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Superior diastolic function with K_{ATP} channel opener diazoxide in a novel mouse Langendorff model

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Abstract

Background: Adenosine triphosphate-sensitive potassium (K_{ATP}) channel openers have been found to be cardioprotective in multiple animal models via an unknown mechanism. Mouse models allow genetic manipulation of K_{ATP} channel components for the investigation of this mechanism. Mouse Langendorff models using 30 min of global ischemia are known to induce measurable myocardial infarction and injury. Prolongation of global ischemia in a mouse Langendorff model could allow the determination of the mechanisms involved in K_{ATP} channel opener cardioprotection.

Methods: Mouse hearts (C57BL/6) underwent baseline perfusion with Krebs-Henseleit buffer (30 min), assessment of function using a left ventricular balloon, delivery of test solution, and prolonged global ischemia (90 min). Hearts underwent reperfusion (30 min) and functional assessment. Coronary flow was measured using an inline probe. Test solutions included were as follows: hyperkalemic cardioplegia alone (CPG, $n = 11$) or with diazoxide (CPG + DZX, $n = 12$).

Results: Although the CPG + DZX group had greater percent recovery of developed pressure and coronary flow, this was not statistically significant. Following a mean of 74 min (CPG) and 77 min (CPG + DZX), an additional increase in end-diastolic pressure was noted (plateau), which was significantly higher in the CPG group. Similarly, the end-diastolic pressure (at reperfusion and at the end of experiment) was significantly higher in the CPG group.

Conclusions: Prolongation of global ischemia demonstrated added benefit when DZX was added to traditional hyperkalemic CPG. This model will allow the investigation of DZX

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mechanism of cardioprotection following manipulation of targeted K_{ATP} channel components. This model will also allow translation to prolonged ischemic episodes associated with cardiac surgery.

Keywords

Cardioplegia; Myocardial protection; Mouse model; Animal model; Diazoxide

Introduction

The adenosine triphosphate-sensitive potassium (K_{ATP}) channel exists in metabolically active tissues throughout the body and provides endogenous myocardial protection via coupling of cell membrane potential to myocardial metabolism (inhibited by ATP and open during times of metabolic stress).^{1–5} Pharmacologic opening of K_{ATP} channels with diazoxide (DZX) prevents detrimental myocardial swelling and reduced contractility in response to different types of stress: metabolic inhibition, exposure to hyperkalemic cardioplegia (CPG), and hypo-osmotic stress in animal^{6–8} and in human cardiac myocytes.⁹ The exact mechanism of protection provided by K_{ATP} channel openers is incompletely defined and is the focus of intensive research.

Mouse Langendorff models with 20–45 min of global ischemia are known to induce myocardial infarction.^{10–14} The benefit of K_{ATP} channel openers over conventional, hypothermic, hyperkalemic CPG is yet to be demonstrated. Previous models failed to emulate the additive injury induced during cardiac surgery, which is composed of prolonged global ischemia and exposure to hyperkalemic CPG. We sought to develop a murine model translatable to this setting and use it to assess the protective effect provided by K_{ATP} channel openers as a component of hypothermic, hyperkalemic CPG.

Methods

All animals received humane care in compliance with the “Principles of Laboratory Animal Care” formulated by the National Society for Medical Research and the “Guide for the Care and Use of Laboratory Animals” published by the US National Institutes of Health.¹⁵ The Animal Care and Use Committee from Washington University approved of this study.

Animals and anesthesia

Adult, male, C57BL/6 mice (25 g) were anesthetized by intraperitoneal injection of 0.4 mL Avertin (3% 2,2,2-tribromoethanol, 1.86% 2-methyl-2-butanol) and 100 units of unfractionated heparin. Sternotomy and rapid cardiectomy were performed. The heart was submersed in an ice-cold Krebs-Henseleit buffer (KHB) while aortic cannulation with a blunted 22-gauge needle was performed using a dissection microscope. Hearts were then perfused with KHB (NaCl 118.5, NaHCO_3 25.0, KCl 3.2, KH_2PO_4 1.2, MgSO_4 1.2, CaCl_2 , and D-glucose 35.0 [concentrations expressed in mmol/L]) at a pH range between 7.40 and 7.48 by bubbling with a gas mixture of 95% O_2 and 5% CO_2 . Perfusate passed through 45-mm polyvinylidene fluoride filters (EMD Millipore Corp, Billerica, MA) and was not recirculated. Hearts were excluded if aortic cannulation time exceeded 5 min. The left

atrium was opened by amputating the pulmonary veins to allow visualization of the mitral apparatus. A balloon was introduced through the mitral valve. All hearts were epicardially paced at 170 beats per minute and surrounded by a warm (37°C) water-jacketed beaker. Coronary perfusion pressure was maintained at 80 mm Hg, determined by the height of the column (see Fig. 1). This pressure has been identified as the zone of autoregulation for coronary vasculature in murine hearts.¹⁶

Experimental protocol

The left ventricular (LV) balloon was connected via a 15-cm long polyethylene tube to a HP1290C pressure transducer (Hewlett Packard, Andover, MA) and a 20-4 amplifier (Gould Instrument Systems Inc, Valley View, OH). The balloon volume was adjusted to an end-diastolic pressure (EDP) of 2.5 mm Hg. Isolated hearts underwent a 30-min baseline period permitting stabilization. Then a pressure–volume curve was obtained by 1.4 μ L balloon volume increments with 90-s intervals between these for stabilization and data collection. The LV balloon volume was returned to the initial volume. Volume increments were precisely administered using a threaded plunger syringe (Hamilton Company, Franklin, MA). Hearts were arrested with test solution and pacing interrupted. A 90-min ischemic injury was sustained with a continuous epicardial drip of KHB maintained at a temperature of 27°C. The test solutions included were as follows: hyperkalemic CPG alone (Plegisol, Pfizer, New York City, NY) (in mmol/L: NaCl, 110; NaHCO₃, 10; KCl, 16; Mg, 32; and CaCl₂, 2.4) or CPG + DZX (Sigma Aldrich, St. Louis, MO) (7-chloro-3-methyl-1,2,4-benzothiadiazine-1,1-dioxide) at a dose of 100 μ mol/L. The DZX dose was chosen based on previous experiments.^{6–9} A stock solution of DZX was made by dissolving in 0.1% dimethyl sulfoxide, a concentration at which the antioxidant effect of dimethyl sulfoxide remains inconsequential.¹⁷ Pressure measurements were continuously recorded throughout the experiment. At the end of the ischemic period, retrograde coronary perfusion and epicardial pacing were resumed, providing reperfusion during 30 min. A second pressure–volume curve was recorded at identical balloon volumes as previously described.

Construction of LV balloons

LV balloons were constructed using silicone to be small, durable, and highly elastic.¹⁸ In brief, a hard candy mold was dipped into silicone dispersion gel (NuSil Tech, Carpinteria, CA) to create a thin layer around the mold, and the gel-covered mold was dried followed by submersion in deionized water to dissolve the candy. The silicone sleeve was removed from the mold by squeezing the bland candy out. The sleeve was then cannulated by a PE 60 tube (Becton Dickinson, Franklin Lakes, NJ) and tied with 4-0 silk to create a water-tight seal. Since presence of air in the circuit could dampen the pressure signal significantly, all the balloons were assessed under magnification for bubbles before use.

Data acquisition and analysis

Left ventricular end-systolic (ESP), EDP, and developed (LVDP) pressures were determined from digitalized data files with the use of LabVIEW 2014 (National Instruments, Austin, TX) set at a sampling rate of 1000 Hz. The software quantifies ESP as the maximum value of each waveform and EDP as the point at which the increasing slope of the pressure waveform exceeds a threshold of 0.5 mm Hg/ms. LVDP was defined as the difference

between ESP and EDP for each data point. Average ESP, EDP, and LVDP were calculated by sampling 10 consecutive beats. Coronary flow rates were measured every 5 min by an inline N-series flow probe (Transonic Systems, Ithaca, NY) and a T206 flow meter (Transonic Systems, Ithaca, NY). Figure 2 illustrates LVDP over a series of balloon volumes in a single experiment from pressure–volume curves before and after injury. The percent recovery of LVDP was defined as the quotient of the area under the curve (AUC) for the reperfusion LVDP (blue) by the AUC for baseline LVDP (orange). AUC was calculated by the use of the trapezoidal rule as depicted in the following equation:

$$100 * \int_{BV_{min}}^{BV_{max}} \frac{LVDP \text{ postreperfusion } dBV}{LVDP \text{ baseline}} \big/ (BV_{max} - BV_{min})$$

where BV_{max} is the maximum postreperfusion-matched balloon volume, and BV_{min} is the minimum postreperfusion-matched balloon volume. The percent recovery of coronary flow rate was calculated in a similar manner to that described for LVDP. Raw values of ESP, EDP, and LVDP were standardized by the baseline measurements for each experiment and described as percent from baseline (see Figs. 3 and 4).

Ventricular compliance was assessed through change in EDP over a series of identical intracavitary balloon volumes as obtained from the pressure–volume curves (Fig. 4). After standardizing for baseline values, mean EDP at each volume was compared between groups. To quantify the impact of preservation of ventricular compliance provided by DZX, a linear regression model was fitted with EDP as the outcome variable using end-diastolic volume and treatment group as covariates.

The investigators were blinded to test groups at the time of performing the experiments. Results are reported as mean $\pm$ standard deviation unless stated otherwise. Univariate comparisons were conducted through Wilcoxon rank-sum test or unpaired Student's *t*-test for continuous variables, as appropriate. *P*-value < 0.05 (two-sided) is used to define statistical significance. All statistical analyses were done with *R* software, version 3.3.2.¹⁹

Waveform morphology

Using intraventricular balloon-recorded pressure measurements during ischemia, a characteristic and reproducible signal became evident, as seen in Figure 3. Recurring landmarks in this waveform were consistently measured and labeled as: zero EDP, ischemic EDP, plateau EDP, third plateau EDP, reperfusion EDP, and final EDP. Zero EDP was measured 1 min before cardioplegic arrest. Ischemic EDP was measured immediately after cessation of ventricular activity from the arrest. Plateau EDP was measured at the point where pressure remained unchanged for 5 min. Reperfusion EDP was measured 1 min before the reinstatement of retrograde aortic perfusion. The third plateau EDP was measured at the highest point after reperfusion, usually occurring within 4–8 min of reperfusion. The final EDP was measured after 30 min of reperfusion. Time to plateau was measured from the start of the ischemic injury to beginning of the plateau in minutes. The $t_{1/2}$ was measured as half of the duration of the slope to reach the plateau in minutes.

Results

In the present study, the effects of a 90-min period of global ischemia on myocardial mechanics were studied in an isolated murine heart Langendorff preparation. Twenty-three experiments were performed, 11 in the control group (CPG) and 12 in the test group (CPG + DZX). Three hearts in the control group and one in the test group failed to recover contractile activity after the ischemic injury ($P = 0.518$). Hearts with failure to recover from injury were included for the comparisons in Table 1. The percentage recovery of LVDP was similar between groups with 22.3 ± 20.8 for CPG and 28.3 ± 21.2 for CPG + DZX ($P = 0.385$). The percentage recovery of coronary flow was similar between groups with 42.5 ± 31.8 for CPG and 54 ± 37.3 for CPG + DZX ($P = 0.559$) (Table 1).

Waveform morphology

Time to plateau was similar between the CPG and CPG + DZX groups: 75 *versus* 77 min ($P = 0.600$). $t_{1/2}$ to plateau was similar between groups: 62 *versus* 69 min ($P = 0.097$). Baseline EDP was similar between groups: 1.0 *versus* 0.7 mm Hg ($P = 0.999$). Ischemic EDP was also similar between groups: 2.5 *versus* 2.5 mm Hg ($P = 0.999$). Plateau EDP was higher in the CPG group: 11.5 *versus* 8.2 mm Hg ($P = 0.042$). Reperfusion EDP was higher in the CPG group: 12.0 *versus* 8.4 mm Hg ($P = 0.016$). The third plateau EDP was higher in the CPG: 33.8 *versus* 18.5 mm Hg ($P = 0.042$). Final EDP was higher in the CPG group: 24.9 *versus* 19 mm Hg ($P = 0.049$) (Table 1).

Preservation of compliance and contractility

Compliance was quantified only in those hearts that recovered from the injury to obtain a second pressure–volume curve. The mean baseline EDP was similar between groups (P -values > 0.05 for all balloon volumes). The mean increase from baseline EDP was greater in the CPG group than that in the CPG + DZX group for three of the five balloon volumes: 686 *versus* 362% at 0 μ L ($P = 0.007$), 871 *versus* 509% at 1.4 μ L ($P = 0.004$), 962 *versus* 631% at 2.8 μ L ($P = 0.062$), 1236 *versus* 743% at 4.2 μ L ($P = 0.027$), and 1391 *versus* 759% at 5.6 μ L ($P = 0.073$) (Fig. 4). In a linear regression model for percent increase from baseline EDP, the coefficient for belonging to the CPG + DZX group was -414.7% ($P < 0.001$) (Table 2).

Contractility was also quantified only for those hearts that recovered from the injury. The mean LVDP was similar between the groups at baseline (all P -values > 0.05). The mean percentage LVDP change from baseline was similar between CPG and CPG + DZX groups: 31 *versus* 28% at 0 μ L ($P = 0.968$), 37 *versus* 38% at 1.4 μ L ($P = 0.840$), 42 *versus* 48% at 2.8 μ L ($P = 0.967$), 42 *versus* 74% at 4.2 μ L ($P = 0.360$), and 39 *versus* 66% at 5.6 μ L ($P = 0.180$) (Fig. 5).

Discussion

This study builds on previous work from our laboratory which explored the cellular mechanisms involved in the cardioprotective effect of K_{ATP} channel openers in murine ventricular^{6–8} and in human atrial myocytes.⁹ The present study suggests that the addition of DZX to hyperkalemic CPG provides reduced diastolic injury in hearts exposed to a prolonged, ischemic injury through preservation of the diastolic pressure–volume

relationship. Other investigators have suggested that reduction in ventricular compliance is secondary to the severity of the ischemic injury.^{20–22} A reduction in ventricular compliance was observed during ischemia in both groups and was more pronounced in the control group (experiencing significantly higher plateau, reperfusion, third plateau, and final EDP values) (Table 1). These findings were also evident in the upward shift in the EDP-volume relationship (Table 2 and Fig. 4).

K_{ATP} channel openers such as DZX have been shown to mimic ischemic preconditioning in multiple animal models.^{1–5} Findings of the present study demonstrate preservation of diastolic function after a prolonged global ischemic period. These two beneficial effects are likely related to separate and independent mechanisms that may or may not include a K_{ATP} channel and require further research. Post-ischemic diastolic dysfunction occurs from uncoupled relaxation-repolarization and is usually associated with myocardial stunning. Contributing factors to stunning may include generation of oxygen-free radicals, intracellular calcium overload, and decreased responsiveness of contractile filaments.²³ In a canine model of cardioplegic arrest and global ischemia, it was determined that these cellular mechanisms lead to an overall increase in ventricular wall stiffness secondary to increased myocardial edema.²⁴ In addition, exposure to hypothermic hyperkalemic CPG in the absence of ischemia is known to induce myocyte swelling.²⁵ We previously determined that DZX prevented myocyte swelling secondary to CPG in isolated rabbit⁷ and human²⁶ myocytes. Finally, Murata et al²⁷ determined that preischemic exposure to DZX attenuated mitochondrial calcium overload during ischemia-reperfusion injury. Hence, we hypothesize that the mechanisms of reduced diastolic injury with DZX may be provided a reduction in free radicals, a reduction in cellular edema, and a reduction in intracellular calcium. However, the specific site and mechanism of action for DZX remain elusive. Myocardial stunning²⁸ and diastolic dysfunction^{29,30} have become ever-more prevalent disease processes in patients undergoing increasingly complicated cardiac surgery procedures, emphasizing the need for interventions to ameliorate these phenomena.

Rationale for prolonged injury

Previously reported murine global ischemia-reperfusion injury models are significantly shorter, ranging from 20 to 45 min followed by varying reperfusion periods.^{11–14} Prolongation of ischemic time was necessary to establish a significant injury given the presence of highly effective hyperkalemic CPG. This study describes a novel global ischemic injury tailored to simulate the pathophysiology of prolonged global cardioplegic arrest during cardiopulmonary bypass. In 2014, the European Society of Cardiology working group issued a position paper highlighting the importance of using complex translational models that simulate realistic clinical scenarios at the benchtop³¹ such as this one. Nonetheless, the clinical implications of these findings should be extrapolated cautiously.

The significant decline of LVDP in both groups (percent recovery of only 22%-28% LVDP) indicates a severe injury during the prolonged global ischemic period despite protection of hyperkalemic CPG with or without DZX. The characteristic waveform produced by a prolonged ischemic injury (Fig. 3) initially corresponds to depletion of phosphocreatine and ATP, leading to ischemic contracture and increased intracavitary pressure.³² Prior studies

suggest that by 60 min of ischemia, anaerobic glycolysis has been significantly reduced, and 94% of ATP has been depleted.^{33,34} We theorize that this corresponds to the stabilization of intracavitary pressure and onset of a plateau during ischemia. On reperfusion, a transient increase in EDP becomes evident (third plateau phase) that may be triggered by a combination of reperfusion injury and the “no-reflow” phenomenon.³⁵

The safety of DZX during cardiac surgery has been briefly explored in human studies. DZX has been investigated as a preconditioning agent³⁶ and as an additive to intermittent warm blood antegrade CPG.³⁷ However, these clinical trials included a relatively healthy group of patients. DZX has not been explored as an additive to hypothermic CPG in high-risk patients (who often require repeat surgery or prolonged cardiopulmonary bypass times). We propose that pharmacologic K_{ATP} channel openers have the potential to prevent stunning during prolonged periods of ischemia, and their use in these situations requires further investigation.

Limitations

This study was performed in isolated murine hearts perfused with an *ex-vivo* perfusion apparatus using crystalloid buffers for oxygen delivery. The absence of hemoglobin and plasma proteins requires providing high levels of partial pressure of oxygen while exposing capillaries to a low oncotic pressure. Coronary disease and myocardial ischemia are multifactorial processes that become more prevalent with older age, whereas the hearts used in this study were from healthy mice. It is likely that these hearts would require prolonged periods of ischemia to achieve a significant injury. Specific indices of ventricular relaxation such as the relaxation time constant (τ) or dP/dt min during isovolumetric relaxation were not measured.

Future directions

Further studies are needed to elucidate the precise mechanism of cardioprotection provided by DZX. Myocardial implications of K_{ATP} channel variation have not been extensively explored, and the precise mechanism of action by which K_{ATP} channel openers provide myocardial protection remains unclear. This model provides an *ex-vivo*, whole organ environment to systematically investigate the role of known channel subunits using targeted gene deletion or subunit-specific antagonists.

Conclusion

The addition of DZX to conventional, hypothermic, hyperkalemic CPG maintained ventricular compliance and provided reduced diastolic injury compared with hyperkalemic CPG alone in a novel, prolonged ischemia mouse Langendorff model.

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Authors' contributions: J.S.L., C.G.N., and C.M.M contributed for study design. C.M.M. and E.M.K were responsible for acquisition of data. J.S.L., A.S.P., and R.B.S helped in analysis and interpretation of data. Drafting of manuscript is performed by J.S.L. and A.S.P. Critical revision is done by J.S.L., C.G.N., and A.S.P.

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REFERENCES

1. Grover GJ, Sleph PG, Parham CS. Nicorandil improves postischemic contractile function independently of direct myocardial effects. *J Cardiovasc Pharmacol.* 1990;15:698–705. [PubMed: 1692928]
2. Auchampach JA, Maruyama M, Cavero I, Gross GJ. Pharmacological evidence for a role of ATP-dependent potassium channels in myocardial stunning. *Circulation.* 1992;86:311–319. [PubMed: 1301066]
3. Galinanes M, Shattock MJ, Hearse DJ. Effects of potassium channel modulation during global ischaemia in isolated rat heart with and without cardioplegia. *Cardiovasc Res.* 1992;26:1063–1068. [PubMed: 1291083]
4. Pignac J, Bourgouin J, Dumont L. Cold cardioplegia and the K⁺ channel modulator aprikalim (RP 52891): improved cardioprotection in isolated ischemic rabbit hearts. *Can J Physiol Pharmacol.* 1994;72:126–132. [PubMed: 8050052]
5. Shigematsu S, Sato T, Abe T, Saikawa T, Sakata T, Arita M. Pharmacological evidence for the persistent activation of ATP-sensitive K⁺ channels in early phase of reperfusion and its protective role against myocardial stunning. *Circulation.* 1995;92:2266–2275. [PubMed: 7554211]
6. Mizutani S, Prasad SM, Sellitto AD, Schuessler RB, Damiano RJ, Lawton JS. Myocyte volume and function in response to osmotic stress: observations in the presence of an adenosine triphosphate-sensitive potassium channel opener. *Circulation.* 2005;112(9 Suppl):I219–I223. [PubMed: 16159820]
7. Mizutani S, Al-Dadah AS, Bloch JB, et al. Hyperkalemic cardioplegia-induced myocyte swelling and contractile dysfunction: prevention by diazoxide. *Ann Thorac Surg.* 2006;81:154–159. [PubMed: 16368355]
8. Al-Dadah AS, Voeller RK, Schuessler RB, Damiano RJ, Lawton JS. Maintenance of myocyte volume homeostasis during stress by diazoxide is cardioprotective. *Ann Thorac Surg.* 2007;84:857–862. [PubMed: 17720390]
9. Sellitto AD, Maffit SK, Al-Dadah AS, et al. Diazoxide maintenance of myocyte volume and contractility during stress: evidence for a non-sarcolemmal K(ATP) channel location. *J Thorac Cardiovasc Surg.* 2010;140:1153–1159. [PubMed: 20804990]
10. Sumeray MS, Yellon DM. Characterisation and validation of a murine model of global ischaemia-reperfusion injury. *Mol Cell Biochem.* 1998;186:61–68. [PubMed: 9774186]
11. Whittington HJ, McAndrew DJ, Cross RL, Neubauer S, Lygate CA. Protective effect of creatine elevation against ischaemia reperfusion injury is retained in the presence of co-morbidities and during cardioplegia. *PLoS One.* 2016;11:e0146429. [PubMed: 26765737]
12. Rossello X, Hall AR, Bell RM, Yellon DM. Characterization of the Langendorff perfused isolated mouse heart model of global ischemia–reperfusion injury. *J Cardiovasc Pharmacol Ther.* 2016;21:286–295. [PubMed: 26353758]
13. Ma Y, Zhang L, Edwards JN, Launikonis BS, Chen C. Growth hormone secretagogues protect mouse cardiomyocytes from in vitro ischemia/reperfusion injury through regulation of intracellular calcium. *PLoS One.* 2012;7:e35265. [PubMed: 22493744]
14. Sharma V, Kearney MT, Davidson SM, Yellon DM. Endothelial insulin resistance protects the heart against prolonged ischemia-reperfusion injury but does not prevent insulin transport across the endothelium in a mouse Langendorff model. *J Cardiovasc Pharmacol Ther.* 2014;19:586–591. [PubMed: 24651518]
15. National Research Council. Guide for the Care and Use of Laboratory Animals. 8th ed Washington, D.C: The National Academies Press; 2011 Available at: <https://grants.nih.gov/grants/olaw/guide-for-the-care-and-use-of-laboratory-animals.pdf>. Accessed September 7, 2017.
16. Sutherland FJ, Shattock MJ, Baker KE, Hearse DJ. Mouse isolated perfused heart: characteristics and cautions. *Clin Exp Pharmacol Physiol.* 2003;30:867–878. [PubMed: 14678252]
17. Man W, Ming D, Fang D, Chao L, Jing C. Dimethyl sulfoxide attenuates hydrogen peroxide-induced injury in cardiomyocytes via heme oxygenase-1. *J Cell Biochem.* 2014;115:1159–1165. [PubMed: 24415199]

18. Miller A, Wright GL. Fabrication of murine ventricular balloons for the Langendorff heart preparation. *J Biotechnol Biomater*. 2011;1.
19. R Core Team. R: a language and environment for statistical computing; 2017 Available at: <https://www.r-project.org/>.
20. Schaff HV, Gott VL, Goldman RA, Frederiksen JW, Flaherty JT. Mechanism of elevated left ventricular end-diastolic pressure after ischemic arrest and reperfusion. *J Am Physiol*. 1981;240:H300–H307.
21. Starnes VA, Hammon JW. The influence of acute global ischemia on left ventricular compliance in the adult and immature dog. *J Surg Res*. 1981;30:281–286. [PubMed: 7230777]
22. Lorell BH, Palacios I, Daggett WM, Jacobs ML, Fowler BN, Newell JB. Right ventricular distension and left ventricular compliance. *Am J Physiol*. 1981;240:H87–98. [PubMed: 7457623]
23. Bolli R, Marban E. Molecular and cellular mechanisms of myocardial stunning. *Physiol Rev*. 1999;79:609–634. [PubMed: 10221990]
24. Tabayashi K, McKeown PP, Miyamoto M, et al. Diastolic mechanical properties of the left ventricle after global ischemia. *J Cardiovasc Surg*. 1991;32:250–258. [PubMed: 2019630]
25. Drewnowska K, Clemon HF, Baumgarten CM. Prevention of myocardial intracellular edema induced by St. Thomas' Hospital cardioplegic solution. *J Mol Cell Cardiol*. 1991;23:1215–1221. [PubMed: 1666412]
26. Maffit SK, Sellitto AD, Al-Dadah AS, Schuessler RB, Damiano RJ, Jr, Lawton JS. Diazoxide maintains human myocyte volume homeostasis during stress. *J Am Hear Assoc*. 2012;1 10.1161/JAHA.112.000778.
27. Murata M, Akao M, O'Rourke B, Marbán E. Mitochondrial ATP-sensitive potassium channels attenuate matrix Ca(2+) overload during simulated ischemia and reperfusion: possible mechanism of cardioprotection. *Circ Res*. 2001;89:891–898. [PubMed: 11701616]
28. Rinaldi CA, Hall RJ. Myocardial stunning and hibernation in clinical practice. *Int J Clin Pract*. 2000;54:659–664. [PubMed: 11221279]
29. Lappas DG, Skubas NJ, Lappas GD, Ruocco E, Tambassis E, Pasque M. Prevalence of left ventricular diastolic filling abnormalities in adult cardiac surgical patients: an intraoperative echocardiographic study. *Semin Thorac Cardiovasc Surg*. 1999;11:125–133. [PubMed: 10378856]
30. Bernard F, Denault A, Babin D, et al. Diastolic dysfunction is predictive of difficult weaning from cardiopulmonary bypass. *Anesth Analg*. 2001;92:291–298. [PubMed: 11159219]
31. Lecour S, Bøtker HE, Condorelli G, et al. ESC working group cellular biology of the heart: position paper: improving the preclinical assessment of novel cardioprotective therapies. *Cardiovasc Res*. 2014;104:399–411. [PubMed: 25344369]
32. Ventura-Clapier R, Veksler V. Myocardial ischemic contracture. Metabolites affect rigor tension development and stiffness. *Circ Res*. 1994;74:920–929. [PubMed: 8156639]
33. Reimer KA, Hill ML, Jennings RB. Prolonged depletion of ATP and of the adenine nucleotide pool due to delayed resynthesis of adenine nucleotides following reversible myocardial ischemic injury in dogs. *J Mol Cell Cardiol*. 1981;13:229–239. [PubMed: 7265245]
34. Reimer KA, Jennings RB, Tatum AH. Pathobiology of acute myocardial ischemia: metabolic, functional and ultrastructural studies. *Am J Cardiol*. 1983;52:72A–81A.
35. Kloner RA, Ganote CE, Jennings RB. The "No-Reflow" phenomenon after temporary coronary occlusion in the dog. *J Clin Invest*. 1974;54:1496–1508. [PubMed: 4140198]
36. Wang X, Wei M, Kuukasjärvi P, et al. Novel pharmacological preconditioning with diazoxide attenuates myocardial stunning in coronary artery bypass grafting. *Eur J Cardiothorac Surg*. 2003;24:967–973. [PubMed: 14643816]
37. Deja MA, Malinowski M, Golba KS, et al. Diazoxide protects myocardial mitochondria, metabolism, and function during cardiac surgery: a double-blind randomized feasibility study of diazoxide-supplemented cardioplegia. *J Thorac Cardiovasc Surg*. 2009;137:997–1004, 1004–2. [PubMed: 19327530]

Fig. 1 –.
Schematic representation of mouse Langendorff apparatus. Apparatus used for prolonged (90 min) global ischemia Langendorff model.

Fig. 2 –.

Depiction of LVDP calculation (area under the curve) for a single experiment. Each isolated mouse heart underwent 90 min of global ischemia and 30 min reperfusion. Left ventricular pressure was measured using identical balloon volumes. Distribution of LVDP over an incremental series of intracavitary balloon volumes in a single experiment. In orange, LV developed pressure before 90 min of ischemia, and blue, after the injury. The quotient of the area under the reperfusion curve by the baseline curve provides the percentage recovery of LVDP after the injury.

Fig. 3 –.

Representative pressure tracing during a single experiment. Each isolated mouse heart underwent 90 min of global ischemia and 30 min of reperfusion. Left ventricular pressure was measured using identical balloon volumes. Zero EDP was measured a minute before cardioplegic arrest. Ischemic EDP was measured immediately after cessation of ventricular activity from the arrest. Plateau EDP was measured at the point where pressure remained unchanged for 5 min. Reperfusion EDP was measured 1 min before the reinstatement of retrograde aortic perfusion. The third plateau EDP was measured at the highest point after reperfusion, usually occurring within 4-8 min of reperfusion. The final EDP was measured after 30 min of reperfusion. Time to plateau was measured from the start of the ischemic injury to beginning of the plateau in minutes. The $t_{1/2}$ was measured as half of the duration of the slope to reach the plateau in minutes.

Fig. 4 –.

Mean EDP for all experiments at each balloon volume. Each isolated mouse heart underwent 90 min of global ischemia and 30 min of reperfusion. Left ventricular pressure was measured using identical balloon volumes. Mean EDP, expressed as a percentage from the baseline measurement, over an incremental series of intracavitary balloon volumes. Error bars represent standard error of the mean. Any reperfusion value above its baseline value represents a loss in ventricular compliance (i.e., change in pressure at the same volume) secondary to the ischemic injury. The control group (CPG) consistently had higher EDP in comparison with the study group (CPG + DZX).

Fig. 5 –.

Mean percentage LVDP for all experiments at each balloon volume. Each isolated mouse heart underwent 90 min of global ischemia and 30 min of reperfusion. Left ventricular pressure was measured using identical balloon volumes. LVDP expressed as a percentage from the baseline measurement over an incremental series of intracavitary balloon volumes. Errors bars represent standard error of the mean. LVDP values were similar between groups both at baseline and after reperfusion (all P -values > 0.05).

Table 1 –

comparison of hemodynamic outcomes following 90 min of global ischemia.

Endpoints	CPG	CPG + DZX	P-value
% Recovery of developed pressure	22.3 ± 20.8	28.3 ± 21.2	0.385
% Recovery of coronary flow	42.5 ± 31.8	54 ± 37.3	0.559
Time to plateau (min)	74.9 ± 9.7	77.2 ± 7.7	0.600
t½ to plateau (min)	61.6 ± 8.2	68.9 ± 9.9	0.097
Baseline EDP (mm Hg)	1.0 ± 2	0.7 ± 1.2	0.999
Ischemic EDP (mm Hg)	2.5 ± 1	2.5 ± 1.3	0.999
Plateau EDP (mm Hg)	11.5 ± 3.6	8.2 ± 2.6 *	0.042 *
Reperfusion EDP (mm Hg)	12.0 ± 3.7	8.4 ± 3.3 *	0.016 *
Third plateau EDP (mm Hg)	33.8 ± 9.3	18.5 ± 7.6 *	0.042 *
Final EDP (mm Hg)	24.9 ± 8.9	19.0 ± 6.3 *	0.049 *

Results reported as mean ± standard deviation.

t½ = half-life; CPG = cardioplegia; DZX = diazoxide; EDP = end-diastolic pressure.

* P-value < 0.05 when compared with CPG.

Table 2 –

Left ventricular compliance assessed using linear regression model for end-diastolic pressure, standardized to baseline performance.

Covariates	Coefficient	SE	P-value
Volume (μL)	98.3	20.1	<0.001
Group			
DZX	Ref.	Ref.	Ref.
DZX + CPG	-414.7	77.2	<0.001

CPG = cardioplegia; DZX = diazoxide; Ref. = reference group; SE = standard error.