Kir1.1 and SUR1 are not implicated as subunits of an adenosine triphosphate-sensitive potassium channel involved in diazoxide cardioprotection

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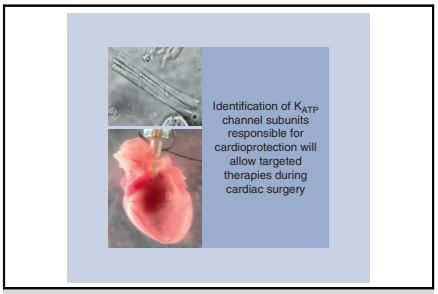
ABSTRACT

Objective: The adenosine triphosphate-sensitive potassium channel opener diazoxide mimics ischemic preconditioning and is cardioprotective. Clarification of diazoxide's site and mechanism of action could lead to targeted pharmacologic therapies for patients undergoing cardiac surgery. Several mitochondrial candidate proteins have been investigated as potential adenosine triphosphate-sensitive potassium channel components. Renal outer medullary potassium (Kir1.1) and sulfonylurea sensitive regulatory subunit 1 have been suggested as subunits of a mitochondrial adenosine triphosphate-sensitive potassium channel. We hypothesized that pharmacologic blockade or genetic deletion (knockout) of renal outer medullary potassium and sensitive regulatory subunit 1 would result in loss of diazoxide cardioprotection in models of global ischemia with cardioplegia.

Methods: Myocyte volume and contractility were compared after Tyrode's physiologic solution (20 minutes), stress (hyperkalemic cardioplegia ± diazoxide, ± VU591 (Kir1.1 inhibitor), N = 9 to 23 each, 20 min), and Tyrode's (20 minutes). Isolated mouse (wild-type, sensitive regulatory subunit 1 [−/−], and cardiac knockout renal outer medullary potassium) hearts were given cardioplegia ± diazoxide (N = 9-16 each) before global ischemia (90 minutes) and 30 minutes reperfusion. Left ventricular pressures were compared before and after ischemia.

Results: Stress (cardioplegia) was associated with reduced myocyte contractility that was prevented by diazoxide. Isolated myocytes were not responsive to diazoxide in the presence of VU591. In isolated hearts, diazoxide improved left ventricular function after prolonged ischemia compared with cardioplegia alone in wild-type and knockout (sensitive regulatory subunit 1 [−/−] and cardiac knockout renal outer medullary potassium) mice.

Conclusions: Isolated myocyte and heart models may measure independent and separate actions of diazoxide. By definitive genetic deletion, these data indicate that sensitive regulatory subunit 1 and renal outer medullary potassium are not implicated in cardioprotection by diazoxide. (JTCVS Open 2023;15:231-41)



Identification of K_{ATP} channel subunits responsible for cardioprotection for clinical use.

CENTRAL MESSAGE

Myocyte and heart models may measure independent actions of diazoxide; however, definitive genetic deletion of SUR1 and ROMK subunits suggests they are not implicated in cardioprotection by diazoxide.

PERSPECTIVE

K_{ATP} channel openers mimic IPC and may be exploited during cardiac surgery to reduce myocardial stunning. The molecular mechanism is unknown, and potential channel subunits SUR1 and ROMK are implicated. By using genetic deletion, we demonstrate they are not involved. Identification of implicated subunits would allow the creation of direct targeted channel openers to be used during cardiac surgery.

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Abbreviations and Acronyms

CKO = cardiac knockout
CPG = hypothermic hyperkalemic cardioplegia
DZX = diazoxide
EDP = end-diastolic pressure
IPC = ischemic preconditioning
K_{ATP} = adenosine triphosphate-sensitive potassium
KHB = Krebs–Henseleit buffer
KO = knockout
LVDP = left ventricular developed pressure
ROMK = renal outer medullary potassium
SUR1 = sulfonylurea sensitive regulatory subunit 1
TYR = Tyrode's physiologic solution
WT = wild-type

Ischemic preconditioning (IPC) is a known beneficial strategy to protect the heart from a subsequent ischemic insult. However, IPC using intermittent ischemia with aortic occlusion is not practical or appropriate in the setting of cardiac surgery. Pharmacologic openers of adenosine triphosphate–sensitive potassium (K_{ATP}) channels mimic IPC by exploitation of the heart's own mechanism of myocardial protection. K_{ATP} channels couple cell membrane potential to myocardial metabolism and are inhibited by ATP and open in the setting of metabolic stress.[1] Large animal models have documented improved recovery after global ischemia with the K_{ATP} channel opener diazoxide (DZX) in cardioplegia and small-sample human trials have documented benefits when K_{ATP} channel openers have been administered before cardiac surgery or in warm cardioplegia.[2-6] K_{ATP} channel openers thus provide an innovative strategy to reduce myocardial stunning after the global ischemia imposed during cardiac surgery.

The precise molecular mechanism of DZX cardioprotection is unknown. Both K_{ATP} channel and non-K_{ATP} channel mechanisms have been explored.[7-11] The molecular basis of sarcolemmal K_{ATP} channels is clear; however, cardioprotection due to K_{ATP} channel opener DZX involves a nonsarcolemmal action and may involve the inhibition of succinate dehydrogenase, thus implicating a possible mitochondrial K_{ATP} channel.[7-13] No responsible mitochondrial K_{ATP} channel has been identified, and involved channel components remain elusive.

Identification of the location and mechanism of action of K_{ATP} channel openers is imperative before their widespread clinical targeting in cardioplegia. To identify potential mitochondrial K_{ATP} channel proteins involved in DZX cardioprotection, we have systematically evaluated multiple known or potential K_{ATP} channel subunits (Kir6.1, Kir6.2, Kir1.1, SUR1) using pharmacologic blockade, genetic deletion, or genetic alteration (gain of function) in multiple mouse models (isolated mitochondria, myocyte, and heart [Langendorff)].[12-19] However, no subunit has thus far been definitively implicated in cardioprotection by diazoxide.

Previous isolated myocyte experiments and proteomic analysis of mitochondria have implicated SUR1 and Kir1.1 subunits, respectively, as potential mitochondrial K_{ATP} channel components.[12,14,18,20] Therefore, we hypothesized that SUR1 and Kir1.1 (ROMK) subunits were the K_{ATP} channel SUR and Kir subunits involved in cardioprotection by DZX and that loss of cardioprotection by DZX would be observed with their inhibition or genetic deletion.

MATERIALS AND METHODS

All animal procedures were approved by the Animal Care and Use Committee at Johns Hopkins University. Animals received humane treatment in abidance with the Principles of Laboratory Animal Care as stated by the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals.[21] Although both sexes of animals were used in all experiments, subgroup analyses of results by sex were not performed because of the lack of power given the small numbers of animals in each group.

Mouse Myocyte Stress Model

Myocyte isolation. Wild-type mice (either sex, C57Bl/6J, Jackson Laboratory) underwent rapid cardiectomy with aortic cannulation for extracellular tissue digestion and cellular isolation, using solutions A and B (detailed next), as described previously.[22] The left ventricle was transferred into solution C (detailed next) and gently dispersed. The cells were allowed to sediment by gravity, and serial washings were performed. The typical yield of viable myocytes was 50% to 80% per isolation. Cells were selected for viability using the following criteria: normal rod shape, smooth edges, sharp borders, clear striations, absence of vacuoles or blebbing, and lack of spontaneous beating. Up to 3 myocytes were used per isolation. Myocytes were used in experiments within 4 hours. Investigators were blinded to the test group during each experiment and during data analysis.

Solution A consisted of 116 mmol/L NaCl, 5.36 mmol/L KCl, 0.97 mmol/L Na_2HPO_4, 1.47 mmol/L KH_2PO_4, 21.10 mmol/L Hepes (N-[2-hydroxyethyl]-piperazine-N′-[4-butanesulfonic acid]), 11.65 mmol/L glucose; 26.50 μmol/L phenol red (Sigma-Aldrich), 3.72 mmol/L $MgCl_2$, 4.40 mmol/L $NaHCO_3$, essential vitamins (100X, 10 mL; Gibco), and amino acids (50X, 20 mL; Gibco). Solution B consisted of solution A plus 10 μM $CaCl_2$ and 1.2 mg/mL collagenase (type 2; Worthington Biochemical; Freehold, NJ). Solution C consisted of solution A plus 5 mg/mL bovine serum albumin (Sigma-Aldrich), 1.25 mg/mL taurine, and 150 μM $CaCl_2$.

DZX (7-chloro-3-methyl-1,2,4-benzothiadiazine-1,1-dioxide; Sigma-Aldrich), a mitochondrial K_{ATP} channel opener, was used at a dose (100 μmol/L) shown to be effective in multiple previous studies.[22-24] VU591 (2,2′-oxybis(methylene) bis(5-nitro-1H-benzo[d]imidazole); Sigma-Aldrich, 20 μmol/L for all studies), is a ROMK (Kir1.1) inhibitor. Hyperkalemic cardioplegia (CPG) solution (Plegisol; Pfizer) consisted of 110 mmol/L NaCl, 10 mmol/L $NaHCO_3$, 16 mmol/L KCl, 32 mmol/L Mg, and 2.4 mmol/L $CaCl_2$, titrated to pH 7.8 with 8.4% $NaHCO_3$ solution.

Experimental protocol. Myocytes were initially perfused with 37 °C Tyrode's solution (TYR; 130 mmol/L NaCl, 5 mmol/L KCl, 2.5 mmol/L $CaCl_2$, 1.2 mmol/L MgSO4, 24 mmol/L $NaHCO_3$, 1.75 mmol/L Na_2HPO_4, and 10 mmol/L glucose), buffered to a pH of 7.4 for baseline volume and contractility measurements (Figure 1, *A*). Myocytes were then perfused for 20 minutes with randomly assigned test

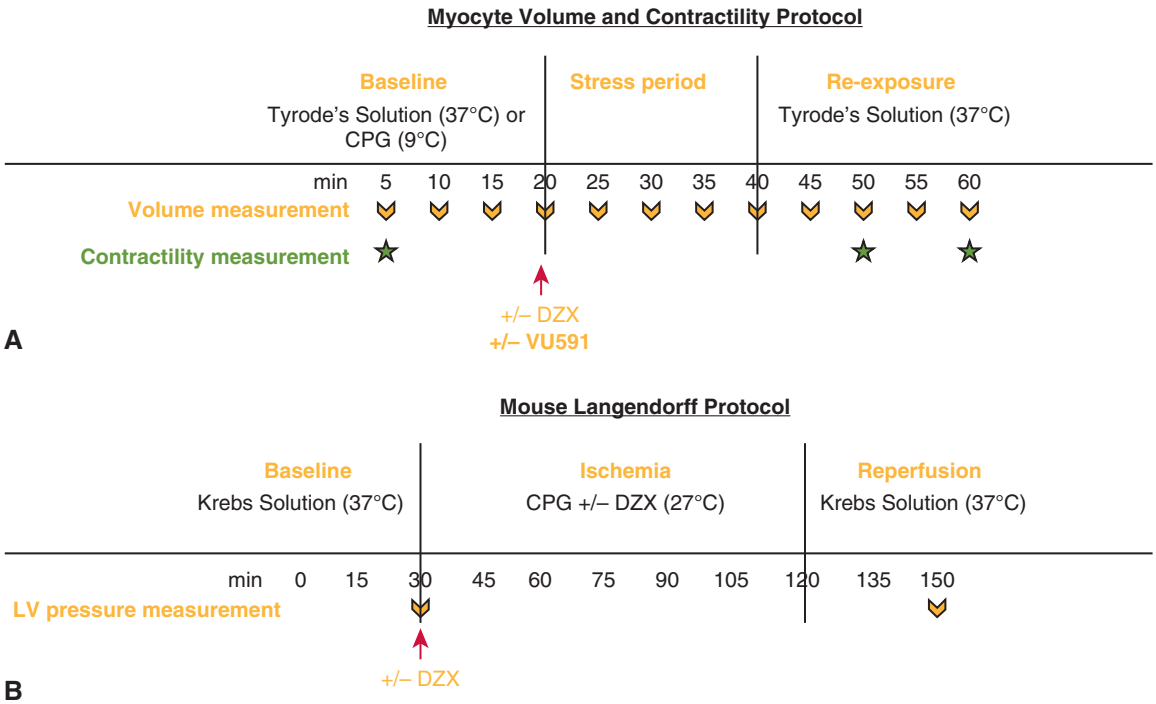

FIGURE 1. Isolated myocyte and heart (Langendorff) experimental protocols. A, Isolated mouse myocytes were exposed to 37 °C control Tyrode's physiologic solution for 20 minutes (baseline), followed by a test/stress period (*red arrow*, 20 minutes) with randomly assigned test solution (n = 9-23 each; Tyrode's solution, Tyrode's + VU591(ROMK inhibitor), 9 °C CPG, 9 °C CPG + DZX, 9 °C CPG + VU591, or 9 °C CPG + DZX + VU591), and then 20 minutes reexposure to Tyrode's. Volume measurements were taken every 5 minutes (*yellow symbols*). Contractility measurements were taken at 5 minutes (baseline) and 10 and 20 minutes after reexposure to Tyrode's (time 50 and 60 minutes) (*green stars*). B, Isolated mouse hearts (WT cKO ROMK littermates, SUR1(−/−), or cKO ROMK) underwent Langendorff baseline perfusion with KHB solution for 30 minutes followed by arrest (*red arrow*), and global ischemia (90 minutes) with test solution (hypothermic hyperkalemic cardioplegia +/− K_{ATP} channel opener diazoxide), and then reperfusion with KHB for 30 minutes. Left ventricular pressures were recorded during baseline (at 30 minutes), and after 30 minutes (time 150) of reperfusion at identical balloon volumes (*yellow symbols*). *CPG*, Cardioplegia; *DZX*, diazoxide; *LV*, left ventricular. A is an adaptation of the figure used in our previous publication.[25]

solution (n = 9-23 each; TYR, TYR + VU591, 9 °C CPG, 9 °C CPG + DZX, 9 °C CPG + VU591, or 9 °C CPG + DZX + VU591). Myocytes were then reexposed to TYR for 20 minutes. The research team was blinded to test group.

The exposure to CPG in this model is considered myocyte stress (during the test solution period), because it is associated with significant myocyte swelling and reduced contractility that is prevented by diazoxide.[23] DZX was administered with CPG because it has been demonstrated to be cardioprotective (prevent significant myocyte swelling and contractility) only when administered at the onset of stress.[25]

Myocyte volume measurement. Myocyte volume was measured as previously described.[22] Isolated myocytes were placed on an inverted microscope stage (model IX51; Olympus). After a 5-minute stabilization, the chamber was perfused (3 mL/min). Chamber temperature was controlled by a water bath system (37 °C; HAAKE; Thermo Electron Karlsruhe, or 9 °C; Polystat; Cole-Parmer). Cell images were displayed on a video monitor using a charge-coupled device camera (IonOptix). Digital images of viable cells were captured using a video frame grabber (Scion) every 5 minutes (Figure 1, *A*). Relative cell volume change was determined as previously described.[22]

Myocyte contractility. Myocyte contractility was measured using a video-based edge detection system (IonOptix) as described previously.[12,26] Cells were paced using a field stimulator (MyoPacer; IonOptix), and data were recorded at baseline and at 10 minute and 20 minute intervals after reexposure to Tyrode's solution (Figure 1, *A*). The following variables were computed using edge-detection software (IonOptix): percentage shortening, peak velocity of shortening, and peak velocity of relengthening. Myocytes that showed less than 7% cell shortening during baseline were excluded.

Statistical analyses. Hypothesis testing was conducted with 1-way analysis of variance. *P* values of pairwise comparisons were adjusted using the Benjamini–Hochberg procedure. Sample size calculations were based on previous work in this model. Studies were designed to detect a 5% ± 4% difference using an $\alpha = 0.05$ and a power of 0.80. Statistical analyses were done using R version 3.5.2 (R Core Team), and illustrations were created using Prism version 8.0.2 (GraphPad Software).

Isolated Mouse Heart (Langendorff) Model of Global Ischemia

Isolated heart preparation. Wild-type mice (C57Bl6/J) were acquired from Jackson Laboratory. The CRISPR/Cas9 strategy for genome editing was used to target ROMK (renal outer medullary potassium) in C57BI6/J mice to create cardiomyocyte-specific knockout for ROMK (ROMK-CKO) mice as previously described.[27] The SUR1(−/−) mice were created by removal of the 1-kbp gene segment containing both promoter and exon 1 sequences of SUR1 gene by re-mediated recombination as previously described.[12,28]

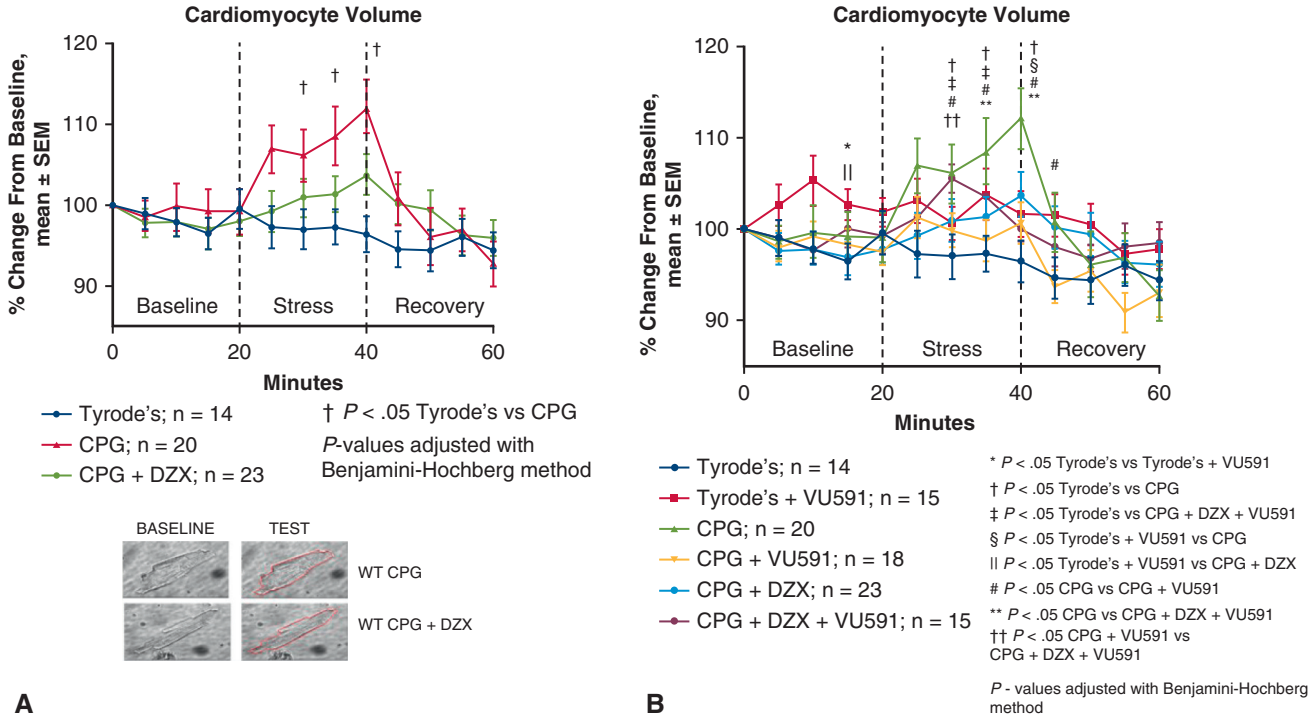

FIGURE 2. Hyperkalemic cardioplegia exposure results in myocyte swelling that is prevented by K_{ATP} channel opener DZX and is lost with the addition of Kir 1.1 inhibitor VU591. Isolated mouse myocytes were exposed to 37 °C control TYR for 20 minutes (baseline), followed by a stress period (20 minutes) with test solution (N = 14-23 for each; Tyrode's physiologic solution, Tyrode's + VU591(ROMK inhibitor), hypothermic hyperkalemic CPG, CPG + K_{ATP} channel opener DZX, CPG + VU591, or CPG + VU591 + DZX) for 20 minutes, then 20 minutes reexposure to Tyrode's. Volume measurements were taken every 5 minutes. Cardioplegia resulted in significant myocyte swelling (A) that was prevented by DZX (†P < .05 Tyrode's vs CPG). Inset depicts WT mouse myocytes at baseline and exposed to CPG (with swelling) or CPG + DZX (no swelling) during test period. The beneficial effect of DZX was lost with the addition of VU591 (B). *SEM*, Standard error of the mean; *CPG*, cardioplegia; *DZX*, diazoxide. *P < .05 TYR versus TYR + VU591, †P < .05 TYR versus CPG, ‡P < .05 TYR versus CPG + DZX + VU591, §P < .05 TYR + VU591 versus CPG, ‖ P < .05 TYR + VU591 versus CPG + DZX, #P < .05 CPG versus CPG + VU591, **P < .05 CPG versus CPG + DZX + VU591, ††P < .05 CPG + VU591 versus CPG + DZX + VU591. Data are represented as mean percent change from baseline ± SEM. Inset in A is an adaptation of the figure used in our previous publication.[12]

Mice (SUR1(−/−), ROMK-cardiac KO (CKO), or WT C57Bl/J (ROMK-CKO littermate), either sex) underwent rapid cardiectomy as previously described.[8,29] The heart was submersed in ice-cold Krebs–Henseleit buffer (KHB) during aortic cannulation. Hearts were perfused in a Langendorff fashion (retrograde via the aorta) with KHB (118.5 mmol/L NaCl, 25.0 mmol/L $NaHCO_3$, 3.2 mmol/L KCl, 1.2 mmol/L KH_2PO_4, 1.2 mmol/L $MgSO_4$, 1.4 $CaCl_2$, and 5.5 mmol/L D-glucose). Hearts were excluded if aortic cannulation time exceeded 5 minutes. A balloon (custom made in our laboratory) was introduced into the left ventricle (LV) and connected to a pressure transducer (HP1290 C; ADInstruments) and an amplifier (20-4; Hugo Sachs Elektronik–Harvard Apparatus). Hearts were epicardially paced at 450 bpm and surrounded by a warm (37 °C) water-jacketed beaker. Coronary perfusion pressure was maintained at 80 mm Hg (the zone of autoregulation for coronary vasculature in murine hearts) by Langendorff column height.

Experimental protocol. Balloon volume was adjusted to an end-diastolic pressure (EDP) of 2.5 mm Hg. During a 30-minute baseline period, LV pressure was measured at increasing 1.4-μL balloon volume increments for baseline data collection (Figure 1, *B*). The LV balloon volume was then returned to the initial volume, and the heart was arrested for 90 minutes using the test solution.

Randomly assigned test solutions included hyperkalemic CPG (Plegisol) (110 mmol/L NaCl, 10 mmol/L $NaHCO_3$, 16 mmol/L KCl, 32 mmol/L Mg, and 2.4 mmol/L $CaCl_2$, titrated to pH 7.8 with 8.4% $NaHCO_3$ solution) or CPG + DZX 100 μmol/L (n = 6-9). After 90 minutes of global ischemia, KHB retrograde perfusion and epicardial pacing were resumed. After 30 minutes, LV pressures were recorded at the identical balloon volumes used during the baseline period as previously described.[8,14,29] Investigators were blinded to the test group during each experiment and during data analysis. DZX was administered before ischemia because it has been demonstrated to be cardioprotective only when administered before stress.[25]

Data acquisition and analysis. LV end-diastolic pressure (EDP), and LV developed pressure (LVDP) were determined from digitalized data files using LabVIEW 2014 (National Instruments) and compared over a series of identical intracavitary balloon volumes.

Coronary flow rates were measured at baseline and every 5 minutes after reperfusion by an inline N-series flow probe and a T206 flow meter (Transonic Systems) and compared as described previously.[29]

Statistical analysis. Coronary flow between groups was examined using repeated measures analysis of variance. Changes in EDP and

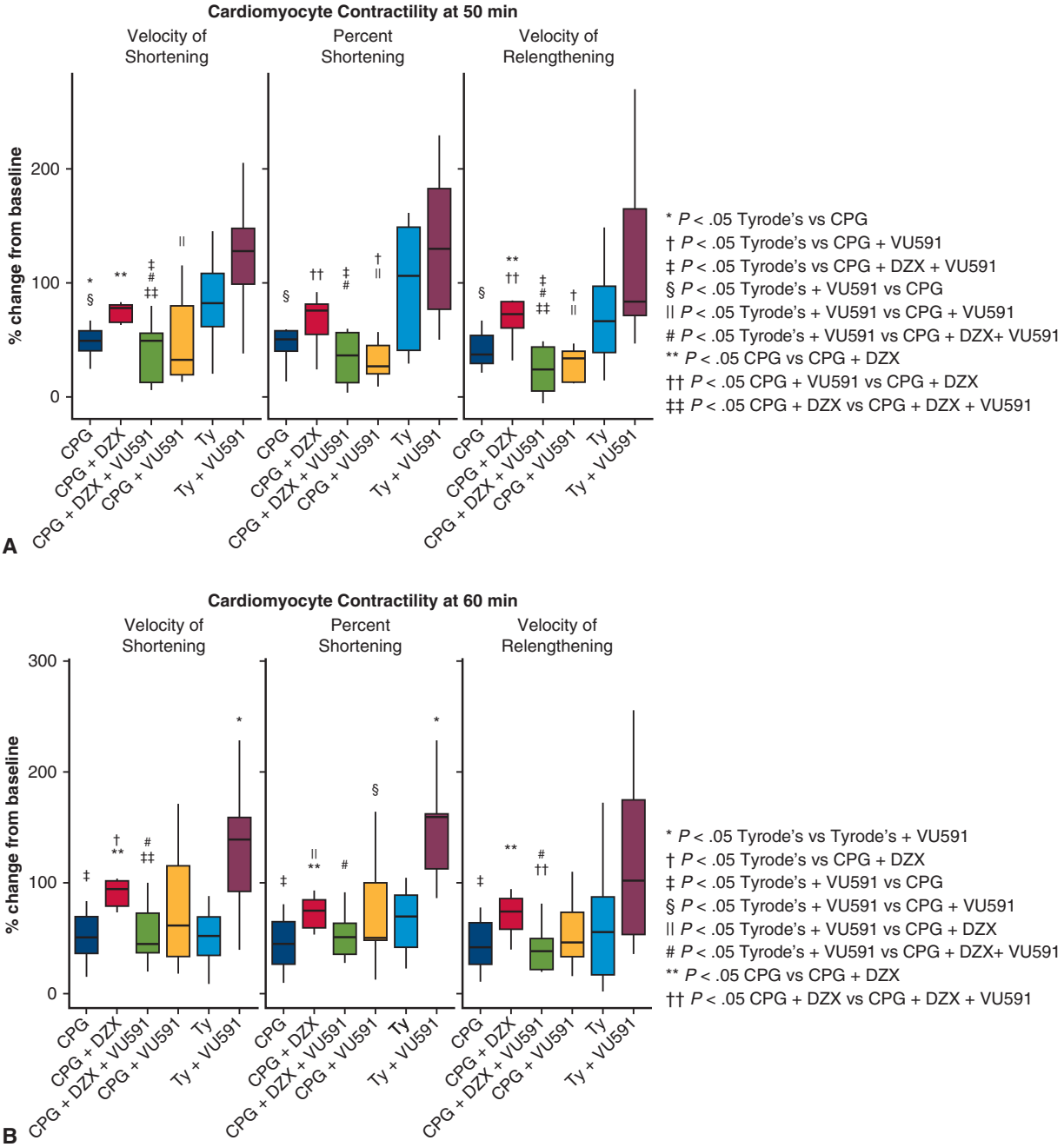

FIGURE 3. Hyperkalemic cardioplegia exposure results in reduced myocyte contractility that is prevented by K_{ATP} channel opener DZX and is lost with the addition of Kir 1.1 inhibitor VU591. Isolated mouse myocytes were exposed to 37 °C control Tyrode's physiologic solution (TYR) for 20 minutes (baseline), followed by a stress period (20 minutes) with test solution (N = 9-19 for each; Tyrode's physiologic solution, Tyrode's + VU591(ROMK inhibitor), hypothermic hyperkalemic CPG, CPG + K_{ATP} channel opener DZX, CPG + VU591, or CPG + VU591 + DZX) for 20 minutes, and then 20 minutes re-exposure to Tyrode's. Contractility (velocity of shortening, percent shortening, or velocity of relengthening) was measured after 50 minutes (A) and 60 minutes (B) reperfusion with Tyrode's. The *middle horizontal line in the box* represents the median percent change from baseline ± interquartile range. The *upper and lower vertical lines* represent the maximum and minimum values of nonoutliers. *CPG*, Cardioplegia; *DZX*, diazoxide; *Ty*, Tyrode's physiologic solution. (A) *P < .05 TYR versus CPG, †P < .05 TYR versus CPG + VU591, ‡P < .05 TYR versus CPG + DZX + VU591, §P < .05 TYR + VU591 versus CPG, ||P < .05 TYR + VU591 versus CPG + VU591, #P < .05 TYR + VU591 versus CPG + DZX + VU591, **P < .05 CPG versus CPG + DZX, ††P < .05 CPG + VU591 versus CPG + DZX, ‡‡P < .05 CPG + DZX versus CPG + DZX + VU591. (B) *P < .05 TYR versus TYR + VU591, †P < .05 TYR versus CPG + DZX, ‡P < .05 TYR + VU591 versus CPG, §P < .05 TYR + VU591 versus CPG + VU591, ||P < .05 TYR + VU591 versus CPG + DZX, #P < .05 TYR + VU591 versus CPG + DZX + VU591, **P < .05 CPG versus CPG + DZX, ‡‡P < .05 CPG + DZX versus CPG + DZX + VU591.

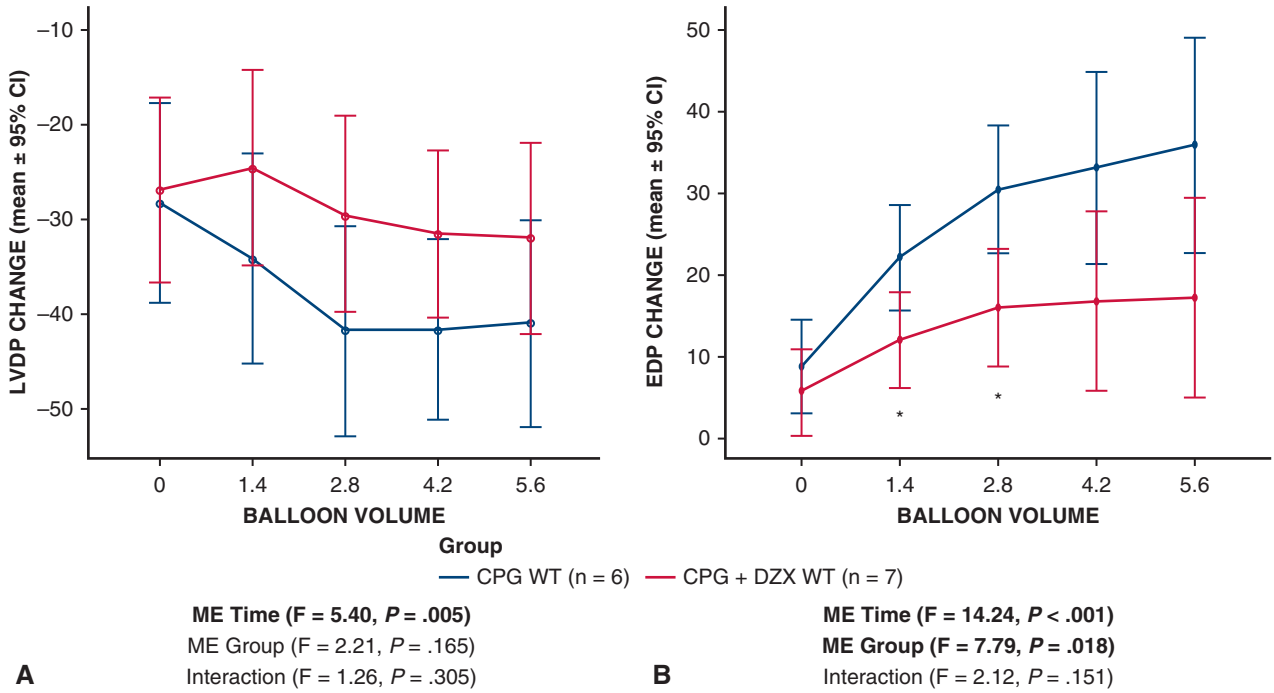

FIGURE 4. Hyperkalemic cardioplegia is associated with reduced left ventricular developed pressure and increased end-diastolic pressure after global ischemia that is prevented by K_{ATP} channel opener DZX in WT mouse hearts. Isolated WT (CKO ROMK littermates) mouse hearts underwent Langendorff perfusion for 30 minutes followed by arrest with test solution (CPG or CPG + DZX) and 90 minutes global ischemia, and reperfusion for 30 minutes. Left ventricular pressure was recorded during baseline and after ischemia at identical balloon volumes (μL). N = 6 to 7 for each. A, Mean change in LVDP (from baseline ± 95% CI) across all balloon volumes following global ischemia. B, Mean change in EDP (from baseline ± 95% CI) across all balloon volumes after global ischemia. ME Time, the main effect of balloon volume (regardless of group), ME Group is the main effect of mouse type groups (collapsed across balloon volumes), and Interaction is the interaction of balloon volume and group to indicate if the pattern of results over different balloon volumes differed for each group. *CPG*, Cardioplegia; *WT*, wild-type; *DZX*, diazoxide; *LVDP*, left ventricular developed pressure; *CI*, confidence interval; *EDP*, end-diastolic pressure; *ME*, main effect; *F*, analysis of variance F test statistic; *$P < .05$ EDP CPG versus CPG + DZX at balloon volumes 1.4 and 2.8 using independent-samples t tests.

LVDP by balloon volume and mouse type groups were examined using repeated measures analysis of variance with Huynh-Feldt correction when the assumption of sphericity was violated. The results of these analyses provided the main effect of balloon volume (regardless of group), the main effect of mouse type groups (collapsed across balloon volumes), and the interaction of balloon volume and group to indicate if the pattern of results over different balloon volumes differed for each group. Pairwise comparisons between the groups at individual balloon volumes were conducted using independent-samples *t* tests. Sample size calculations were based on previous work in this model. Studies were designed to detect a 5% ± 4% difference using an $\alpha = 0.05$ and a power of 0.80. Analyses were conducted with IBM SPSS Statistics, Version 28.0 (IBM Corp).

RESULTS

Myocyte Volume

Myocyte volume over time is shown in Figure 2. Similar to previous results, stress (hyperkalemic cardioplegia) was associated with myocyte swelling that was prevented by DZX (Figure 2, *A*). Of note, VU591 (ROMK inhibitor) alone prevented myocyte swelling when added to CPG ($P < .05$ CPG + VU591 vs CPG). However, VU591 added to CPG + DZX was associated with loss of cardioprotective benefit of either compound alone ($P < .05$ CPG + DZX + VU591 vs CPG + DZX) (Figure 2, *B*).

Myocyte Contractility

Myocyte contractility after test solution is shown in Figure 3. Similar to previous results, myocytes demonstrated significant decline in all 3 parameters of contractility after exposure to CPG ($P < .05$ CPG vs TYR). The addition of DZX to CPG prevented this reduction in contractility ($P < .05$ CPG + DZX vs CPG). VU591 alone improved contractility when added to both CPG ($P < .05$ CPG + VU591 vs CPG) and TYR ($P < .05$ TYR + VU591 vs TYR), respectively. The observed benefit of DZX was lost in the presence of VU591 ($P < .05$ CPG + DZX + VU591 vs CPG + DZX).

Coronary Flow in Isolated Hearts After Global Ischemia

Coronary flow was not different between groups at any time point (baseline or during reperfusion) (Figure E1).

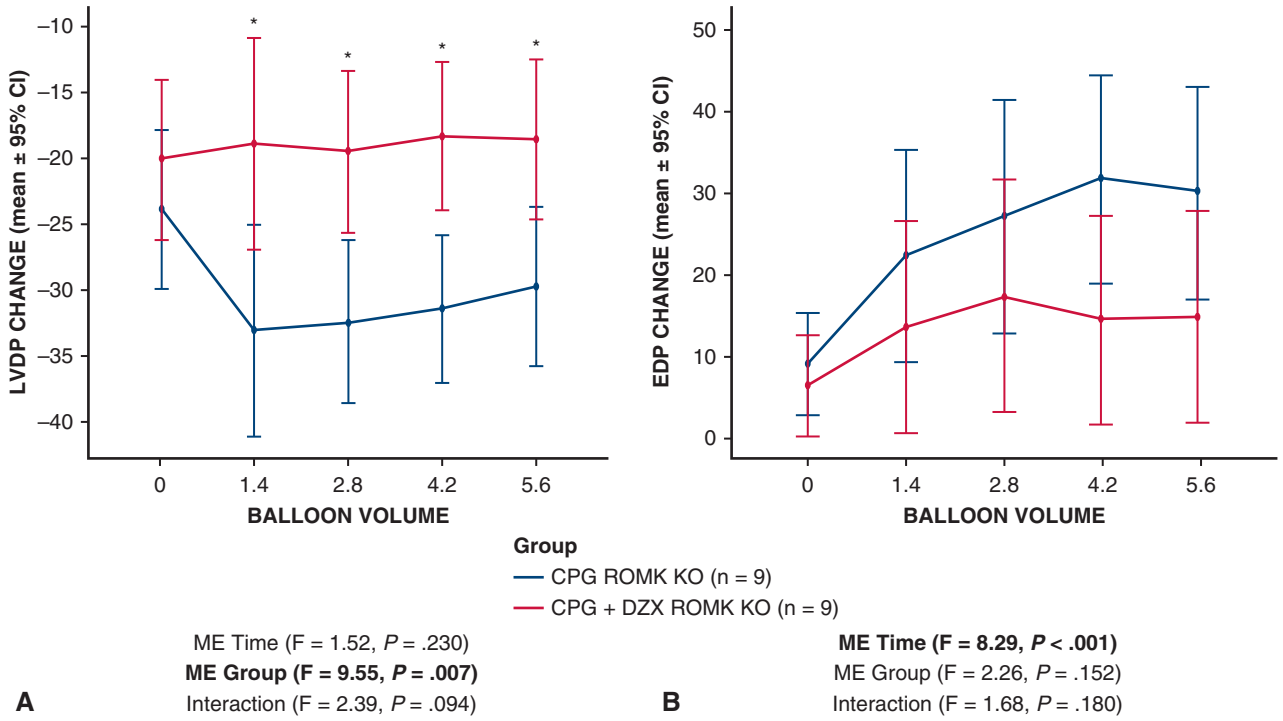

FIGURE 5. Hyperkalemic cardioplegia is associated with reduced left ventricular developed pressure and increased end-diastolic pressure after global ischemia that is prevented by K_{ATP} channel opener DZX in mouse hearts with cardiac knockout of renal outer medullary potassium subunit. Isolated CKO ROMK mouse hearts underwent Langendorff perfusion for 30 minutes followed by arrest with test solution (CPG or CPG + DZX) and 90 min global ischemia, and reperfusion for 30 minutes. Left ventricular pressure was recorded during baseline and after ischemia at identical balloon volumes (μL). N = 9 for each. A, Mean change in LVDP (from baseline ± 95% CI) across all balloon volumes after global ischemia. B, Mean change in EDP (from baseline ± 95% CI) across all balloon volumes following global ischemia. ME Time is the main effect of balloon volume (regardless of group), ME Group is the main effect of mouse type groups (collapsed across balloon volumes), and Interaction is the interaction of balloon volume and group to indicate if the pattern of results over different balloon volumes differed for each group. *CPG*, Cardioplegia; *DZX*, diazoxide; *LVDP*, left ventricular developed pressure; *CI*, confidence interval; *EDP*, end-diastolic pressure; *ME*, main effect; *F*, analysis of variance F test statistic. **P* < .05 LVDP CPG versus CPG + DZX at balloon volumes 1.4, 2.8, 4.2, and 5.6 using independent-samples t tests.

Left Ventricular Developed Pressure and End-Diastolic Pressure After Global Ischemia

Response to diazoxide in cardiac knockut ROMK (Kir1.1) mice. WT hearts (from CKO ROMK littermates) had the expected response to DZX as they exhibited improved LVDP and reduced EDP after prolonged global ischemia ($P < .05$ DZX + CPG vs CPG) (Figure 4). Unexpectedly, ROMK CKO hearts were also responsive to DZX and had improved LVDP and reduced EDP after ischemia ($P < .05$ DZX + CPG vs CPG) (Figure 5).

Response to diazoxide in SUR1(−/−) mice. SUR1(−/−) mice were also responsive to DZX and had improved LVDP and reduced EDP after ischemia when DZX was added to CPG, versus CPG alone ($P < .05$ DZX + CPG vs CPG) (Figure 6).

DISCUSSION

We have attempted to systematically identify K_{ATP} channel components involved in DZX cardioprotection, towards the goal of developing a K_{ATP} targeted therapeutic drug to provide myocardial protection during cardiac surgery.[12-19] Although others have suggested alternate mitochondrial proteins encoded by CCDC51 and ABCB8 genes, with unknown function, as proteins involved in a mitochondrial K_{ATP} channel,[30] we have focused on the investigation of known or potential K_{ATP} channel subunits.[12-19]

Our previous work in isolated mouse myocytes and via a proteomic analysis of the mitochondrial membrane, has implicated the SUR1 and ROMK proteins as potential subunits of a cardioprotective mitochondrial K_{ATP} channel sensitive to diazoxide.[12,14,18,20] However, by using genetically engineered mouse hearts in the current study, we have definitively demonstrated that the SUR1 and ROMK subunits are not implicated in cardioprotection mediated by diazoxide.

Both the putatively ROMK1-specific inhibitor VU591 and the KO of SUR1 led to loss of DZX protection against experimental CPG in isolated myocytes.[12,14] This apparent discrepancy in results between the myocyte model and the Langendorff model may be related to unique mechanisms

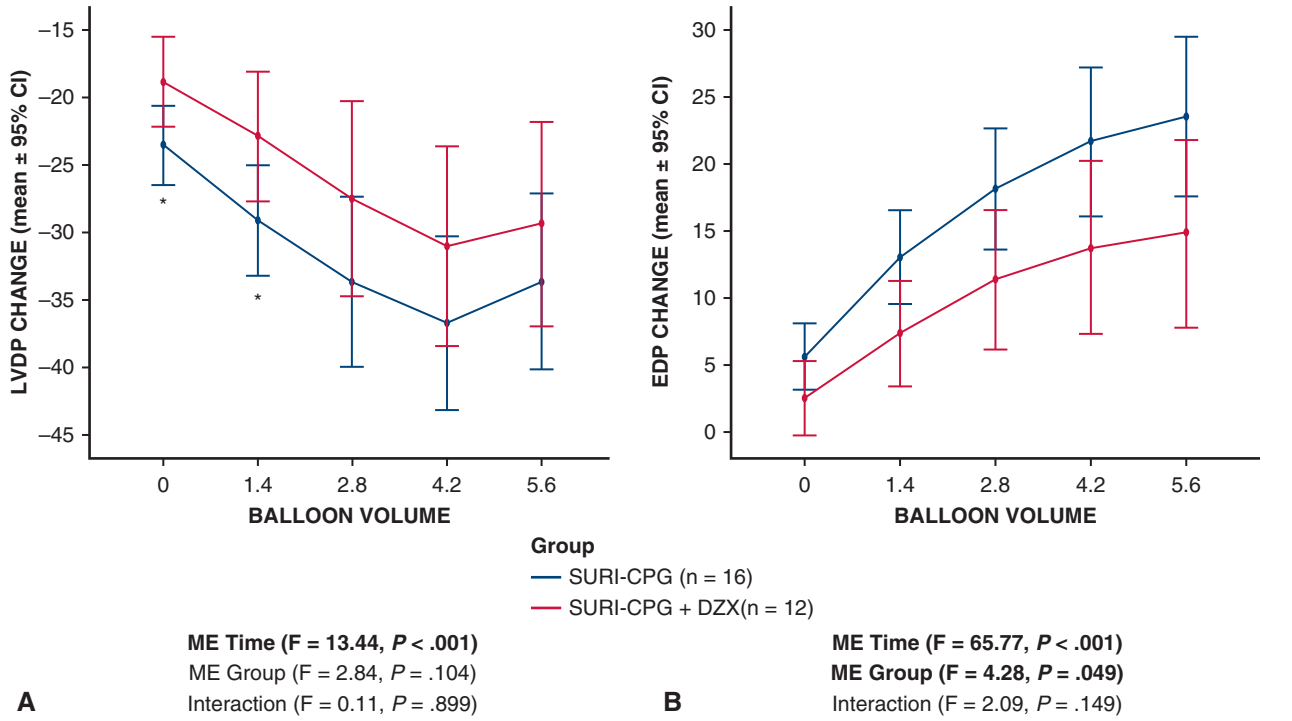

FIGURE 6. Hyperkalemic cardioplegia is associated with reduced left ventricular developed pressure and increased end diastolic pressure after global ischemia that is prevented by K_{ATP} channel opener DZX in mouse hearts with global knockout of sulfonylurea sensitive regulatory subunit 1. Isolated sulfonylurea sensitive regulatory subunit 1 global knock out (SUR1(−/−)) mouse hearts underwent Langendorff perfusion for 30 min followed by arrest with test solution (CPG or CPG + DZX) and 90 minutes global ischemia, and reperfusion for 30 minutes. Left ventricular pressure was recorded during baseline and after ischemia at identical balloon volumes (μL). N = 12 to 16 for each. A, Mean change in LVDP (from baseline ± 95% CI) across all balloon volumes after global ischemia. B, Mean change in EDP (from baseline ± 95% CI) across all balloon volumes after global ischemia. ME Time is the main effect of balloon volume (regardless of group), ME Group is the main effect of mouse type groups (collapsed across balloon volumes), and Interaction is the interaction of balloon volume and group to indicate if the pattern of results over different balloon volumes differed for each group. *SUR1*, Sulfonylurea sensitive regulatory subunit 1; *CPG*, cardioplegia; *DZX*, diazoxide; *LVDP*, left ventricular developed pressure; *CI*, confidence interval; *EDP*, end-diastolic pressure; *ME*, main effect; *F*, analysis of variance F test statistic. **P* < .05 LVDP CPG versus CPG + DZX at balloon volumes 0 and 1.4 using independent-samples t tests. **P* < .05 EDP CPG versus CPG + DZX at balloon volume 1.4 using independent-samples *t* tests.

at separate locations or to the known non-specificity of pharmacologic K_{ATP} channel inhibitors.[31] We and others have questioned the selectivity and efficacy of K_{ATP} channel inhibitors, as well as variable drug efficacy with different metabolic states.[16,18,23,24,26,32]

Non-K_{ATP} channel mechanisms have also been suggested to underlie the cardioprotection of diazoxide, and we and others have documented that the mechanism is at a nonsarcolemmal location. For example, DZX can inhibit succinate dehydrogenase at high concentrations and inhibitors targeting Complex II can also protect hearts.[7-10,12,13,33] Inhibition of electron transport chain at Complex II, succinate dehydrogenase, could thus contribute to cardioprotection.

In previous experiments with mitochondria-targeted S-nitrosylating agent, a cardioprotective effect was noted with mitochondria-targeted S-nitrosylating agent alone with CPG, and this was lost with the addition of DZX.[8] The same phenomenon was noted in the current experiments with Kir1.1 inhibitor VU591 (cardioprotection with VU591 alone that was lost with the addition of DZX). This may suggest an upstream or downstream effect at the mitochondrial level that is vital for DZX action at Complex II (succinate dehydrogenase).

Future work will be necessary to complete the systematic evaluation of *known* K_{ATP} channel components and the investigation of non-K_{ATP} channel mechanisms of pharmacologic channel openers to increase the knowledge of the mechanism of action of these drugs. Recognition of differential K_{ATP} channel makeup in different tissues has led to understanding and successful treatment of several human diseases. Neonatal diabetes and congenital hyperinsulinism are effectively treated with K_{ATP} channel inhibitors and K_{ATP} channel openers, respectively. A variety of selective channel openers have been developed to avoid nonselective systemic side effects.[34] The determination of a selective channel opener implicated in myocardial protection would

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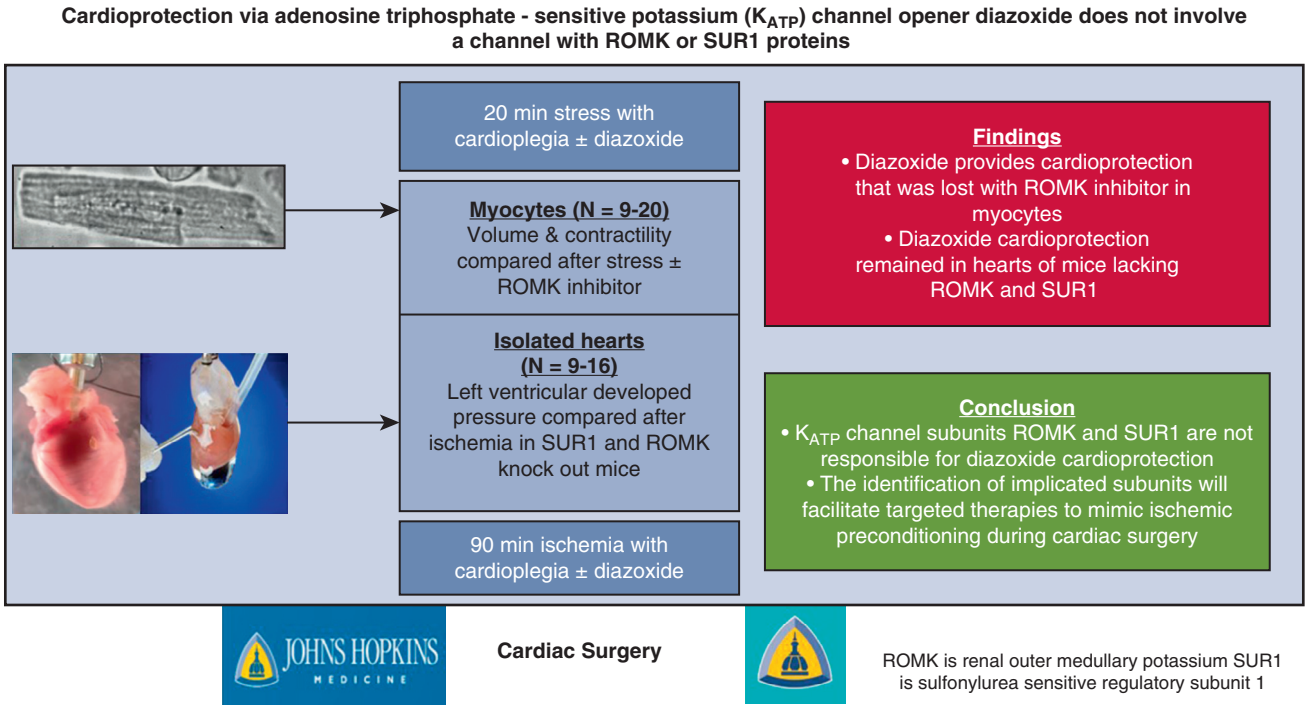

FIGURE 7. Cardioprotection via K_{ATP} channel opener DZX does not involve a channel with ROMK or SUR1 proteins. Isolated myocytes and hearts underwent stress (20 minutes exposure to hyperkalemic cardioplegia or 90 minutes exposure to ischemia with hyperkalemic cardioplegia, respectively) with or without diazoxide. DZX alone provides cardioprotection (reduced myocyte swelling and maintenance of contractility in myocytes and preserved left ventricular developed pressure in hearts). The addition of a ROMK inhibitor prevented DZX cardioprotection in myocytes, however, genetic deletion of channel components ROMK and SUR1 did not prevent DZX cardioprotection in hearts. *ROMK*, Renal outer medullary potassium; *SUR1*, sulfonylurea sensitive regulatory subunit 1.

be an exciting and clinically useful tool to exploit the cell's innate cardioprotection for the prevention of myocardial stunning. Such knowledge would facilitate the development of targeted therapies to provide improved cardioprotection during cardiac surgery without unwanted systemic side effects or crossover drug–induced pathology.[35] Therapy targeted to specific mechanisms could potentially provide safe strategies to mimic ischemic preconditioning, reduce myocardial stunning, and minimize injury following the global ischemia that accompanies cardiac surgery (Figure 7).

Study Limitations

We have implied that pharmacologic channel blockade may be nonspecific and that genetic deletion or modification is definitive. However, it is important to note that genetic deletion may be associated with unexpected biological effects due to channel compensation in individual animals.[35]

CONCLUSIONS

The prolonged global ischemia model[29] used was designed to mimic the clinical situation of global arrest during cardiac surgery. Caution should be taken in extrapolation of these results to the clinical situation. The prolonged global ischemia model is longer than the traditional mouse infarct model; therefore, measuring infarct size is not informative in this model, and the functional results noted cannot be correlated with structural myocardial changes.

Conflict of Interest Statement

The authors reported no conflicts of interest.

The *Journal* policy requires editors and reviewers to disclose conflicts of interest and to decline handling or reviewing manuscripts for which they may have a conflict of interest. The editors and reviewers of this article have no conflicts of interest.

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Key Words: basic science, ion channels, ischemia, preconditioning

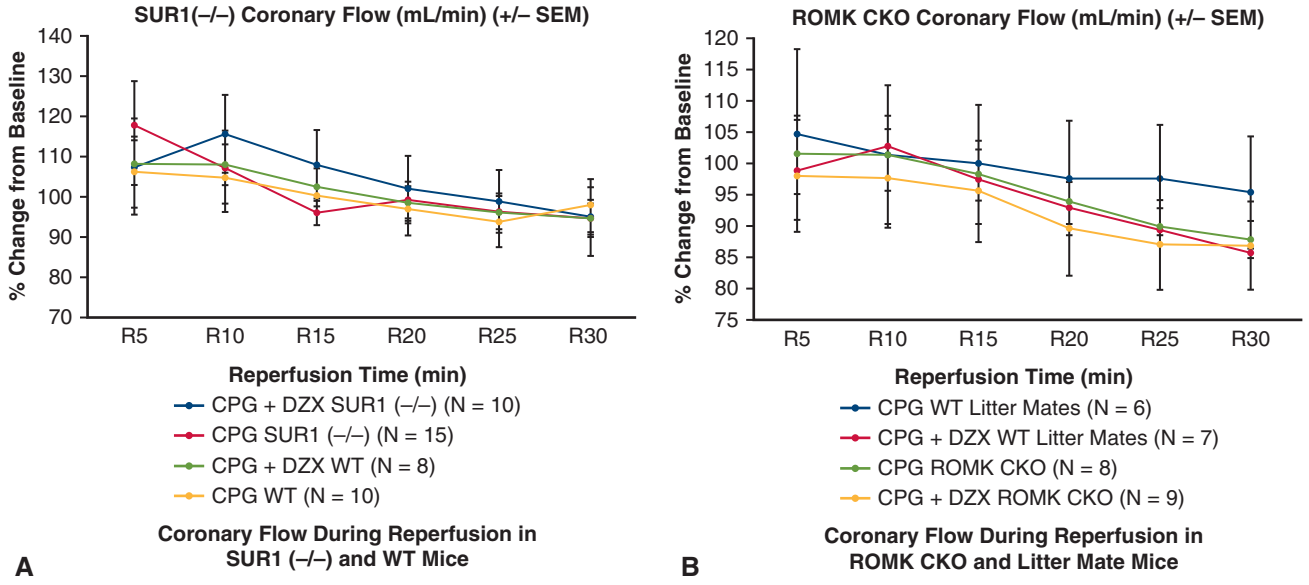

FIGURE E1. Coronary flow was not different between experimental groups after ischemia and reperfusion. Isolated mouse hearts (SUR1(−/−), CKO ROMK, or CKO ROMK littermates) underwent Langendorff perfusion for 30 minutes followed by arrest with test solution (CPG or CPG + DZX) and 90 minutes global ischemia and 30 minutes reperfusion. Coronary flow (mL/min) was measured by an inline flow probe and is represented as mean +/− SEM, N = 6 to 15 for each. A, Coronary flow in WT and SUR1(−/−) mice during reperfusion. B, Coronary flow in CKO ROMK and CKO ROMK WT littermates during reperfusion. There were no significant differences between the groups. *SUR1*, Sulfonylurea sensitive regulatory subunit 1; *SEM*, standard error of the mean; *ROMK*, renal outer medullary potassium; *CKO*, cardiac knockout; *CPG*, cardioplegia; *DZX*, diazoxide; *WT*, wild-type.