An Open Sarcolemmal Adenosine Triphosphate-Sensitive Potassium Channel Is Necessary for Detrimental Myocyte Swelling Secondary to Stress

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Background—Stress (exposure to hyperkalemic cardioplegia, metabolic inhibition, or osmotic) results in significant myocyte swelling and reduced contractility. In contrast to wild-type mice, these detrimental consequences are not observed in mice lacking the Kir6.2 subunit of the sarcolemmal ATP-sensitive potassium (sKATP) channel after exposure to hyperkalemic cardioplegia. The hypothesis for this study was that an open sKATP channel (Kir6.2 and SUR2A subunits) is necessary for detrimental myocyte swelling to occur in response to stress.

Methods and Results—To investigate the role of the sKATP channel in stress-induced myocyte swelling, high-dose pharmacological sKATP channel blockade and genetic deletion (knockout of Kir6.2 subunit) were used. Myocytes were exposed sequentially to Tyrode control (20 minutes), test (stress) solution (20 minutes), and Tyrode control (20 minutes). To evaluate pharmacological channel blockade, myocytes were exposed to hyperkalemic cardioplegia (stress) with and without a KATP channel blocker. To evaluate the effects of genetic deletion, wild-type and sKATP knockout (Kir6.2(−/−)) myocytes were exposed to metabolic inhibition (stress). Myocyte volume was recorded using image-grabbing software. Detrimental myocyte swelling was prevented by high-dose sKATP channel blockade (glibenclamide or HMR 1098) but not mitochondrial KATP channel blockade (5-hydroxydecanoate) during exposure to hyperkalemic cardioplegia. Genetic deletion of the sKATP channel prevented significant myocyte swelling in response to metabolic inhibition.

Conclusions—KATP channel openers prevent detrimental myocyte swelling and reduce contractility in response to stress through an unknown mechanism. Paradoxically, the present data support a role for sKATP channel activation in myocyte volume derangement in response to stress. (Circulation. 2011;124[suppl 1]:S70–S74.)

Keyword(s): muscle cells · surgery · heart arrest induced · potassium

M yocytes exposed to 3 different stresses (hypothermic hyperkalemic cardioplegia, hyposmotic stress, or metabolic inhibition) exhibit significant swelling and associated reduced contractility.1–3 This response has been documented in 3 species (rabbit, mouse, and human) and may reflect one mechanism of myocardial stunning.3 These detrimental consequences are eliminated by the addition of an ATP-sensitive potassium(KATP) channel opener, diazoxide, in all 3 species in response to all 3 stresses,1–3 leading us to hypothesize that diazoxide, or the KATP channel, may play a role in myocyte volume homeostasis. This beneficial mechanism of action of diazoxide has been localized to a nonsarcolemmal KATP channel location using whole-cell voltage clamping and genetic deletion of subunits of the sarcolemmal KATP (sKATP) channel.4

Interestingly, our laboratory observed that significant myocyte swelling was not observed in mice lacking the Kir6.2 subunit of the sKATP channel compared to wild-type (WT) mice after exposure to cardioplegic stress.5 It is well-known that sKATP channels contain Kir6.2 subunits, that KATP channels couple the cell’s energetic state with its activity,6 and that KATP channel openers mimic ischemic preconditioning and are cardioprotective in multiple animal models.7–11 Their role in cellular volume homeostasis is undefined. Paradoxically, an open sKATP channel may be necessary for myocyte swelling and reduced contractility to occur secondary to stress. The hypothesis for the present study was that the sKATP channel is necessary for the detrimental myocyte swelling to occur in response to stress. Specifically, myocytes with a closed or absent sKATP channel (through high-dose pharmacological blockade or genetic deletion of the Kir6.2 subunit, respectively) will not exhibit significant swelling during exposure to stress.

Methods

Isolation of Mouse Ventricular Myocytes

All animal procedures were approved by the Animal Studies Committee at Washington University School of Medicine, and all animals
received humane care in compliance with the Guide to Care and Use of Laboratory Animals. Ventricular myocytes were isolated from mice (either sex, aged 6 weeks to 5 months, 25 to 30 g body weight) as previously described. Rapid cardiectomy was performed in the anesthetized (2.2.2 tribromoethanol [Avertin] 2.5% IP) mouse (WT or Kir6.2 knockout [KO]), the aorta was cannulated using a 28-gauge needle and attached to a Langendorff apparatus, and the aorta was perfused with various solutions for extracellular tissue digestion and cellular isolation (solution A for 5 minutes followed by solution B for 12 minutes.). The left ventricle then was removed and transferred to solution C where it was gently dispersed by glass pipette. The isolated myocytes were allowed to centrifuge by gravity, and serial washings were performed every 10 minutes for 30 minutes. Cells were used in experiments within 5 hours. Yield of viable myocytes was 65% to 75% per mouse. A maximum of 2 myocytes were used per mouse.

Solution A consisted of NaCl, 116 mmol/L; KCl, 5.36 mmol/L; Na,HPO₄, 0.97 mmol/L; KH,PO₄, 1.47 mmol/L; HEPES, 21.10 mmol/L; glucose, 11.65 mmol/L; phenol red (Sigma Chemical Co; St Louis, MO), 26.50 µg/mL; MgCl₂, 3.72 mmol/L; NaHCO₃, 4.40 mmol/L; essential vitamins (100X, 10 mL; Gibco; Grand Island, NY); and amino acids (50X, 20 mL; Gibco). Solution B consisted of solution A plus CaCl₂, 10 µmol/L and collagenase 1.2 mg/mL (type 2; Worthington Biochemical Corporation; Freehold, NJ). Solution C consisted of solution A plus bovine serum albumin (Sigma), 5 mg/mL; taurine, 1.25 mg/mL; and CaCl₂, 150 µmol/L.

**Myocyte Volume Imaging**

Cells were assessed for viability using the following criteria: normal rod shape, smooth edges, sharp borders, and clear striations; absence of vacuoles or blebbing; and lack of spontaneous beating. An aliquot of isolated myocytes was placed on a slide on a glass-bottom chamber on an inverted microscope scope (Leitz; Wetzlar, Germany). After a 5-minute stabilization period, the chamber was perfused at a rate of 3 mL/min with 37°C Tyrode solution (NaCl, 130 mmol/L; KCl, 5 mmol/L; CaCl₂, 2.5 mmol/L; MgSO₄, 1.2 mmol/L; NaHCO₃, 24 mmol/L; Na,HPO₄, 1.75 mmol/L; glucose, 10 mmol/L) buffered to pH 7.4 using 5% O₂ and 5% CO₂. Chamber temperature was controlled by a water bath system (Thermo Haake; Karlsruhe, Germany). Cell images were displayed on a video monitor using a charge-coupled device camera (KPM1U: Hitachi Denshi; Tokyo, Japan). Digital images of cells were captured using a video frame grabber (Scion Corporation; Frederick, MD) and were traced using Scion Image software. Length, width, and area were recorded. To calculate cell volume, it was assumed that changes in cell width and thickness were proportional. Relative cell volume change was determined as previously described with the following formula:

\[
\text{volume/area} = \frac{\text{area} \times \text{width}}{\text{area} \times \text{width}}
\]

For channel blockade experiments, mouse myocytes were exposed to control Tyrode or test solution (stress of hypothermic hyperkalemic cardioplegia). Test solution consisted of Plegisol hyperkalemic cardioplegic solution (Abbott Laboratories; Chicago, IL), which contains NaCl, 110 mmol/L; KCl, 16 mmol/L; MgCl₂, 16 mmol/L; and CaCl₂, 1.2 mmol/L, equilibrated with 95% O₂ and 5% CO₂, and titrated to pH 7.4 with 10% NaHCO₃ solution alone or with a pharmacological channel inhibitor (HMR 1098, 10 and 20 µmol/L) to cardioplegia did not alter the observed significant myocyte swelling secondary to cardioplegia alone (Figures 1 and 2). The addition of high-dose HMR 1098 (40 µmol/L) or glibenclamide (40 µmol/L) to cardioplegia prevented the significant myocyte swelling observed with cardioplegia alone (Figure 2).

**Discussion**

Myocyte volume derangement has been demonstrated to be inversely proportional to contractility, supporting a potential mechanism of myocardial stunning. The administration of pharmacological Kₐtp channel openers has been documented to maintain myocyte volume homeostasis and contractility in response to stress, suggesting a role of the Kₐtp channel in Kir6.2 transcript has been previously confirmed by reverse-transcription polymerase chain reaction and whole-cell membrane currents in the KO mice.
volume regulation and a potential for the use of channel openers in the treatment or prevention of myocardial stunning.\textsuperscript{1–3}

The sK\textsubscript{ATP} channel was first identified by Noma\textsuperscript{15} in the sarcolemma of cardiac myocytes. The sK\textsubscript{ATP} channel is inhibited by intracellular ATP and activated by ADP, indicating a unique link to cellular metabolism, and with the highest density of all sarcolemmal channels in the heart (\approx 2000 to 3000 per cell), this channel shortens the action potential during ischemia and protects the heart from calcium overload.\textsuperscript{16} In addition, a mitochondrial K\textsubscript{ATP} channel is proposed, but the existence of a mitochondrial channel and the underlying protein subunits remains controversial.\textsuperscript{17,18}

Administration of K\textsubscript{ATP} channel openers has been demonstrated to limit ischemic injury, preserve myocardial function, mimic ischemic preconditioning, and alleviate the effect of myocardial stunning in multiple animal models.\textsuperscript{7–11} Various hypotheses (regarding the proposed mechanism of cardioprotection provided by K\textsubscript{ATP} channel openers) implicate both the sarcolemmal and purported mitochondrial K\textsubscript{ATP} channels as well as K\textsubscript{ATP} channel-independent effects of the pharmacological openers themselves.
Opening the sKATP channel allows K⁺ efflux from the cell through sarcolemmal channels, which hyperpolarizes the cell membrane, shortens the action potential duration, and permits arrest at a more physiological (negative) membrane potential. This shortening can prevent Ca²⁺ influx during the plateau phase of the action potential, decreases contractility, and thereby conserves ATP. Opening of the sKATP channel also may be responsible for the phenomenon of ischemic preconditioning because the use of pharmacological channel openers provides similar benefit to traditional ischemic preconditioning, and these benefits have been inhibited by pharmacological channel blockers. Genetic deletion or alteration of the Kir6.2 subunit of the sKATP channel results in both a loss of the ischemic preconditioning benefit and a loss of adaptation to stress. In addition, a reduction in functional sKATP Channel number leads to a lack of action potential shortening during metabolic inhibition, a reduction in tolerance to metabolic stress, and increased Ca²⁺ loading during metabolic inhibition.

However, it has also been suggested that the cardioprotection provided by KATP channel openers may be attributed to the opening of the purported mitochondrial KATP channel rather than to the sKATP channel. In this case, opening the mitochondrial KATP channel is proposed to increase mitochondrial matrix volume, activate the respiratory chain and provide more available ATP, preserve the segregation of adenine nucleotides that normally exists between the mitochondrial and cytosolic compartments, maintain the mitochondrial inner membrane potential by allowing K⁺ influx (into the matrix) and H⁺ efflux, or maintain mitochondrial structural integrity.

In the present study, significant myocyte swelling in mouse myocytes after exposure to hyperkalemic cardioplegia is consistent with previous work. Previously, this swelling was prevented by the KATP channel opener diazoxide with or without the presence of high-dose sKATP or mitochondrial KATP channel blockade, and this cardioprotective effect was demonstrated to depend on the SUR1 subunit of a KATP channel but independent of the sKATP channel. In the present study, high-dose sKATP blockade (with glibenclamide or HMR 1098) alone prevented the detrimental myocyte swelling secondary to stress (without the presence of a channel opener). High-dose mitochondrial KATP channel blockade (with 5-HD) was not associated with a similar prevention of edema, implicating the sarcolemmal location for this separate phenomenon. Similar observations were not seen in the myocytes exposed to stress and low-dose sKATP channel blockade because of the high density of sKATP channels on the myocyte.

Similarly, WT mouse myocytes exhibited significant swelling in response to exposure to metabolic inhibition, which is consistent with previous findings in rabbit myocytes. Similar to previous findings after exposure to hyperkalemic stress, myocytes from mice lacking the Kir6.2 subunit of the sKATP channel did not exhibit significant myocyte swelling after exposure to metabolic inhibition. These data suggest that the sKATP channel must be present for this detrimental myocyte swelling to occur and that it plays some role in volume derangement in response to stress.

Previous work using whole-cell voltage clamp demonstrated that K⁺ current measured directly at the sKATP channel is not elicited by the stress of hyperkalemic cardioplegia, but this does not exclude the possibility of another ion flux through the sKATP channel during stress or a role for this channel in volume regulation. The sKATP channel might serve as a nonspecific pore for other ions (such as Na⁺), leading to swelling during stress. The role of the sKATP channel in myocyte volume regulation thus requires further investigation.

Figure 3. A, WT myocytes demonstrate significant swelling during exposure to MI. Cells were exposed to normal Tyrode solution for baseline volume measurement (20 minutes) followed by test solution (20 minutes) and reexposure to Tyrode solution (20 minutes). WT myocyte volume remained unchanged when exposed to NT. WT myocyte volume significantly increased when exposed to MI. B, Kir6.2 knockout myocytes do not swell during exposure to MI. Cells were exposed to normal Tyrode solution for baseline volume measurement (20 minutes) followed by test solution (20 minutes) and by reexposure to Tyrode solution (20 minutes). Kir6.2 knockout myocyte volume remained unchanged when exposed to NT. Kir6.2 knockout myocyte volume also remained unchanged when exposed to MI. MI indicates metabolic inhibition; NT, control Tyrode solution; WT, wild type.
Paradoxically, these data taken together support the idea that an open or present sKATP channel is required for detrimental myocyte swelling secondary to stress. Thus, the detrimental mechanism of swelling is likely a separate phenomenon (from that of the prevention of swelling) because the 2 phenomena occur in different cellular locations (detrimental swelling depends on an open or present sKATP channel, whereas the beneficial prevention of swelling by the KATP channel opener diazoxide depends on an action independent of the sKATP channel). Thus, the prevention of detrimental myocyte swelling secondary to stress may be accomplished by diazoxide (through an action dependent on SUR1) or by the elimination of (genetic or high-dose pharmacological blockade) the sKATP channel.

A maximum of 2 viable myocytes per each animal were used for the described experiments. Myocytes were randomized to treatment group, but data from the same animal may be correlated (ie, may not be independent observations). The use of a repeated-measures ANOVA statistical analysis, therefore, may result in bias, and this is an inherent limitation of the present study.

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Disclosures

None.

References


