Diazoxide Cardioprotection Is Independent of Adenosine Triphosphate-Sensitive Potassium Channel Kir6.1 Subunit in Response to Stress

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BACKGROUND: The sarcolemmal adenosine triphosphate-sensitive potassium channel (sK_{ATP}), composed primarily of potassium inward rectifier (Kir) 6.2 and sulfonylurea receptor 2A subunits, has been implicated in cardiac myocyte volume regulation during stress; however, it is not involved in cardioprotection by the adenosine triphosphate-sensitive potassium channel (K_{ATP}) channel opener diazoxide (7-chloro-3-methyl-1,2,4-benzothiadiazine-1,1-dioxide [DZX]). Paradoxically, the sK_{ATP} channel subunit Kir6.2 is necessary for detrimental myocyte swelling secondary to stress. The Kir6.1 subunit can also contribute to K_{ATP} channels in the heart, and we hypothesized that this subunit might play a role in myocyte volume regulation in response to stress.

STUDY DESIGN: Isolated cardiac myocytes from either wild-type mice or mice lacking the Kir6.1 subunit (Kir6.1[−/−]) were exposed to control Tyrode's solution (TYR) for 20 minutes, test solution (TYR, hypothermic hyperkalemic cardioplegia [CPG], or CPG + K_{ATP} channel opener DZX [CPG + DZX]) for 20 minutes, followed by TYR for 20 minutes. Myocyte volume and contractility were measured and analyzed.

RESULTS: Both wild-type and Kir6.1(−/−) myocytes demonstrated substantial swelling during exposure to stress (CPG), which was prevented by DZX. Wild-type myocytes also demonstrated reduced contractility during stress of CPG that was prevented by DZX. However, Kir6.1(−/−) myocytes did not show reduced contractility during stress.

CONCLUSIONS: These data indicate that K_{ATP} channel subunit Kir6.1 is not necessary for DZX's maintenance of cell volume during the stress of CPG. The absence of Kir6.1 does not affect the contractile properties of myocytes during stress, suggesting the absence of Kir6.1 improves myocyte tolerance to stress via an unknown mechanism. (J Am Coll Surg 2015;221:319−325. © 2015 by the American College of Surgeons)

Adenosine triphosphate-sensitive potassium channels (K_{ATP}) in the heart are inhibited by ATP and are open during times of stress, providing a "unique electrical transducer of the metabolic state of the cell."[1,2] Pharmacologic opening of K_{ATP} channels provides cardioprotection and mimics ischemic preconditioning in multiple animal models[3-10] and in human myocytes.[11] Paradoxically, the cell surface (sarcolemmal) K_{ATP} channel (sK_{ATP}) has been implicated in myocyte swelling secondary to stress, and deletion of the sK_{ATP} channel subunit potassium inward rectifier (Kir) 6.2 provides resistance to myocyte swelling secondary to stress.[9,12]

Cell surface K_{ATP} channels are composed of 4 pore-forming subunits (Kir6.2 or Kir6.1) and 4 of the ATP binding cassette family of membrane proteins (sulfonylurea receptor [SUR] 1 or SUR2).[13,14] The genes encoding these subunits have been cloned, which allows for genetic manipulation.

The K_{ATP} channel opener diazoxide (7-chloro-3-methyl-1,2,4-benzothiadiazine-1,1-dioxide [DZX]) does not provide

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Abbreviations and Acronyms

CPG = hypothermic hyperkalemic cardioplegia
DZX = diazoxide
K_{ATP} = adenosine triphosphate–sensitive potassium channel
Kir = potassium inward rectifier
sK_{ATP} = sarcolemmal adenosine triphosphate–sensitive potassium channel
SUR = sulfonylurea receptor
TYR = Tyrode's solution
WT = wild type

cardioprotection via the sK_{ATP} channel (composed of Kir6.2 and SUR2A), and the location and mechanism of action of DZX remain elusive.[15] Proposed mechanisms include non-K_{ATP} channel or mitochondrial K_{ATP} channel location of action.[16,17] Using an isolated myocyte model of myocardial stunning, we have provided evidence that the cardioprotection provided by DZX might involve the inhibition of succinate dehydrogenase (independent of K_{ATP} channel) and requires the K_{ATP} channel subunit SUR1.[15,18,19] However, the pore-forming subunits involved have yet to be identified.

The current study was conducted to determine whether the K_{ATP} channel subunit Kir6.1 has any role in detrimental myocyte swelling secondary to stress or in the cardioprotection afforded by DZX.

METHODS

All animal procedures were approved by the Washington University Animal Studies 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.

Kir6.1 knockout mice were generated as described previously by Miki and colleagues[20] by replacing a part of intron 2 and exon 3 of KCNJ8 in the 129Sv background, and then backcrossing to mouse strain C57BL6.

Mouse myocyte isolation

Ventricular myocytes were isolated from both wild-type (WT) and Kir6.1(−/−) mice[20] (aged 6 weeks to 5 months and weighing 15 to 30 g) as described previously.[18] Mice were anesthetized with 2.5% Avertin intraperitoneally. Heparin (0.1 mL) was administered intraperitoneally. Rapid cardiectomy was performed and solution A

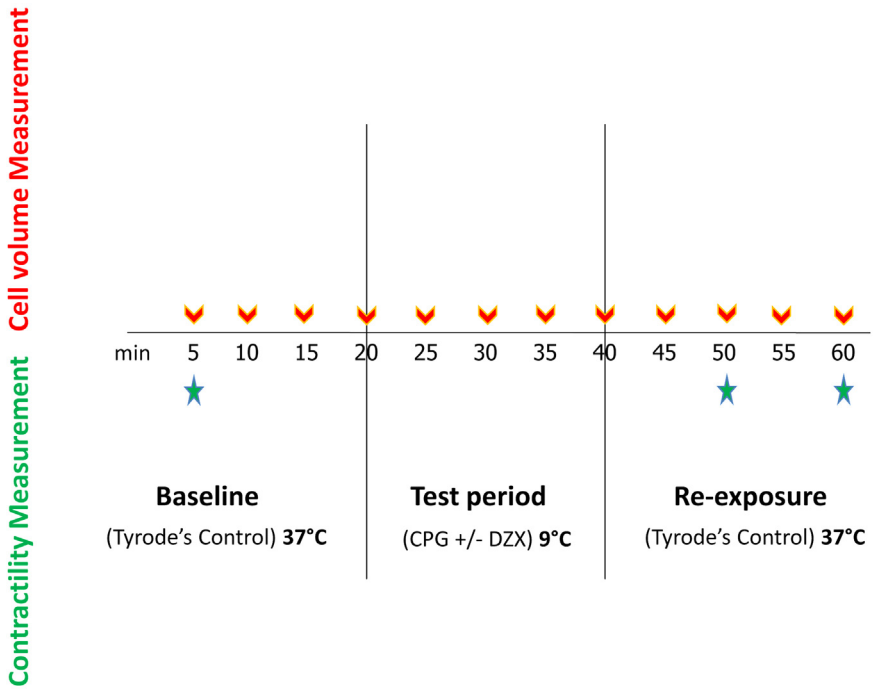

Figure 1. Experimental protocol. Myocytes were exposed to 37°C control Tyrode's physiologic solution (TYR) for 20 minutes, followed by stress test solution (per Methods) for 20 minutes, then 20 minutes re-exposure to TYR. Volume measurements were taken every 5 minutes (red V symbol). Contractility measurements were taken at times 5 minutes (baseline) and at 10 and 20 minutes after re-exposure to TYR (time 50 and 60 minutes) (green stars).