Cardioprotective Benefits of Adenosine Triphosphate-Sensitive Potassium Channel Opener Diazoxide Are Lost with Administration after the Onset of Stress in Mouse and Human Myocytes

M Burhan Janjua, MD, Carol M Makepeace, BS, Melissa M Anastacio, MD, Richard B Schuessler, PhD, Colin G Nichols, PhD, Jennifer S Lawton, MD, FACS

BACKGROUND: Adenosine triphosphate-sensitive (K_{ATP}) potassium channel opener diazoxide (DZX) maintains myocyte volume and contractility during stress via an unknown mechanism when administered at the onset of stress. This study was performed to investigate the cardioprotective potential of DZX when added after the onset of the stresses of hyperkalemic cardioplegia, metabolic inhibition, and hypo-osmotic stress.

STUDY DESIGN: Isolated mouse ventricular and human atrial myocytes were exposed to control Tyrode's solution (TYR) for 10 to 20 minutes, test solution for 30 minutes (hypothermic hyperkalemic cardioplegia [CPG], CPG + 100uM diazoxide [CPG+DZX], metabolic inhibition [MI], MI+DZX, mild hypo-osmotic stress [0.9T], or 0.9T + DZX), with DZX added after 10 or 20 minutes of stress, followed by 20 minutes of re-exposure to TYR (±DZX). Myocyte volume (human + mouse) and contractility (mouse) were compared.

RESULTS: Mouse and human myocytes demonstrated significant swelling during exposure to CPG, MI, and hypo-osmotic stress that was not prevented by DZX when administered either at 10 or 20 minutes after the onset of stress. Contractility after the stress of CPG in mouse myocytes significantly declined when DZX was administered 20 minutes after the onset of stress ($p < 0.05$ vs TYR). Contractility after hypo-osmotic stress in mouse myocytes was not altered by the addition of DZX.

CONCLUSIONS: To maintain myocyte volume homeostasis and contractility during stress (hyperkalemic cardioplegia, metabolic inhibition, and hypo-osmotic stress), K_{ATP} channel opener diazoxide requires administration at the onset of stress in this isolated myocyte model. These data have potential implications for any future clinical application of diazoxide. (J Am Coll Surg 2014; 219:803−813. © 2014 by the American College of Surgeons)

Exposure to stress (hypothermic hyperkalemic cardioplegia [CPG], metabolic inhibition [MI], and hypo-osmotic stress) results in isolated myocyte swelling and reduced contractility in animal and human myocytes.[1-4] These detrimental changes in isolated myocytes have been prevented by the administration of adenosine triphosphate-sensitive potassium (K_{ATP}) opener diazoxide when administered at the onset of stress (via an unknown mechanism).[1-4] Observed volume derangements and resultant functional derangements have been described to be inversely related.[2] These changes have been hypothesized to represent 1 mechanism of myocardial stunning.

The efficacy of K_{ATP} channel openers as cardioprotective agents has been well established when these agents have been administered before or at the onset of ischemia (as in ischemic preconditioning or in cardioplegia solutions).[5-9] Any clinical use of diazoxide would therefore require its administration before or at the onset of

Disclosure Information: Nothing to disclose.

Support: Dr Lawton was supported by NIH RO1 HL098182-01A1, NIH 5T32HL007776 (MBJ), and the Barnes Jewish Hospital Foundation.

Abstract presented at the American College of Surgeons 99th Annual Meeting, Washington, DC, October 2013, and the American Heart Scientific Sessions, Dallas, TX, November 2013.

Received March 6, 2014; Revised April 28, 2014; Accepted May 6, 2014.

Division of Cardiothoracic Surgery, Department of Surgery (Janjua, Makepeace, Anastacio, Scheussler, Lawton) and the Department of Cell Biology and Physiology (Nichols), Washington University School of Medicine, St Louis, MO.

Correspondence address: Jennifer S Lawton, MD, FACS, Washington University School of Medicine, 660 S Euclid Ave, Campus Box 8234, St Louis, MO 63110. email: lawtonj@wustl.edu

© 2014 by the American College of Surgeons
Published by Elsevier Inc.

http://dx.doi.org/10.1016/j.jamcollsurg.2014.05.010
ISSN 1072-7515/14

Abbreviations and Acronyms

CPG = hypothermic hyperkalemic cardioplegia
DZX = diazoxide
K_{ATP} = adenosine triphosphate-sensitive potassium channel
MI = metabolic inhibition
TYR = Tyrode's solution

myocardial ischemia. Ideally, diazoxide would also provide benefit when administered during late ischemia or during reperfusion. This would tremendously increase diazoxide's future clinical applicability to include that of any form of ongoing myocardial stress.

Previous work using K_{ATP} channel opener DZX as a cardioprotective agent with administration during ischemia or before reperfusion has demonstrated inconsistent findings, and myocyte volume and contractility responses to stress have not been studied.[10-13] This study was performed to determine myocyte volume and contractility responses to the stresses of hyperkalemic cardioplegia, metabolic inhibition, and hypo-osmotic stress, with the cardioprotective K_{ATP} channel opener DZX administered after the onset of stress or after stress has ended.

METHODS

All animal procedures were approved by the Animal Studies Committee and all animals received humane care in compliance with the *Guide to Care and Use of Laboratory Animals*.[14] Human myocyte experiments were approved by the Human Studies Committee in accordance with Institutional Review Board approval. All patients gave informed consent for participation in the study.

Mouse myocyte isolation

Ventricular myocytes were isolated from mice of either sex (age 6 weeks to 5 months and weighing 15 to 30 g) as previously described.[15] Mice were anesthetized with 2.5% 2,2,2-Tribromoethanol intraperitoneally. Heparin (0.1 mL) was administered intraperitoneally. Rapid cardiectomy was performed and solution A (as defined below) was perfused through the aorta for 5 minutes. The heart was then perfused at 37°C for 12 to 20 minutes with solution B (as defined below). Ventricles were removed and minced and placed into solution C (as defined below), and gently dispersed by glass pipette. Cells were allowed to centrifuge by gravity, and serial washings were performed every 10 minutes for 15 to 20 minutes. Cells were used within 5 hours and randomized to test solution via random number selection. A typical yield of viable myocytes was 65% to 75%.

Solution A consisted of (in mmol/L, except as noted) NaCl, 116; KCl, 5.36; Na_2HPO_4, 0.97; KH_2PO_4, 1.47; HEPES (*N*-[2-hydroxyethyl] piperazine-*N′*-[4-butanesulfonic acid]), 21.10; glucose, 11.65; phenol red (Sigma), 26.50 μmol/L; $MgCl_2$, 3.72; $NaHCO_3$, 4.40; essential vitamins (100×, 10 mL, GIBCO); 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). Solution C consisted of solution A plus 5 mg/mL bovine serum albumin (Sigma); 1.25 mg/mL taurine; and 150 μmol/L $CaCl_2$.

Human myocyte isolation

Human myocytes were isolated as previously described.[3] Tissue specimens (right or left atrium) were collected during elective cardiac surgery and placed into 37°C oxygenated calcium-free Tyrode's solution (TYR) (in mmol/L): NaCl, 130; KCl, 5; KH_2PO_4, 0.4; $MgCl_2$, 3; HEPES (N-[2-hydroxethyl]piperazine-N′-[4-butanesulfnonic acid]), 5; taurine, 15; glucose, 10; creatine, 5.7 (pH adjusted to 7.3 by 20% NaOH titration); Na_2EGTA, 0.1; and nitrilotriacetic acid, 6 (Sigma) and taken immediately to the laboratory. Specimens were placed in fresh oxygenated 37°C calcium-free TYR with 0.1 mmol/L Na_2EGTA and 6 mmol/L nitrilotriacetic acid, and minced.

Minced tissue was transferred to 20 mL of 37°C calcium-free TYR with 6 mmol/L nitrilotriacetic acid and agitated in a 37°C water bath at 100 rpms for 12 min to remove extracellular calcium.

Tissue was then transferred to 10 mL of 37°C calcium-free TYR with 1,000 mg/L bovine serum albumin (Sigma), 925 mg/L collagenase type II (Worthington Biomedical), and 250 mg/L protease (Sigma) and agitated in a 37°C water bath at 100 rpm for 45 to 50 minutes. The supernatant was discarded and the pellet resuspended in 15 mL of 37°C calcium-free TYR with 1,000 mg/L bovine serum albumin (Sigma) and 925 mg/L collagenase type II (Worthington Biomedical) and agitated in a 37°C water bath at 100 rpm for 18 minutes, and this was then repeated. The supernatant was discarded and the pellet resuspended in a 37°C cell isolation solution (KB solution) containing (in mmol/L): potassium glutamate, 120; KCl, 10; KH_2PO_4, 10; $MgSO_4$, 1.8; K_2EGTA, 0.5; taurine, 10; HEPES, 10; and glucose, 20; and triturated to separate the cells. After filtering, large debris was resuspended in KB solution. Cells were used within 6 hours and randomly assigned to test solution via random number selection. A typical yield of viable myocytes was 30%.

Experimental protocol

Myocytes were exposed to 37°C control TYR for 20 (CPG stress groups) or 10 minutes (MI stress group and hypo-osmotic stress group) to obtain baseline volume (Fig. 1). Any changes in cell volume secondary to the

Download English Version:

https://daneshyari.com/en/article/4291501

Download Persian Version:

https://daneshyari.com/article/4291501

Daneshyari.com