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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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Abstract

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 10–20 min, test solution for 30 min (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 min stress, followed by 20 min 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 min after the onset of stress. Contractility following the stress of CPG in mouse myocytes significantly declined when DZX was administered 20 min after the onset of stress ($p < 0.05$ vs. TYR). Contractility following hypo osmotic stress in mouse myocytes was not altered by the addition of DZX.

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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.

Introduction

Exposure to stress (hypothermic hyperkalemic cardioplegia (CPG), metabolic inhibition (MI), and hypo osmotic) 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 one mechanism of myocardial stunning.

The efficacy of K_{ATP} channel openers as cardioprotective agents has been well established when these agents have been administered *prior to or at the onset* of ischemia (as in ischemic preconditioning or in cardioplegia solutions).^{5–9} Any clinical utilization of diazoxide would therefore require its administration *prior to 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 utilizing K_{ATP} channel opener DZX as a cardioprotective agent with administration *during ischemia or prior to reperfusion* has demonstrated inconsistent findings and myocyte volume and contractility responses to stress have not been studied.^{10–13}

The present 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 the Institutional Review Board approval. All patients gave informed consent for participation in the study.

Mouse Myocyte Isolation

Ventricular myocytes were isolated from mice either sex (age 6 weeks – 5 months and 15–30g weight) as previously described.¹⁵ Mice were anesthetized with 2.5% Avertin 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 min. The heart was then perfused at 37°C for 12–20 min 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 min for 15–20 min. 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) 116 NaCl; 5.36 KCl; 0.97 Na₂HPO₄; 1.47 KH₂PO₄; 21.10 HEPES (N-[2-hydroxyethyl] piperazine-N'-[4-butanefulfonic acid]); 11.65 glucose; 26.50 µmol/L phenol red (Sigma, St. Louis, MO); 3.72 MgCl₂; 4.40 NaHCO₃; essential vitamins (100×, 10 mL, GIBCO, Grand Island, NY); and amino acids (50×, 20 mL, GIBCO, Grand Island, NY). Solution B consisted of solution A plus 10 µmol/L CaCl₂; 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 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 TYR (in mmol/L): NaCl 130, KCl 5, KH₂PO₄ 0.4, MgCl₂ 3, HEPES (N-[2-hydroxyethyl]piperazine-N'-[4-butanefulfonic acid]) 5, taurine 15, glucose 10, and creatine 5.7 (pH adjusted to 7.3 by 20% NaOH titration), Na₂EGTA 0.1, and nitrilotriacetic acid 6 (NTA, Sigma, St. Louis, MO) 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 NTA, and minced.

Minced tissue was transferred to 20 mL of 37°C calcium-free TYR with 6 mmol/L NTA 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 1000 mg/L bovine serum albumin (Sigma, St. Louis, MO), 925 mg/L collagenase type II (Worthington Biomedical, Freehold, NJ), and 250 mg/L protease (Sigma, St. Louis, MO) and agitated in a 37°C water bath at 100 rpms for 45–50 min. The supernatant was discarded and the pellet resuspended in 15 mL of 37°C calcium-free TYR with 1000 mg/L bovine serum albumin (Sigma, St. Louis, MO) and 925 mg/L collagenase type II (Worthington Biomedical) and agitated in a 37°C water bath at 100 rpms for 18 min, 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 min (MI stress groups and Hypo osmotic stress groups) to obtain baseline volume (Figure 1). Any changes in cell volume secondary to the isolation would be evident during this period. Myocytes were then exposed to test solution (30 min). Test solutions included (N = # in mouse, # in human, selectively): Control TYR 37°C (N= 7–15 for all), CPG (N=10,15), CPG + 100µmol/L DZX administered after 10 min of stress (N= 10,15), CPG + DZX after 20 min of stress (N=14,15), CPG + DZX only in re-exposure (N=11,15), CPG + DZX after 10 min of stress + DZX throughout re-exposure (N=10,15), CPG + DZX after 20 min of stress + DZX throughout re-exposure (N=11,15), MI (N=7,6), MI + 100µmol/L DZX administered after 10 min of stress (N=7,6), MI + DZX after 20 min of stress (N=8,6), MI + DZX only in re-exposure (N=7,6), MI + DZX after 10 min of stress + DZX throughout re-exposure (N=7,6), MI + DZX after 20 min 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 + 100µmol/L DZX (N=6,6), 0.9T + DZX administered after 10 min of stress (N=6,6), 0.9T + DZX after 20 min of stress (N=4,6), 0.9T + DZX only in re-exposure (N=6,6), 0.9T + DZX after 10 min of stress + DZX throughout re-exposure (N=6,6), or 0.9T + DZX after 20 min of stress + DZX throughout re-exposure (N=5,6). Following exposure to test solution, myocytes were exposed to Tyrode's solution for 20 min.

CPG 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. 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 130mmol/L D-mannitol for 65mmol/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 130mmol/L with 97.5 mmol/L of D-mannitol for 65mmol/L of NaCl in TYR. The 0.9T solution was utilized as this degree of hypoosmotic stress resulted in significant cellular volume change that was prevented by DZX in previous experiments.⁴ Solution osmolality was measured for every experiment with a vapor pressure osmometer (Wescor, Inc. Elitech, Utah USA).

Diazoxide (7-chloro-3-methyl-1,2,4-benzothiadiazine-1,1-dioxide [DZX]; Sigma, St. Louis, MO) dose of 100 µmol/L was utilized as it was effective in ameliorating cell swelling secondary to stress in previous studies.^{1–4} 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.¹⁶

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, Japan) as previously described.¹⁵ After 5 min, 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, Milton, MA) and volume measured every 5 min as previously described (Figure 1).¹⁵

Mouse Myocyte Contractility

Myocyte contractility was measured using a video-based edge detection system (IonOptix, Milton, MA). Cells were paced using a field stimulator (MyoPacer; IonOptix, Milton, MA) 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 min of stimulation, data were obtained from 12–30 consecutive beats and averaged.

Parameters of contractility included: percentage of cell shortening (PS), maximal velocity of shortening (VS), and percentage of cell relengthening (PR) as previously described.¹⁵ Contractility was measured at baseline and after 10 and 20 min of re-exposure to TYR (Figure 1). Cells that showed less than 7% cell shortening at baseline were excluded.

Statistical Analysis

All data are presented as mean $\pm$ 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., San Jose, CA).

Results

Mouse Myocyte Volume During Stress of Cardioplegia

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

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

Human Myocyte Volume During Stress of Cardioplegia

Myocytes demonstrated significant differences compared to TYR for the first 2 time points of baseline period in the CPG group and for the first baseline time point in the CPG + DZX

at 10 min group. Myocytes in all other groups demonstrated no significant volume change from baseline during exposure to TYR (Figure 3).

Myocytes demonstrated significant swelling when exposed to CPG ($p < 0.05$ vs. TYR). This swelling was not prevented by DZX when administered either at 10 or 20 min after the onset of stress (Figure 3).

Myocyte volume remained significantly increased for only the first 5 min of the re-exposure period in the CPG and the CPG + DZX at 20 min groups. All other groups demonstrated return to and maintenance of baseline volume by the first 5 min 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 (Figure 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 min + DZX in re-exposure group until 10 min into the stress period. This swelling was not prevented by DZX when administered either at 10 or 20 min after the onset of stress (Figure 4). Myocyte volume remained significantly increased for only the first 5 min of the re-exposure to TYR period in the MI and MI + DZX at 20 min groups. All other groups demonstrated return to and maintenance of baseline volume by the first 5 min 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 (Figure 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 min and the MI + DZX in re-exposure only groups until 5 min of stress. Myocyte swelling was not prevented by DZX when administered either at 10 or 20 min after the onset of stress in all groups except in the MI + DZX at 10 min + 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 min of the re-exposure period in the MI group. All other groups demonstrated return to and maintenance of baseline volume by the first 5 min 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 (Figure 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 20min. of stress. This swelling was not prevented by DZX when administered at any time point (Figure 6).

Myocyte volume returned to levels that were not significantly different from baseline after 5 min. re-exposure to 1T 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 (Figure 7). Myocytes demonstrated significant swelling when exposed to 0.9T TYR solution throughout stress ($p=0.00-0.01$ 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 min. ($p=0.45$) after the onset of stress compared to 0.9T (Figure 7).

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

Mouse Myocyte Contractility During Stress of Cardioplegia

Myocytes demonstrated a decline in contractility following exposure to CPG (Figure 8). This decline in contractility was significant in at least one measure of contractility in groups CPG + DZX at 20 min and in CPG + DZX at 20 min + 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 min following re-exposure to TYR (Figure 8).

Mouse Myocyte Contractility During Hypo osmotic Stress

Myocytes demonstrated a decline in contractility following exposure to 0.9T solution ($p=0.87$) (Figure 9). This decline in contractility was not altered by the addition of DZX at any time point (Figure 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) that is prevented by the administration of K_{ATP} channel opener diazoxide *at the onset of stress*.¹⁻⁴ The consistent relationship between myocyte swelling and reduced contractility suggests myocyte swelling may be one mechanism of myocardial stunning. The present study was performed to determine if diazoxide 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 the present 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 min *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 upon re-exposure to TYR likely indicates a return of volume homeostatic mechanisms and suggests that the mechanism of DZX does not involve the reduction of reactive oxygen species or other mechanisms that are known to reduce reperfusion injury.

A decline in contractility was noted in all groups following cardioplegia and hypo osmotic stress; however, this was only statistically significant in two groups at 10 min following stress ($p=0.02$ velocity of shortening and $p=0.02$ peak velocity of re-lengthening in TYR vs. CPG + DZX at 20 min; and $p=0.048$ velocity of shortening in TYR vs. CPG + DZX in 20 min + DZX in re-exposure). All groups exposed to CPG had improvement in contractility by 20 min. after stress. This is in contrast to previous work (with a shorter stress period) in which a statistically significant decline in contractility was demonstrated in the cardioplegia group following 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 the present study.

Previous investigators using a variety of models have documented conflicting results with diazoxide administration after the onset of stress, ischemia, or hypoxia.^{10–13} In an intact rabbit heart model of 30 min regional ischemia and 3 hours reperfusion, DZX during ischemia was found to provide no benefit to infarct size.¹⁰ In a model of rat cultured myocytes subjected to 12 hours hypoxia and 12 hours reoxygenation, the 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 min global ischemia and 2 hours reperfusion, DZX after 10 min of ischemia demonstrated decreased infarct size; however, DZX administration after 25 min of ischemia did not reduce infarct size.¹² Administration of DZX 10 min before and throughout a 90 min hypoxic period in isolated human trabeculae demonstrated improved recovery of contractility compared to controls; however, no benefit was demonstrated when DZX was given only as a preconditioning agent or during reoxygenation alone.¹³ Thus, 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 *prior to* stress, previous work by our laboratory and others has documented the benefit of DZX when administered *at the onset* of stress.^{1–4} The data from the present study also suggest that the mechanism of action of diazoxide 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.^{15,17–19} 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 utilized a 20 min stress period.¹⁻² The stress period for the current study was lengthened to 30 min 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 to 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 utilized 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.

Future Directions

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

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Abbreviations

K_{ATP}	Adenosine triphosphate – sensitive potassium channel
CPG	Hypothermic hyperkalemic cardioplegia
DMSO	Dimethyl sulfoxide
DZX	Diazoxide
MI	Metabolic inhibition
0.9T	Hypo osmotic stress
PR	Percentage of relengthening
PS	Percentage of cell shortening
TYR	Tyrodé's solution
VS	Velocity of cell shortening

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Experimental Protocol

Figure 1.

Schemata of experimental protocol. Myocytes were exposed to 37°C control TYR for 20 min (CPG stress groups) or 10 min (MI stress groups and Hypo osmotic stress groups), followed by stress test solution (per methods) for 30 min, then 20 min re-exposure to Tyrode's solution. Volume measurements were taken every 5 min (V symbol). Contractility measurements were taken at times 5 min and 60 and 70 min (stars). Diazoxide was introduced at 3 different time points during the protocol (arrows) +/- continuation throughout re-exposure to Tyrode's (brackets). CPG, xxx; DZX, diazoxide.

Figure 2.

Diazoxide does not prevent mouse myocyte swelling secondary to stress of cardioplegia. Isolated myocytes were exposed to control Tyrode's physiologic solution (TYR) for 20 min (time 5–20), stress (CPG, CPG + DZX at 10 or 20 min) for 30 min (time 25–50), followed by TYR (+/- DZX) for 20 min (time 55–70). Data are represented as mean $\pm$ SEM. All groups demonstrated significant myocyte swelling vs. TYR ($p < 0.05$) at all time points during stress. Unfilled symbols represent $p < 0.05$ vs. Tyrode's solution. CPG, xxx; DZX, diazoxide.

Figure 3.

Diazoxide does not prevent human myocyte swelling secondary to stress of cardioplegia. Isolated myocytes were exposed to control Tyrode's physiologic solution (TYR) for 20 min (time 5–20), stress (CPG, CPG + DZX at 10 or 20 min) for 30 min (time 25–50), followed by TYR (+/- DZX) for 20 min (time 55–70). Data are represented as mean $\pm$ SEM. All groups demonstrated significant myocyte swelling vs. TYR ($p < 0.05$) at all time points during stress. Unfilled symbols represent $p < 0.05$ vs. Tyrode's. CPG, xxx; DZX, diazoxide

Figure 4.

Diazoxide does not prevent mouse myocyte swelling secondary to stress of metabolic inhibition. Isolated myocytes were exposed to control Tyrode's physiologic solution (TYR) for 10 min (time 5–10), stress (MI, MI + DZX at 10 or 20 min) for 30 min (time 15–40), followed by TYR (+/– DZX) for 20 min (time 45–60). Data are represented as mean $\pm$ SEM. All groups demonstrated significant myocyte swelling vs. TYR ($p < 0.05$) at all time points during stress (except the DZX at 10 min + re-exposure to TYR group at the first time point of stress). Unfilled symbols represent $p < 0.05$ vs. Tyrode's. * represents $p < 0.05$ vs. MI + DZX in re-exposure. Ψ represents $p < 0.05$ vs. MI + DZX in 20 min + DZX in re-exposure. + represents $p < 0.05$ vs. MI + DZX in 10 min + DZX in re-exposure. CPG, xxx; DZX, diazoxide; MI, metabolic inhibition.

Figure 5.

Diazoxide does not prevent human myocyte swelling secondary to stress of metabolic inhibition. Isolated myocytes were exposed to control Tyrode's physiologic solution (TYR) for 10 min (time 5–10), stress (MI, MI + DZX at 10 or 20 min) for 30 min (time 15–40), followed by TYR (+/– DZX) for 20 min (time 45–60). Data are represented as mean $\pm$ SEM. All groups demonstrated significant myocyte swelling vs. TYR ($p < 0.05$) at all time points during stress except the MI + DZX at 10 min + DZX in re-exposure group for the last time point of stress. Unfilled symbols represent $p < 0.05$ vs. Tyrode's. + represents $p < 0.05$ vs. MI + DZX in 10 min + DZX in re-exposure. DZX, diazoxide; MI, metabolic inhibition

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 min (time 5–10), stress (0.9T, 0.9T + DZX at 10 or 20 min) for 30 min (time 15–40), followed by 1T TYR (+/- DZX) for 20 min (time 45–60). 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 min (time 5–10), stress (0.9T, 0.9T + DZX at 10 or 20 min) for 30 min (time 15–40), followed by 1T TYR (+/- DZX) for 20 min (time 45–60). 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.

Figure 8.

Diazoxide does not preserve myocyte contractility following stress of cardioplegia. Isolated mouse myocytes were exposed to control Tyrode's physiologic solution (TYR) for 20 min (time 5–20), stress (CPG, CPG + DZX at 10 or 20 min) for 30 min (time 25–50), followed by TYR (+/- DZX) for 20 min (time 55–70). Data are represented as mean and error bars have been removed for clarity. All groups demonstrated a reduction in myocyte contractility vs. TYR following stress and this was statistically significant in the CPG + DZX in 20 min. and the CPG + DZX in 20 min + DZX in re-exposure groups. All groups demonstrated significant improvement in contractility between 10 and 20 min following re-exposure to TYR (time 60 min vs. 70 min). * $p < 0.05$ vs. TYR. CPG, xxx; DZX, diazoxide; MI, metabolic inhibition.

Figure 9.

Diazoxide does not preserve myocyte contractility following hypoosmotic stress. Isolated mouse myocytes were exposed to control 1T Tyrode's physiologic solution (TYR) for 10 min (time 5–10), stress (0.9T, 0.9T + DZX at 10 or 20 min) for 30 min (time 15–40), followed by 1T TYR (+/- DZX) for 20 min (time 45–60). Data are represented as mean and error bars have been removed for clarity. All groups demonstrated a non statistically significant reduction in myocyte contractility vs. 1T TYR following stress ($p=0.87$). DZX, diazoxide.