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Increased tolerance to stress in cardiac expressed gain-of-function of adenosine triphosphate–sensitive potassium channel subunit Kir6.1

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Abstract

Background: The adenosine triphosphate–sensitive potassium (K_{ATP}) channel opener diazoxide (DZX) prevents myocyte volume derangement and reduced contractility secondary to stress. K_{ATP} channels are composed of pore-forming (Kir6.1 or Kir6.2) and regulatory (sulfonylurea receptor, SUR1 or SUR2) subunits. Gain of function (GOF) of Kir6.1 subunits has been implicated in cardiac pathology in Cantu syndrome in humans (cardiomegaly, lymphedema, and pericardial effusions). We hypothesized that GOF of Kir6.1 subunits would result in altered myocyte response to stress.

Materials and methods: Isolated cardiac myocytes from wild type (WT) and transgenic Kir6.1GOF mice were exposed to Tyrode's physiologic solution for 20 min, test solution (Tyrode's or stress [hyperkalemic cardioplegia {CPG, known myocyte stress}] +/- K_{ATP} channel opener DZX), followed by Tyrode's for 20 min. Myocyte volume and contractility were measured and compared.

Results: WT myocytes demonstrated significant swelling in response to stress, but significantly less swelling was seen in Kir6.1GOF myocytes. DZX prevented swelling secondary to CPG in WT but resulted in a nonsignificant reduction in swelling in Kir6.1GOF myocytes. Both WT and Kir6.1GOF myocytes demonstrated a reduction in contractility during stress, although this was only significant in Kir6.1GOF myocytes. DZX was not associated with an improvement in contractility in Kir6.1GOF myocytes following stress.

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The authors reported no proprietary or commercial interest in any product mentioned or concept discussed in this article.

Conclusions: Similar to previous results in Kir6.1(−/−) myocytes, Kir6.1GOF myocytes demonstrate resistance (less volume derangement) to stress of cardioplegia. Understanding the role of Kir6.1 in myocyte response to stress may aid in the treatment of patients with Cantu syndrome and warrants further investigation.

Keywords

Potassium channel; Myocardial stress; Diazoxide; Cantu syndrome

Introduction

Adenosine triphosphate-sensitive potassium (K_{ATP}) channels are found in metabolically active tissues throughout the body and couple metabolic dynamics with transmembrane electrical activity.¹ K_{ATP} channels provide endogenous myocardial protection via coupling of cell membrane potential to myocardial metabolism.² It is well established that the pharmacologic openers of K_{ATP} channels mimic ischemic preconditioning in multiple animal models.^{3–13}

An isolated myocyte model of myocardial stunning has documented that myocytes demonstrate significant swelling and associated reduction of contractility when exposed to three different stresses: hypothermic hyperkalemic cardioplegia (CPG), hypoosmotic stress, or metabolic inhibition.^{3,4,14} These detrimental consequences may be prevented by the addition of K_{ATP} channel opener diazoxide (DZX) in rabbit, mouse, and human myocytes.^{14–16}

Canonical K_{ATP} channels are heterooctamers composed of 4 pore-forming subunits (potassium inward rectifier [Kir]6.2 or Kir6.1) and four of the ATP-binding cassette family of membrane proteins (sulfonylurea receptor, SUR1 or SUR2).¹⁷ The sulfonylurea receptor subunit represents the site for blockade by sulfonylureas and stimulation by potassium channel openers and ADP. The Kir6.x subunit is the location for inhibition by ATP.^{17,18} The genes encoding these subunits have been cloned, which allows for genetic manipulation and specific evaluation of the exact mechanism of DZX. Using genetic deletion, we have demonstrated that cardioprotection afforded by DZX requires the regulatory K_{ATP} subunit SUR1 but does not appear to result from direct activation of sarcolemmal K_{ATP} channels (which are predominately composed of Kir6.2 and SUR2A).¹⁹ We have also noted that genetic deletion of either the Kir6.1 or Kir6.2 subunit confers an increased tolerance to stress via an unknown mechanism.^{5,13}

Mutations in the K_{ATP} channel Kir6.1 or SUR2 subunits (both located on human chromosome 12p) in humans cause Cantu syndrome, which is characterized by hypertrichosis, coarse facial features, persistent ductus arteriosus, skeletal abnormalities, and lymphedema and a host of cardiac complications, including cardiomegaly, hypercontractility, and pericardial effusions.^{20,21} In smooth muscle-expressing transgenic mice, the phenotype of Kir6.1GOF (gain of function) was characterized by smooth muscle relaxation and vasodilation and is consistent with some of the human characteristics of Cantu syndrome (lymphedema, patent ductus arteriosus, and pericardial effusions).²² In cardiac muscle-expressing transgenic mice, the cardiac phenotype is complex, but includes

hypercontractility due to enhanced L-type Ca^{2+} channel activity, but again this reiterates findings of Cantu syndrome.²³ The consequences of myocardial Kir6.1GOF for myocardial responses to stress have not been studied in animals or humans. The aim of this investigation was to determine the response to stress and to K_{atp} channel opener DZX in Kir6.1GOF myocytes. The results of such investigations will guide future therapies in patients with Cantu syndrome and other clinically significant K_{ATP} channelopathies.

Material and methods

All animal procedures were approved by the Washington University Animal Care and Use Committee, and all animals received humane care in compliance with the “Guide to Care and Use of Laboratory Animals” prepared by the Institutes for Laboratory Animal Research.²⁴

Cardiac-specific Kir6.1GOF mice were generated as previously described²¹ and confirmed by PCR on mouse-tail DNA using green fluorescent protein-specific oligonucleotide primers.²²

Mouse myocyte isolation

Ventricular myocytes were isolated from wild type (WT) and Kir6.1GOF mice (age 6 wk-5 mo and 15–30 g weight) as previously described.¹³ Mice were anesthetized by intraperitoneal injection with 2.5% Avertin mixed with Heparin (0.1 mL). After adequate anesthesia was confirmed, rapid cardiectomy was performed and the aorta was cannulated. Solution A (as defined in the following section) was perfused through the aorta for 5 min followed by solution B (as defined in the following section) for 12–20 min at 37°C. Atria were removed and ventricular tissue was minced in solution C (as defined in the following section) and then gently dispersed by glass pipette. Cells were allowed to centrifuge by gravity, and serial washings were performed every 10 min for 1520 min. A typical yield of viable myocytes was 65% to 75%. Viable cells were used within 5 h after isolation and kept in solution C at room temperature before the experimental protocol.

Solution A consisted of (in mmol/L, except as noted) 116 NaCl, 5.36 KCl, 0.97 Na_2HPO_4 , 1.47 KH_2PO_4 , 21.10 HEPES (N-[2-hydroxyethyl] piperazine-N'-[4-butanesulfonic acid]), 11.65 glucose, 26.50 $\mu\text{mol/L}$ phenol red (Sigma, St. Louis, MO), 3.72 MgCl_2 , 4.40 NaHCO_3 , essential vitamins (100 $\times$, 10 mL; GIBCO, Grand Island, NY), and amino acids (50 $\times$, 20 mL, GIBCO; Grand Island, NY). Solution B consisted of solution A plus 10 mmol/L CaCl_2 and 1.2 mg/mL collagenase (Type 2; Worthington Biochemical Corporation; Freehold, NJ). Solution C consisted of solution A plus 5 mg/mL bovine serum albumin (Sigma, St. Louis, MO), 1.25 mg/mL taurine, and 150 $\mu\text{mol/L}$ CaCl_2 .

Myocyte volume measurement

Myocytes were visualized on an inverted microscope stage (IX-51, Olympus, Japan) as previously described.¹⁴ After 5 min, the chamber was perfused at a rate of 3 mL/min with Tyrode's physiologic solution (TYR; in mmol/L): NaCl 130, KCl 5, CaCl_2 2.5, MgSO_4 1.2, NaHCO_3 24, Na_2HPO_4 1.75, and glucose 10 (buffered to a pH of 7.4 using 95% O_2 to 5%

CO₂). After viability was confirmed, myocyte images were captured using video-based edge detection software (IonOptix, Milton, MA).

Both WT and Kir6.1GOF myocytes were exposed to 37°C TYR for 20 min to obtain baseline volume. Any changes in cell volume secondary to the isolation would be evident during this period. Myocytes were then exposed to test solution (20 min) including: TYR 37° C, hypothermic hyperkalemic cardioplegia (CPG) at 9° C, or cardioplegia + DZX (CPG + DZX), followed by TYR 37°C for 20 min. Volume was measured every 5 min as previously described.¹⁴ Fourteen to 20 myocytes were observed for each group.

CPG consisted of (in mmol/L) NaCl 110, NaHCO₃ 10, KCl 16, MgCl₂16, and CaCl₂1.2 and was equilibrated with 95% O₂ to 5% CO₂ and titrated to the pH of 7.3 with 10% NaHCO₃ solution.

DZX (7-chloro-3-methyl-1,2,4-benzothiadiazine-1,1-dioxide [DZX]; Sigma, St. Louis, MO) dose of 100 mmol/L was used as it was effective in ameliorating cell swelling secondary to stress in previous studies.¹⁹ A stock solution of DZX was made by dissolving DZX in 0.1% dimethyl sulfoxide, at which concentration dimethyl sulfoxide has no effect on cell volume.

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Myocyte contractility

Myocyte contractility was measured using a video-based edge detection system (IonOptix, Milton, MA) as previously described.¹⁴ Cells were paced using a field stimulator (Myo-Pacer; IonOptix, Milton, MA) at a voltage of 10% above threshold at a frequency of 1 Hz with a 5-ms duration to avoid the occurrence of fusion beats. After myocytes underwent 5 min of stimulation, data were obtained from 12–30 consecutive beats and averaged. Contractility was measured at baseline and after 10 and 20 min of reexposure to TYR. Parameters of contractility including peak velocity of shortening, and peak velocity of relengthening (PR) were analyzed as previously described.¹⁴ Cells that showed less than 7% cell shortening at baseline were excluded.

Statistical analysis

All data were presented as mean ± standard error of the mean and normalized to baseline. A Shapiro–Wilk test was used to test for normality. Cell volume was normally distributed so a repeated-measures analysis of variance was used for sequential time-based measurements for each test solution against its own baseline and control values. Multiple comparisons between groups were done using contrasts with Bonferroni correction. Contractility data were normally distributed, and analysis of variance was done at 50 and 60 min time points. Multiple post hoc comparisons were made using Fishers' least significant difference test. All reported probability values are two sided and adjusted. Probability values < 0.05 were considered significant. Statistical analysis was performed using Systat software, version 13 (Systat Software Inc, San Jose, CA).

Results

Myocyte volume during stress of cardioplegia

There were no significant differences in myocyte volume between groups (WT or Kir6.1GOF) during baseline exposure to physiologic Tyrode's solution (Fig. 1). When exposed to CPG (stress) at 20 min, both WT and Kir6.1GOF myocytes swelled significantly ($P < 0.05$ versus control for both). However, the Kir6.1GOF myocytes demonstrated significantly less swelling compared with WT myocytes ($P < 0.05$ WT CPG versus Kir6.1GOF CPG). Similar to previous results, DZX significantly reduced swelling secondary to CPG in WT myocytes ($P = 0.000$ – 0.006 WT CPG versus WT CPG + DZX). In contrast, Kir6.1GOF myocytes exposed to CPG demonstrated a nonsignificant reduction in swelling with the addition of DZX ($P = 0.335$ – 0.860 Kir 6.1GOF CPG versus Kir6.1GOF CPG + DZX).

Myocyte contractility during stress of cardioplegia

Myocyte contractility was similar in Kir6.1GOF myocytes versus WT at both 50 and 60 min (Figs. 2 and 3). Both WT and Kir6.1GOF myocytes demonstrated a reduction in contractility following stress (CPG) in all measures of contractility (PS, S, PR); however, this was only statistically significant after 50 min and only in PR in Kir6.1GOF myocytes ($P = 0.036$ Kir6.1GOF TYR versus Kir6.1GOF CPG).

There was no statistically significant difference in contractility between CPG and CPG + DZX groups in Kir6.1GOF myocytes after stress.

Discussion

The K_{ATP} channel opener DZX consistently maintains volume homeostasis and is typically found to maintain contractility in response to multiple stresses in animal and human myocytes, but the specific cardioprotective mechanism of DZX remains elusive.^{14–16} Pharmacologic and genetic manipulation of implicated K_{ATP} channel subunits in DZX cardioprotection have demonstrated the involvement of the K_{ATP} channel subunit SUR1 in protection but have not implicated Kir6.1 or Kir6.2 subunits.^{5,13,19} In contrast, deletion of Kir6.1 or Kir6.2 appears to confer an increased tolerance to stress (reduced myocyte swelling in response to stress of CPG, 7% and 3.2%, respectively, compared with WT).^{5,13,16} The present study indicates that Kir6.1GOF myocytes swell in response to the stress of CPG, but they also demonstrate an increased tolerance to stress (reduced myocyte swelling, 6%, in response to stress of CPG compared with WT). The finding that both GOF and loss-of-function (LOF) of this subunit should confer increased stress tolerance is initially counter-intuitive. However, although there is no obvious cardiac compensation for Kir6.1 knockout,²⁶ Kir6.1GOF,²³ or Kir6.2GOF,²⁷ both result in complex secondary cardiac effects including significant enhancement of L-type Ca^{2+} channel activity such that naive expectations of opposing effects of Kir6.1 LOF and GOF on contractility or stress response are unlikely to be met. Although Kir6.2 has been implicated in myocyte volume regulation (as a component of sarcolemmal K_{ATP} channels), further investigation is necessary to

determine whether any compensatory mechanisms play a role in the increased tolerance to stress in both LOF and GOF animals.¹⁶

The predominant K_{ATP} channel pore-forming subunit in the heart is Kir6.2, however, Kir6.1 subunit has also been found in cardiac tissue.^{21,28,29} The K_{ATP} channel subunit Kir6.1 generates channels in vascular smooth muscle (VSM) cells and endothelial cells, and channel activation leads to vaso-dilation.^{22,30} Although the current experiments were conducted in cardiac-specific Kir6.1GOF mice, VSM-specific transgenic Kir6.1GOF mice demonstrate lower blood pressure,²² associated reduced VSM cell contractility, reduced sensitivity to ATP, and enhanced K⁺ conductance in response to K_{ATP} channel openers.²²

Genetic mutations of K_{ATP} channel subunits are associated with documented human channelopathies, many of which are treated with the pharmacologic administration of K_{ATP} channel openers or inhibitors.²¹ Cantu syndrome in humans can result from mutations in Kir6.1 (encoded by KCNJ8) or SUR2 (encoded by ABCC9), resulting in GOF in K_{ATP} channels composed of these subunits.^{11,21} Cantu syndrome is a rare autosomal dominant disorder characterized by hypertrichosis, osteochondrodysplasia, cardiomegaly, craniosynostosis, facial dysmorphism, cardiomegaly, left ventricular hypertrophy, patent ductus arteriosus, pericardial effusion, and lower extremity edema.^{21,31} Many of the cardiovascular features are likely to arise from enhanced vascular K_{ATP} channel activity, but myocardial features, including cardiomegaly, are not trivially explained. No specific treatment options for these patients are currently available.²⁰ The findings of enhanced stress tolerance observed in the present study may ultimately be related to these features and may help to develop appropriate therapies.

Conclusions

In a mouse model of the human channelopathy of Cantu Syndrome, Kir6.1GOF myocytes demonstrate resistance to stress and lack of responsiveness to the protective effects of the K_{ATP} channel opener DZX. Further study into the mechanism of resistance is warranted to help characterize response to stress in Cantu Syndrome patients and to help inform new therapeutic options for these patients.

Limitations

Isolated myocytes were used because they allow for repeated observations and the independent evaluation of one stress at a time. This model is not intended to mimic the clinical situation of ischemia and reperfusion. Caution should therefore be taken before any extrapolation to the clinical setting.

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Fig. 1 –.

Isolated myocyte volume. Kir6.1GOF myocytes demonstrate significant swelling (*versus* control) secondary to stress (CPG), although this swelling is significantly less than that observed in WT myocytes secondary to stress. Isolated myocytes (WT or Kir6.1GOF) were exposed to control TYR 37°C for 20 min (time 5 to 20 min), stress (hypothermic hyperkalemic cardioplegia [CPG] at 9°C, or CPG+ DZX [CPG + DZX]) for 20 min (time 20 to 40 min), followed by TYR 37°C for 20 min (time 40 to 60 min). Myocyte volume was measured every 5 min and normalized to baseline. Data are represented as mean $\pm$ SEM. * P < 0.05 *versus* WT TYR, + P < 0.05 *versus* Kir6.1GOF TYR, Ψ p < 0.05 *versus* WT CPG. Kir = potassium inward rectifier.

Fig. 2 –.

Isolated myocyte contractility after stress at time 50 min. Kir6.1GOF myocytes demonstrate reduced contractility after stress. Isolated myocytes (WT or Kir6.1GOF) were exposed to control TYR 37°C for 20 min (time 5 to 20 min), stress (hypothermic hyperkalemic cardioplegia [CPG] at 9°C or CPG + DZX [CPG + DZX]) for 20 min (time 20 to 40 min), followed by TYR 37°C for 20 min (time 40 to 60 min). Myocyte contractility was measured at baseline and at time 50 min (after stress and 10 min of reexposure to TYR) and normalized to baseline. Data are represented as mean $\pm$ SEM. * $P < 0.05$ versus Kir6.1GOF TYR, $\Psi p < 0.05$ versus WT CPG + DZX. Kir = potassium inward rectifier; CPG = cardioplegia.

Fig. 3 –.

Isolated myocyte contractility after stress at time 60 min. Contractility remains stable after exposure to stress in WT and Kir6.1GOF myocytes. Isolated myocytes (WT or Kir6.1GOF) were exposed to control TYR 37°C for 20 min (time 5 to 20 min), stress (hypothermic hyperkalemic cardioplegia [CPG] at 9°C or CPG + DZX [CPG + DZX]) for 20 min (time 20 to 40 min), followed by TYR 37°C for 20 min (time 40 to 60 min). Myocyte contractility was measured at baseline and at time 60 min (after stress and 20 min of reexposure to TYR) and normalized to baseline. Data are represented as mean $\pm$ SEM. Kir = potassium inward rectifier.