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

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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. 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). Observed volume derangements and resultant functional derangements have been described to be inversely related. 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). Any clinical use of diazoxide would therefore require its administration before or at the onset of...
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 KATP 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.10,11 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 KATP 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; Na2HPO4, 0.97; KH2PO4, 1.47; HEPES (N-[2-hydroxyethyl] piperazine-N’-[4-butanesulfonic acid]), 21.10; glucose, 11.65; phenol red (Sigma), 26.50 μmol/L; MgCl₂, 3.72; NaHCO₃, 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 CaCl₂ 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 CaCl₂.

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; KH₂PO₄, 0.4; MgCl₂, 3; HEPES (N-[2-hydroxyethyl] piperazine-N’-[4-butanesulfonic acid]), 5; taurine, 15; glucose, 10; creatine, 5.7 (pH adjusted to 7.3 by 20% NaOH titration); Na₂EGTA, 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 Na₂EGTA 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 rpm 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; KH₂PO₄, 10; MgSO₄, 1.8; K₂EGTA, 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

Abbreviations and Acronyms

CPG = hypothermic hyperkalemic cardioplegia
DZX = diazoxide
KATP = adenosine triphosphate-sensitive potassium channel
MI = metabolic inhibition
TYR = Tyrode’s solution

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isolation would be evident during this period. Myocytes were then exposed to test solution (30 minutes). Test solutions included (n = number in mouse, number in human, respectively):

Control TYR 37°C (n = 7 to 15 for all); CPG (n = 10, 15); CPG + 100 μmol/L DZX administered after 10 minutes of stress (n = 10, 15); CPG + DZX after 20 minutes of stress (n = 14, 15); CPG + DZX only in re-exposure (n = 11, 15); CPG + DZX after 10 minutes of stress + DZX throughout re-exposure (n = 10, 15); CPG + DZX after 20 minutes of stress + DZX throughout re-exposure (n = 11, 15); MI (n = 7, 6); MI + 100 μmol/L DZX administered after 10 minutes of stress (n = 7 6); MI + DZX after 20 minutes of stress (n = 8, 6); MI + DZX only in re-exposure (n = 7, 6); MI + DZX after 10 minutes of stress + DZX throughout re-exposure (n = 7, 6); MI + DZX after 20 minutes of stress + DZX throughout re-exposure (n = 7, 6); altered control 1T solution (n = 6, 6); Hypo-osmotic stress (0.9T) (n = 6, 6); 0.9T + 100 μmol/L DZX (n = 6, 6); 0.9T + DZX administered after 10 minutes of stress (n = 6, 6); 0.9T + DZX after 20 minutes of stress (n = 4, 6); 0.9T + DZX only in re-exposure (n = 6, 6); 0.9T + DZX after 10 minutes of stress + DZX throughout re-exposure (n = 6, 6); or 0.9T + DZX after 20 minutes of stress + DZX throughout re-exposure (n = 5, 6).

After exposure to test solution, myocytes were exposed to Tyrode’s solution for 20 minutes.

Cardioplegia consisted of (in mmol/L): NaCl, 110; NaHCO3, 10; KCl, 16; MgCl2, 16; and CaCl2, 1.2; and was equilibrated with 95% O2 to 5% CO2 and titrated to the pH of 7.3 with 10% NaHCO3 solution. Metabolic inhibition (MI) solution consisted of (in mmol/L): NaCl, 130; KCl, 5; CaCl2, 2.5; MgSO4, 1.2; NaHCO3, 24; 2 deoxyglucose, 10; and NaCN, 2.

Altered control 1T solution was made by substituting 130 mmol/L D-mannitol for 65 mmol/L NaCl in normal TYR solution (which consists of [in mmol/L]): NaCl, 130; KCl, 5; CaCl2, 2.5; MgSO4, 1.2; NaHCO3, 24; Na2HPO4, 1.75; and glucose, 10 [buffered to a pH of 7.4 using 95% O2 to 5% CO2] for hypo-osmotic stress experiments). Hypo-osmotic stress (0.9T) solution was made by substituting 130 mmol/L with 97.5 mmol/L of D-mannitol for 65 mmol/L of NaCl in TYR. The 0.9T solution was used because this degree of hypo-osmotic stress resulted in significant cellular volume change that was prevented by DZX in previous experiments.

Solution osmolarity was measured for every experiment with a vapor pressure osmometer (Wescor, Inc).

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

Mouse and human myocyte volume measurement

Myocytes were used on the day of isolation and were not cultured. Myocytes were visualized on an inverted microscope stage (IX-51, Olympus) as previously described. After 5 minutes, the chamber was perfused at a rate of 3 mL/min with TYR (in mmol/L): NaCl, 130; KCl, 5; CaCl2, 2.5; MgSO4, 1.2; NaHCO3, 24; Na2HPO4, 1.75; and glucose, 10 (buffered to a pH of 7.4 using 95% O2 to 5% CO2) or altered control TYR for hypo-osmotic stress experiments. After viability was confirmed, myocyte images were captured using video-based edge detection software (IonOptix) and volume was measured every 5 minutes, as previously described (Fig. 1).

Mouse myocyte contractility

Myocyte contractility was measured using a video-based edge detection system (IonOptix). Cells were paced using a field stimulator (MyoPacer; IonOptix) at a voltage of 105 above threshold at a frequency of 1HZ with a 5-ms duration to avoid the occurrence of fusion beats. After 5 minutes of stimulation, data were obtained from 12 to 30 consecutive beats and averaged.
Parameters of contractility included: percentage of cell shortening, maximal velocity of shortening, and percentage of cell relengthening, as previously described. Contractility was measured at baseline and after 10 and 20 minutes of re-exposure to TYR (Fig. 1). Cells that showed less than 7% cell shortening at baseline were excluded.

Statistical analysis
All data are presented as mean ± standard error of the mean, normalized to baseline. 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. Probability values < 0.05 were considered significant. A Shapiro-Wilks test was used to test for normality. Statistical analysis was performed using Systat v13 (Systat Software, Inc).

RESULTS
Mouse myocyte volume during stress of cardioplegia
Myocytes demonstrated no significant volume change from baseline during exposure to TYR (Fig. 2). Myocytes demonstrated significant swelling when exposed to CPG (p < 0.05 vs TYR). This swelling was not prevented by DZX when administered at either 10 or 20 minutes after the onset of stress (Fig. 2).

Myocyte volume remained significantly increased during the entire 20-minute re-exposure to TYR period in the CPG and the CPG + DZX at 20 minutes groups. Myocyte volume in the CPG + DZX at 10 minutes + DZX during re-exposure group returned to baseline volume by 5 minutes of re-exposure to TYR. Myocyte volume returned to baseline levels after 10-minute re-exposure to TYR in the CPG + DZX at 10 minutes, CPG + DZX only in re-exposure, and the CPG + DZX at 20 minutes + DZX in re-exposure groups.

Human myocyte volume during stress of cardioplegia
Myocytes demonstrated significant differences compared with TYR for the first 2 time points of the baseline period in the CPG group and for the first baseline time point in the CPG + DZX at 10 minutes group. Myocytes in all other groups demonstrated no significant volume change from baseline during exposure to TYR (Fig. 3). Myocytes demonstrated significant swelling when exposed to CPG (p < 0.05 vs TYR). This swelling was not prevented by...
Diazoxide (DZX) when administered at either 10 or 20 minutes after the onset of stress (Fig. 3).

Myocyte volume remained significantly increased for only the first 5 minutes of the re-exposure period in the CPG and the CPG + DZX at 20 minutes groups. All other groups demonstrated return to and maintenance of baseline volume by the first 5 minutes of re-exposure to TYR.

**Mouse myocyte volume during stress of metabolic inhibition**

Myocytes demonstrated no significant volume change from baseline during exposure to TYR (Fig. 4). Myocytes in all groups demonstrated significant swelling when exposed to MI (p < 0.05 vs TYR). This swelling did not reach statistical significance (vs TYR) in the MI + DZX at 10 minutes + DZX in the re-exposure group until 10 minutes into the stress period. This swelling was not prevented by DZX when administered at either 10 or 20 minutes after the onset of stress (Fig. 4).

Myocyte volume remained significantly increased for only the first 5 minutes of the re-exposure to TYR period in the MI and MI + DZX at 20 minutes groups. All other groups demonstrated return to and maintenance of baseline volume by the first 5 minutes of re-exposure to TYR.

**Human myocyte volume during stress of metabolic inhibition**

Myocytes demonstrated no significant volume change from baseline during exposure to TYR (Fig. 5). Myocytes demonstrated significant swelling when exposed to MI in all groups (p < 0.05 vs TYR). This swelling did not reach statistical significance (vs TYR) in the MI + DZX at 20 minutes and the MI + DZX in re-exposure only groups until 5 minutes of stress. Myocyte swelling was not prevented by DZX when administered either at 10 or 20 minutes after the onset of stress in all groups except in the MI + DZX at 10 minutes + DZX during re-exposure group, which demonstrated return to baseline level at the last time point of stress.

Myocyte volume remained significantly increased for the first 15 minutes of the re-exposure period in the MI group. All other groups demonstrated return to and maintenance of baseline volume by the first 5 minutes of re-exposure to TYR.
Mouse myocyte volume during hypo-osmotic stress

Myocytes demonstrated no significant volume change from baseline during exposure to 1T TYR solution (Fig. 6). Myocytes demonstrated swelling when exposed to 0.9T solution vs 1T TYR solution. This swelling was statistically significant in all groups except the 0.9T and the 0.9T + DZX groups by 20 minutes of stress. This swelling was not prevented by DZX when administered at any time point (Fig. 6). Myocyte volume returned to levels that were not significantly different from baseline after 5-minute re-exposure to 1T TYR solution in all test groups.

Human myocyte volume during hypo-osmotic stress

Myocytes demonstrated no significant volume change from baseline during exposure to 1T TYR solution (Fig. 7). Myocytes demonstrated significant swelling when exposed to 0.9T TYR solution throughout stress (p = 0.00 to 0.01 for all stress time points vs 1T TYR). This swelling was reduced when DZX was introduced at the onset of stress (p = 0.94); however, it was not prevented by DZX when administered either at 10 (p = 0.71) or 20 minutes (p = 0.45) after the onset of stress compared with 0.9T (Fig. 7).

Myocyte volume remained significantly increased for the entire re-exposure period in the 0.9T TYR group and only for the first 5 minutes of re-exposure in the 0.9T TYR + DZX at 20 minutes group. All other groups demonstrated return to and maintenance of baseline volume by the first 5 minutes of re-exposure to 1T TYR.

Mouse myocyte contractility during stress of cardioplegia

Myocytes demonstrated a decline in contractility after exposure to CPG (Fig. 8). This decline in contractility was significant in at least 1 measure of contractility in groups CPG + DZX at 20 minutes and in CPG + DZX at 20 minutes + DZX during re-exposure to TYR (p < 0.05 vs TYR). Contractility significantly improved in all groups when comparisons were made between 10 and 20 minutes after re-exposure to TYR (Fig. 8).

Mouse myocyte contractility during hypo-osmotic stress

Myocytes demonstrated a decline in contractility after exposure to 0.9T solution (p = 0.87) (Fig. 9). This
decline in contractility was not altered by the addition of DZX at any time point (Fig. 9).

DISCUSSION

Isolated myocytes demonstrate significant swelling and reduced contractility in response to stress (exposure to hypothermic hyperkalemic cardioplegia, metabolic inhibition, or hypo-osmotic solution), which is prevented by the administration of K<sub>ATP</sub> channel opener DZX at the onset of stress. The consistent relationship between myocyte swelling and reduced contractility suggests myocyte swelling may be one mechanism of myocardial stunning. This study was performed to determine if DZX would provide similar benefits when administered after the onset of stress, after the onset of stress and throughout re-exposure to normal physiologic solution, or throughout re-exposure to normal physiologic solution only.

In this study, hypothermic hyperkalemic cardioplegia, metabolic inhibition, and hypo-osmotic stress resulted in significant myocyte swelling and reduced contractility in animal and human myocytes; however, diazoxide did not prevent these changes when administered after the onset of stress. Only in human myocytes exposed to metabolic inhibition was a return to baseline volume noted at the last time point of stress when diazoxide was administered at 10 minutes after the onset of stress. Perhaps a benefit of DZX could be demonstrated with longer stress periods, particularly in human myocytes.

Interestingly, there was no difference in return to baseline volume between groups with DZX in the re-exposure period and groups without. The return to baseline volume in most groups on re-exposure to TYR likely indicates a return of volume homeostatic mechanisms and suggests that the mechanism of DZX does not involve reduction of reactive oxygen species or other mechanisms that are known to reduce reperfusion injury.

A decline in contractility was noted in all groups after cardioplegia and hypo-osmotic stress; however, this was only statistically significant in 2 groups at 10 minutes after stress (p = 0.02, velocity of shortening and p = 0.02, peak velocity of relengthening in TYR vs CPG + DZX at 20 minutes; and p = 0.048, velocity of shortening in TYR vs CPG + DZX in 20 minutes + DZX in re-exposure). All groups exposed to CPG had improvement in contractility by 20 minutes after stress. This is in
Figure 6. Diazoxide does not prevent mouse myocyte swelling secondary to hypo-osmotic stress. Isolated myocytes were exposed to control 1T Tyrode’s physiologic solution (TYR) for 10 minutes (time 5 to 10 minutes), stress (0.9T, 0.9T + DZX at 10 or 20 minutes) for 30 minutes (time 15 to 40 minutes), followed by 1T TYR (±DZX) for 20 minutes (time 45 to 60 minutes). Data are represented as mean ± SEM. All groups demonstrated a rapid return to baseline volume during re-exposure to 1T TYR. Unfilled symbols represent $p < 0.05$ vs 1T Tyrode’s. DZX, diazoxide.

Figure 7. Diazoxide does not prevent human myocyte swelling secondary to hypo-osmotic stress. Isolated myocytes were exposed to control 1T Tyrode’s physiologic solution (TYR) for 10 minutes (time 5 to 10 minutes), stress (0.9T, 0.9T + DZX at 10 or 20 minutes) for 30 minutes (time 15 to 40 minutes), followed by 1T TYR (±DZX) for 20 minutes (time 45 to 60 minutes). Data are represented as mean ± SEM. All groups demonstrated statistically significant myocyte swelling at various time points during stress ($p < 0.05$ vs 1T TYR). Unfilled symbols represent $p < 0.05$ vs 1T Tyrode’s. * represents $p < 0.05$ vs 0.9T. DZX, diazoxide.
contrast to previous work (with a shorter stress period) in which a statistically significant decline in contractility was demonstrated in the cardioplegia group after stress.\(^1\)

The presence of DZX during the re-exposure to TYR period provided no additional benefit to contractility, similar to the work of other investigators.\(^13\) As stated above, intrinsic reparative mechanisms other than the presence of DZX during the re-exposure period were likely responsible for the observations noted in this study.

Previous investigators using a variety of models have documented conflicting results with DZX administration after the onset of stress, ischemia, or hypoxia.\(^10-13\) In an intact rabbit heart model of 30-minute regional ischemia and 3-hour reperfusion, DZX during ischemia was found to provide no benefit to infarct size.\(^10\) In a model of rat cultured myocytes subjected to 12-hour hypoxia and 12-hour reoxygenation, administration of DZX at reoxygenation or during oxidative stress demonstrated decreased cell death, preserved cellular and mitochondrial integrity, and decreased mitochondrial dysfunction.\(^11\) In an isolated rabbit heart model of 30 minutes of global ischemia and 2 hours of reperfusion, DZX after 10 minutes of ischemia demonstrated decreased infarct size; however, DZX administration after 25 minutes of ischemia did not reduce infarct size.\(^12\) Administration of DZX 10 minutes before and throughout a 90-minute hypoxic period in isolated human trabeculae demonstrated improved recovery of contractility compared with that in controls; however, no benefit was demonstrated when DZX was given only as a preconditioning agent or during reoxygenation alone.\(^13\) So, consistent benefit has not been demonstrated when DZX has been administered after the onset of stress or during reperfusion, and observations in isolated animal and human myocytes are lacking.

**Determination of the mechanism of diazoxide: timing and location**

Unlike cardioprotective mechanisms related to ischemic preconditioning, which require activation before stress, previous work by our laboratory and others has documented the benefit of DZX when administered at the onset of stress.\(^14\) The data from this study also suggest that the mechanism of action of DZX is one that requires
activation at the onset of stress, and no benefit is gained by its administration only during the time of re-exposure to physiologic solutions.

The $K_{ATP}$ channel opener DZX may provide myocyte benefit at a sarcolemmal $K_{ATP}$ channel, at a purported mitochondrial $K_{ATP}$ channel, or at a location independent of a $K_{ATP}$ channel. Previous work supports a location of action at the mitochondrial level. Until a mitochondrial $K_{ATP}$ channel is identified, the precise mechanism of action of DZX remains elusive and its investigation requires indirect methods. Elucidation of the mechanism of action of $K_{ATP}$ channel opener diazoxide will facilitate its potential exploitation for clinical use in the future.

**Limitations**

Previous work in our laboratory using this model used a 20-minute stress period. The stress period for this study was lengthened to 30 minutes in order to examine multiple time points of DZX introduction. The baseline period for the stress of MI was shortened due to the higher loss of viable myocytes noted throughout the prolonged experimental period compared with the CPG stress groups. It is for this reason that contractility could not be consistently measured in the metabolic inhibition groups. In addition, human myocyte contractility has not been accomplished in our laboratory despite years of work in this area.

Isolated myocytes were used because they allow for repeated observations and the independent evaluation of 1 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.

**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. Future work will investigate the effects of the timing of administration of diazoxide in a regional infarct in vivo model.

**Author Contributions**

Study conception and design: Nichols, Lawton
Acquisition of data: Janjua, Makepeace, Anastacio

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**Figure 9.** Diazoxide does not preserve myocyte contractility after hypo-osmotic stress. Isolated mouse myocytes were exposed to control 1T Tyrode’s physiologic solution (TYR) for 10 minutes (time 5 to 10 minutes), stress (0.9T, 0.9T + DZX at 10 or 20 minutes) for 30 minutes (time 15 to 40 minutes), followed by 1T TYR (±DZX) for 20 minutes (time 45 to 60 minutes). Data are represented as means, and error bars have been removed for clarity. All groups demonstrated a nonstatistically significant reduction in myocyte contractility vs 1T TYR after stress ($p = 0.87$). DZX, diazoxide.
Analysis and interpretation of data: Janjua, Makepeace, Schuessler, Lawton
Drafting of manuscript: Janjua, Lawton
Critical revision: Nichols, Lawton

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