W mercury lamp. A GFP-specific filter was used for fluorescence images. An Olympus DP72 CCD camera and DP72 BSW software were used to acquire images. Fluorescence images were taken at the same light intensity, aperture setting and exposure time.

2.5 Statistical analysis

Results are expressed as mean $\pm$ SEM. Comparisons between two groups are made using the 2-sided Student's t-test and between multiple groups using analysis of variance (ANOVA) and appropriate *post hoc* tests. A *p* value <.05 is considered significant.

3. Results

3.1 Selective myocardial expression of the potassium impermeable Kir6.1AAA pore-forming mutant is tracked by elimination of green fluorescence

Tg[α MHC-Cre] mice are crossed with Tg[CX1-eGFP-Kir6.1AAA] mice to produce Tg[α MHC-Kir6.1AAA] with selective myocardial expression of Kir6.1AAA, tracked by elimination of eGFP expression (Fig. S1). Previous studies have confirmed myocardial-

specific expression of the Kir6.1AAA protein in Tg[α MHCKir6.1AAA] mice [8]. Here, WT hearts (Fig. S1C) show faint autofluorescence of the great vessels (40X views). In contrast, hearts from Tg[CX1-eGFP-Kir6.1AAA] mice show bright green fluorescence of both myocardium and the great vessels (Fig. S1A, inset and rightmost column). Hearts from Tg[α MHC-Kir6.1AAA] mice show green fluorescence in the great vessels (40X views), but elimination of green fluorescence in the myocardium (40X and inset), consistent with the myocardial-specific expression of α MHC (Fig. S1B).

3.2 Transgenic expression of Kir6.1AAA significantly reduces the K_{ATP} channel current density

Cardiomyocytes isolated from hearts of Tg[α MHC-Kir6.1AAA] and control mice were exposed to the mitochondrial uncoupler, 2,4-dinitrophenol (DNP), and the specific K_{ATP} channel opener, pinacidil. Expression of the Kir6.1AAA transgene is associated with a reduction in whole cell K_{ATP} channel current, normalized to cell size, of ~85% (WT: 18.1 ± 2.3 pA/pF, Tg[CX1-eGFP-Kir6.1AAA]: 17.4 ± 1.7 pA/pF, Tg[α MHC-Kir6.1AAA]: 2.5 ± 0.6 pA/pF, Fig. S2A and B, * $p < .05$). Similarly, the number of K_{ATP} channels/patch is reduced by ~80% in the presence of the Kir6.1AAA transgene, compared with littermate controls (WT: 4.7 ± 0.7 , Tg[CX1-eGFP-Kir6.1AAA]: 4.6 ± 0.5 , Tg[α MHC-Kir6.1AAA]: 0.9 ± 0.2 , Fig. S2C and E, $p < .05$), despite no differences in average pipette tip size (Fig. S2D, $p = NS$). These data are similar to our previously published findings [1].

3.3 Reduction in functional K_{ATP} channels results in the same magnitude, but a significantly slower time-course, of APD shortening in response to hypoxia

MAP duration at 90% repolarization (APD₉₀) was measured from a single, stable position on the left ventricular epicardium of isolated hearts from Tg[α MHC-Kir6.1AAA] and control mice, while pacing at a fixed cycle length. The change in APD₉₀ of each heart (Δ APD₉₀) compared to its own baseline duration is plotted as a function of time before and after a change in the heart perfusate to the hypoxic solution (Fig. 1A). In hearts from control mice, the Δ APD₉₀ follows a time-course in response to hypoxia characterized by an initial lengthening, followed by a continuous decrease until a new steady-state duration is achieved at about one minute. However, in hearts from Tg[α MHC-Kir6.1AAA] mice, the Δ APD₉₀ demonstrates a similar initial increase, but has a slower decreasing phase, although eventually achieving the same steady-state as in controls. As a result, the half-time of achieving the new steady state Δ APD₉₀ is significantly longer in hearts from Tg[α MHC-Kir6.1AAA] mice (WT: 45.5 ± 6.3 sec, Tg[CX1-eGFP-Kir6.1AAA]: 38.8 ± 3.6 sec, Tg[α MHC-Kir6.1AAA]: 60.9 ± 4.7 sec, Fig. 1C, * $p < .05$). The area under the curve (AUC), which subtracts the effects of positive and negative APD₉₀ deflections from baseline, is significantly smaller in hearts from Tg[α MHC-Kir6.1AAA] mice than controls (WT -1.48 ± 0.23 sec², Tg[CX1-eGFP-Kir6.1AAA] -1.62 ± 0.10 sec², Tg[α MHC-Kir6.1AAA] $-.87 \pm 0.23$ sec², Fig. 1D, * $p < .05$). Of note, there is no difference in coronary flow at baseline or during hypoxia in hearts from Tg[α MHC-Kir6.1AAA] mice compared with WT or Tg[CX1-eGFP-Kir6.1AAA] controls (2.95 ± 0.22 , 3.15 ± 0.79 , 3.2 ± 0.41 ml/min baseline and 2.55 ± 0.5 , 2.4 ± 0.6 , and 3.0 ± 0.8 ml/min during hypoxia, respectively, $p = NS$), consistent with limitation of dominant-negative Kir6.1AAA expression to the myocardium (Fig. S1), without effect on vascular K_{ATP} channels [32]. As an additional control, Δ APD₉₀ was also tracked in WT hearts perfused with the specific K_{ATP} channel blocker glyburide at a dose of $10 \mu M$, sufficient to completely block cardiac K_{ATP} channel current [34]. This also reveals an initial increase in Δ APD₉₀ in response to hypoxia, but is then followed by only a slight dip towards baseline, before continuing to lengthen until reaching a steady-state well above baseline (Fig. 1A). This is associated with a very positive AUC (1.32 ± 0.14 sec², Fig. 1D). Consistent with the effect of glyburide on increasing vasoconstriction through blockade of Kir6.1-containing vascular smooth muscle K_{ATP} channels, the coronary flow is reduced both at baseline and

during exposure to hypoxia (1.4 ± 1.2 ml/min baseline and 1.3 ± 0.9 ml/min during hypoxia, $n=5$, $p < .05$ compared to WT without glyburide treatment).

4. Discussion

K_{ATP} channels are membrane protein complexes vital for preservation of myocardial energy balance under normal and disease conditions [1-3,5,6,12,14,15]. Such a basic physiologic role in cardiac adaptation is consistent with the high expression of these channels in cardiomyocytes [2,3,5,6,14,15,21,24]. Here we use myocardial-specific expression of a dominant-negative K_{ATP} channel subunit to reduce the number of functional K_{ATP} channels on the membrane of cardiomyocytes, with a consequent reduction in the inducible K_{ATP} channel current. This reduction is associated with a slowed time-course of APD shortening in isolated hearts in response to the metabolic stress imposed by hypoxia. This study is in agreement with previous studies that indicate a deficit in functional K_{ATP} channels results in both a limitation of APD shortening and increased myocardial oxygen consumption under heart rate acceleration, proarrhythmia and vulnerability to injury [1-3,8-10,12-14,35,36].

As such, the AUC (Fig. 1D), which quantifies the difference between positive and negative areas of APD_{90} deflection from baseline, can be taken as a measure of the efficiency of energy conservation in response to metabolic challenge, with more negative values representing overall greater responsiveness of APD shortening and thus improved dynamics of energy economy [1]. In the current study, we see that, despite the same steady-state degree of APD shortening in response to hypoxia, there is a smaller AUC in K_{ATP} channel deficient hearts. This reduction in dynamic control of energy efficiency could be expected to result in increased myocardial vulnerability to injury and dysfunction, likely contributing to the reduced exercise tolerance and increased mortality in these mice [8]. Furthermore, the achievement of the same degree of steady-state APD shortening in hearts with normal and ~80-85% reduced expression of functional K_{ATP} channels provides direct experimental support for models predicting that opening a small percentage of normally expressed K_{ATP} channels is sufficient to dramatically reduce APD [5,20,24,30]. In addition, the present data provide important detail about the time-course of shortening, indicating that the level of membrane K_{ATP} channel expression may impact cardioprotection through the rapidity of APD response, and thus efficiency of cardiac energetic conservation, under severe metabolic challenge.

The complex time-course of APD changes in response to hypoxia as measured here using MAPs in intact murine hearts (Fig. 1A), is similar to data obtained using sharp intracellular electrodes in isolated guinea-pig cardiomyocytes exposed to the mitochondrial uncoupler DNP [37]. In that study, the APD also initially increased following metabolic inhibition, before a phase of dramatic shortening. The initial APD increase was attributed to inhibition of the electrogenic sodium pump caused by ATP depletion, while the subsequent dramatic APD decrease was attributed to the simultaneous pronounced increase in outward current with a reversal potential consistent with a predominant potassium ion component. A similar phenomenon was more recently reported using the patch clamp technique, also in isolated guinea-pig ventricular myocytes in response to DNP [38], and in a study recording MAPs obtained through a suction electrode on the apex of intact ferret hearts exposed to cyanide [39]. The similarities in time-course of APD changes between these studies support that the phenomenon is unlikely to be a technical artifact, or to be species specific, although the magnitude of the effect would be difficult to compare between studies and could have a technical or species-related component. The effect may be more pronounced in the current study due to our beat-to-beat recording and higher metabolic rate (37°C and paced at 130ms C.L vs. cooler temperatures and/or slower pacing rates in the other studies). The differences demonstrated here in the time-course of APD adjustment in Tg[$\alpha\text{MHC-Kir6.1AAA}$] hearts

cannot be attributed to mitochondrial K_{ATP} channels as no expression of the Kir6.1AAA transgene has been found in the mitochondria [8].

Given the persistence of GFP fluorescence in the great vessels of Tg[α MHC-Kir6.1AAA] hearts (Fig. S1), and the preserved coronary flow, we also rule out disruption of K_{ATP} channel function in the vasculature as a cause for the altered APD response to hypoxia in Tg[α MHCKir6.1AAA] mice. In contrast, glyburide treated WT hearts, in which both myocardial and vascular K_{ATP} channels would be blocked [34], exhibit both reduced coronary flow and loss of APD shortening (Fig. 1A, B). Interestingly, not only does perfusion with glyburide result in an absence of APD shortening with hypoxia, but it is also associated with a slow increase in APD over time. This results in a markedly positive AUC, and would be expected to represent an energetically very unfavorable outcome. Indeed, we previously showed that oxygen consumption with heart rate acceleration is significantly increased in hearts perfused with glyburide, coincident with loss of APD shortening [1]. Those data, in addition to the data reported here with hypoxia, as well as numerous other studies [34], indicate a negative effect of glyburide on cardioprotective K_{ATP} channel current. This would suggest that this drug, used extensively for the treatment of diabetes due to its blockade of pancreatic β -cell K_{ATP} channel isoforms and related increase in insulin secretion, would have a detrimental and potentially dangerous effect on cardiac stress resistance. However, clinical studies have shown inconsistent results in this regard [40], likely due to the finding that the clinically desirable effect of glyburide inhibition of pancreatic K_{ATP} channel isoforms is irreversible, while the potentially harmful inhibition of cardiac channels is only transient [34].

The finding that cardiac K_{ATP} channel expression has an impact on the APD rate of response to metabolic challenge lends insight into heart diseases manifested by reduced K_{ATP} channel expression, such as some forms or stages of heart failure [11,12,41]. While reduced K_{ATP} channel expression may not impact the maximum K_{ATP} channel-dependent APD change, loss of prompt APD responsiveness may exacerbate metabolic deficits in the already energetically challenged myocardium. It is possible that disease states such as heart failure may also result in heterogeneous loss of K_{ATP} expression such that parts of the myocardium respond more slowly to changes in heart rate or oxygen delivery resulting in a time-dependent dispersion of refractoriness contributing to an increased propensity to arrhythmias [42-44]. Thus, understanding mechanisms and effects of K_{ATP} channel expression regulation may provide novel strategies for combating the energetic and electrical derangements that contribute to progression of myocardial dysfunction.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- Sarcolemmal K_{ATP} channels are essential for cardiac resistance to stress
- K_{ATP} channel activation shortens action potential duration (APD), conserving energy
- Reduction in cardiac K_{ATP} channels does not alter magnitude of APD shortening
- Cardiac K_{ATP} channel number defines rate of APD shortening under hypoxia
- Sarcolemmal K_{ATP} channel expression defines cardiac energetic efficiency

Figure 1. Reduction in functional K_{ATP} channels translates into slowed APD shortening in response to hypoxia

A. Summary plots of beat-to-beat MAP duration shortening (ΔAPD_{90}) following initiation of hypoxia in isolated, perfused hearts from WT ($n=4$), Tg[CX1-eGFPKir6.1AAA] ($n=3$), and Tg[α MHC- α MHC-Kir6.1AAA] vs. WT and Tg[CX1-eGFP-Kir6.1AAA] controls). Also graphed is the response in glyburide ($10\mu M$) treated hearts from WT mice ($n=5$, $*p<.05$ vs. WT hearts not treated with glyburide). Symbols with error bars mark the mean $\pm$ S.E for each 40th APD. **B.** Representative examples of MAPs recorded from WT, Tg[CX1-eGFP-Kir6.1AAA], Tg[α MHC-Kir6.1AAA] and glyburide treated WT hearts paced at 130 ms cycle length. MAPs at baseline and after 30 and 60 seconds of hypoxia are superimposed for comparison. Horizontal dotted lines indicate 90% repolarization. **C.** Summary statistics showing the half time ($t_{1/2}$) of the ΔAPD_{90} response ($*p<.05$ for Tg[α MHCKir6.1AAA] vs. combined WT and Tg[CX1-eGFP-Kir6.1AAA] controls). **D.** Summary statistics showing the area under the curve (AUC) of the ΔAPD_{90} time course ($*p<.05$ for Tg[α MHCKir6.1AAA] and glyburide-treated WT vs. combined WT and Tg[CX1-eGFP-Kir6.1AAA] controls).