Diazoxide maintenance of myocyte volume and contractility during stress: Evidence for a non-sarcolemmal K_{ATP} channel location

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Objective: Animal and human myocytes demonstrate significant swelling and reduced contractility during exposure to stress (metabolic inhibition, hyposmotic stress, or hyperkalemic cardioplegia), and these detrimental consequences may be inhibited by the addition of diazoxide (adenosine triphosphate-sensitive potassium channel opener) via an unknown mechanism. Both SUR1 and SUR2A subunits have been localized to the heart, and mouse sarcolemmal adenosine triphosphate-sensitive potassium channels are composed of SUR2A/Kir6.2 subunits in the ventricle and SUR1/Kir6.2 subunits in the atria. This study was performed to localize the mechanism of diazoxide by direct probing of sarcolemmal adenosine triphosphate-sensitive potassium channel current and by genetic deletion of channel subunits.

Methods: Sarcolemmal adenosine triphosphate-sensitive potassium channel current was recorded in isolated wild-type ventricular mouse myocytes during exposure to Tyrode's solution, Tyrode's + 100 μmol/L diazoxide, hyperkalemic cardioplegia, cardioplegia+diazoxide, cardioplegia+100 μmol/L pinacidil, or metabolic inhibition using whole-cell voltage clamp (N = 7–12 cells per group). Ventricular myocyte volume was measured from SUR1(-/-) and wild-type mice during exposure to control solution, hyperkalemic cardioplegia, or cardioplegia + 100 μmol/L diazoxide (N = 7–10 cells per group).

Results: Diazoxide did not increase sarcolemmal adenosine triphosphate-sensitive potassium current in wild-type myocytes, although they demonstrated significant swelling during exposure to cardioplegia that was prevented by diazoxide. SUR1(-/-) myocytes also demonstrated significant swelling during exposure to cardioplegia, but this was not altered by diazoxide.

Conclusions: Diazoxide does not open the ventricular sarcolemmal adenosine triphosphate-sensitive potassium channel but provides volume homeostasis via an SUR1-dependent pathway in mouse ventricular myocytes, supporting a mechanism of action distinct from sarcolemmal adenosine triphosphate-sensitive potassium channel activation. (J Thorac Cardiovasc Surg 2010;140:1153-9)

The adenosine triphosphate-sensitive potassium (K_{ATP}) channel opener diazoxide (DZX) is cardioprotective and mimics ischemic preconditioning in animal models.[1] We previously documented significant swelling of isolated myocytes and associated reduced contractility secondary to exposure to standard hypothermic hyperkalemic cardioplegia (CPG) solution in human and other species.[2,3] These detrimental consequences were ameliorated by the addition of DZX.[2,3] Because DZX is a K_{ATP} channel opener, we hypothesized that K_{ATP} channels may play a role in myocyte volume homeostasis and that myocyte swelling may be one mechanism of myocardial stunning.[4] Myocyte swelling and reduced contractility are also observed after exposure to hyposmotic *and* ischemic stress.[4,5] Notably, DZX ameliorates both the structural and functional derangements secondary to *all 3 stresses*: hyperkalemic CPG, mild hyposmotic stress, and ischemic stress.[2-5] The addition of 5-hydroxydecanoate, a claimed mitochondrial K_{ATP} (mK_{ATP}) channel blocker, or HMR 1098, a claimed sarcolemmal K_{ATP} (sK_{ATP}) channel blocker, to ischemic stress and hyperkalemic CPG did not alter the beneficial observations noted with DZX alone.[2,4] The cardioprotective mechanism of DZX remains unknown.

DZX has been described as an mK_{ATP} channel opener, with ''selectivity'' toward the mK_{ATP} channel and only weak sK_{ATP} channel activation at high doses.[6] However, controversy regarding the specificity of K_{ATP} channel openers and blockers suggests that pharmacologic manipulation of the K_{ATP} channel, using potassium channel openers or sulfonylurea receptor (SUR) blockers, may be inadequate to definitively confirm ion flux across the sK_{ATP} channel,[7-9] and it remains unclear whether DZX provides cardioprotection

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Abbreviations and Acronyms

CPG = cardioplegia
DZX = diazoxide
K_{ATP} = adenosine triphosphate-sensitive potassium
KO = knockout
MI = metabolic inhibition
mK_{ATP} = mitochondrial K_{ATP}
NT = normal Tyrode's solution
sK_{ATP} = sarcolemmal K_{ATP}
SUR = sulfonylurea receptor
WT = wild-type

via the sK_{ATP} channel, the mK_{ATP} channel, or a K_{ATP} channel-independent mechanism.

K_{ATP} channels are composed of a Kir inward rectifier channel-forming subunit and a sulfonylurea sensitive regulatory subunit, SUR (Figure 1).[10] It is clear that sK_{ATP} channels require both Kir6.2 and SUR2A subunits in the ventricle, but Kir6.2 and SUR1 in the atria.[11] Both SUR1 and SUR2A are expressed in mouse heart,[11,12] and there is evidence that SUR2 and SUR1 subtypes are both present in cultured neonatal rat ventricular myocytes.[13] Mice lacking the SUR1 subunit seem to tolerate ischemia/reperfusion injury better than wild-type (WT) mice in a model of left anterior descending coronary artery ligation.[12] Although there is some evidence for SUR1 subunits in the ventricle of various species, K_{ATP} currents in mouse ventricle are unaltered in mice lacking the SUR1 subunit.[11]

We initially hypothesized that the mechanism of action of DZX might involve opening the sK_{ATP} channel (SUR2A/Kir6.2). Previous work documented a beneficial effect of K_{ATP} channel opener DZX in ventricular myocytes that was unaltered by the pharmacologic inhibition of the K_{ATP} channel.[2,4] The first part of this study was designed to definitively investigate the action of DZX by the *direct* measurement of sK_{ATP} channel current in WT mice using whole-cell voltage clamp.

After demonstrating that sK_{ATP} (SUR2A/Kir6.2) channel activity was not observed in ventricular myocytes from WT mice during exposure to DZX, and knowing that SUR1 subunits have been documented in ventricular tissue,[11-13] we next hypothesized that DZX might act via SUR1 subunits of an alternative K_{ATP} channel or some other channel in ventricular cells. The second part of this study was therefore designed to determine whether ventricular myocytes lacking the SUR1 subunit would be responsive to DZX during stress.

This study was designed to elucidate the location of action of DZX by the *direct* measurement of sK_{ATP} channel activity (in WT mice) and by response to stress in ventricular myocytes from WT mice and mice lacking the SUR1 subunit. The elucidation of DZX's mechanism of action in this model will facilitate its future clinical use.

MATERIALS AND METHODS

Myocyte Isolation

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 used for all experiments and isolated from adult mice (either WT or SUR1 knockout [KO], either sex, 6 weeks to 5 months, 25–30 g body weight) as previously described.[14] Rapid cardiectomy was performed in the anesthetized (2.5% tribromoethanol [Avertin; Sigma, St. Louis, Mo]) mouse, and the aorta was cannulated using a 28-gauge needle. The heart was attached to a Langendorff apparatus, and solution A was perfused through the aorta for 5 minutes. The heart was then perfused at 37°C for 12 minutes with solution B. The left ventricle was removed and transferred into solution C, where it was gently dispersed by glass pipette at room temperature. The cells were allowed to centrifuge by gravity, and serial washings were performed every 10 minutes for a 30-minute period. Cells were used in experiments within 5 hours after isolation. A typical yield of viable myocytes was 65% to 75% per mouse.

Solution A consisted of (in millimoles per liter, except as noted) 116 NaCl; 5.36 KCl; 0.97 Na_2HPO_4; 1.47 KH_2PO_4; 21.10 HEPES; 11.65 glucose; 26.50 μmol/L phenol red (Sigma); 3.72 $MgCl_2$; 4.40 $NaHCO_3$; essential vitamins (100×, 10 mL; GIBCO, Grand Island, NY); and amino acids (50×, 20 mL; GIBCO). Solution B consisted of solution A plus 10 μmol/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), 1.25 mg/mL taurine, and 150 μmol/L $CaCl_2$.

The DZX (7-chloro-3-methyl-1,2,4-benzothiadiazine-1,1-dioxide; Sigma) dose of 100 μmol/L was used because it was effective in ameliorating cell swelling secondary to stress (hyperkalemic CPG, hyposmotic stress, and metabolic inhibition [MI]) in previous studies.[2-5] A stock solution of DZX was made by dissolving the reagent in 0.1% dimethyl sulfoxide, at which concentration dimethyl sulfoxide has no effect on cell volume.[15]

Cells were selected 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.[15] After isolation, acceptable myocytes were randomly assigned to a test solution group. A maximum of 2 cells were used per each animal.

Electrophysiology in Wild-Type Myocytes

After isolation, WT myocytes were placed in a recording chamber containing normal Tyrode's solution (NT). Macroscopic currents in isolated ventricular myocytes were recorded using standard whole-cell voltage-clamp recording techniques.[16] Patch-clamp electrodes (1–3 MΩ when filled with electrode solution) were fabricated from soda lime glass microhematocrit tubes (Kimble 73813, Kimble Glass Co, Vineland, NJ). Electrode solution contained the following (in millimoles/liter): 140 KCl, 10 HEPES, and 10 EGTA (pH 7.3–7.4). Cell capacitance and series resistance were determined using a 5- to 10-mV hyperpolarizing square pulse from a holding potential of –70 mV after establishment of the whole cell recording configuration. PClamp 9.2 software and DigiData 1322 (both from Molecular Devices, Sunnyvale, Calif) were used to generate command pulses and collect data. Data were filtered at 5 kHz. A 4-second ramp from –110 and 40 mV was used to isolate and detect sK_{ATP} channel current only.[17]

Experimental protocol

Isolated WT myocytes were exposed to NT for baseline measurement for 1 to 2 minutes, followed by exposure to test solution (5–10 minutes), and followed by NT for 5 to 10 minutes. Test solutions included NT (n = 8 cells), NT + 100 μmol/L DZX (n = 7 cells), hyperkalemic CPG in the form of St Thomas' solution (CPG, Plegisol, Abbott Laboratories, North