Mitochondrial $K_{ATP}$ channel inhibition blunts arrhythmia protection in ischemic exercised hearts

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Quindry JC, Schreiber L, Hosick P, Wrieden J, Irwin JM, Hoyt E. Mitochondrial $K_{ATP}$ channel inhibition blunts arrhythmia protection in ischemic exercised hearts. Am J Physiol Heart Circ Physiol 299: H175–H183, 2010. First published April 30, 2010; doi:10.1152/ajpheart.01211.2009.—The mechanisms responsible for anti-arrhythmic protection during ischemia-reperfusion (IR) in exercised hearts are not fully understood. The purpose of this investigation was to examine whether the ATP-sensitive potassium channels in the mitochondria (mito $K_{ATP}$) and sarcolemma (sarc $K_{ATP}$) provide anti-arrhythmic protection in exercised hearts during IR. Male Sprague-Dawley rats were randomly assigned to cardioprotective treadmill exercise or sedentary conditions before IR (1 = 20 min, R = 30 min) in vivo. Subsets of exercised animals received pharmacological inhibitors for mito $K_{ATP}$ (5-hydroxydecanoate) or sarc $K_{ATP}$ (HMR1098) before IR. Blinded analysis of digital ECG tracings revealed that mito $K_{ATP}$ inhibition blunted the anti-arrhythmic effects of exercise, while sarc $K_{ATP}$ inhibition did not. Endogenous antioxidant enzyme activities for total, CuZn, and Mn superoxide dismutase, catalase, and glutathione peroxidase from ischemic and perfused ventricular tissue were not mitigated by IR, although oxidative stress was elevated in sedentary and mito $K_{ATP}$-inhibited hearts from exercised animals. These findings suggest that the mito $K_{ATP}$ channel provides anti-arrhythmic protection as part of exercise-mediated cardioprotection against IR. Furthermore, these data suggest that the observed anti-arrhythmic protection may be associated with preservation of redox balance in exercised hearts.

cardioprotection; ischemia-reperfusion; myocardial; oxidative stress; preconditioning

CARDIOVASCULAR DISEASE (CVD) is the leading cause of morbidity and mortality in industrialized countries (3). Injurious processes related to both ischemia and reperfusion, collectively termed ischemia-reperfusion (IR) injury, are among the most prevalent manifestations of CVD (8). IR injury reflects the ever-changing bioenergetic environment of the myocardium and involves progressive levels of damage, beginning with ventricular arrhythmias in the moments following coronary occlusion. In accordance, the scientific and medical communities have sought for decades to uncover endogenous mechanisms of protection as a means of countermeasure development against IR injury, including arrhythmias. As such, preemptive activation of known, and yet to be identified, protective mechanisms precondition the myocardium against acute IR (8). Cardiac preconditioning via exercise is among the most potent and consistent stimuli for eliciting resistance to IR injury (reviewed in Ref. 42). To date, the cellular mechanisms responsible for cardiac preconditioning in exercised hearts are not fully understood.

Early work to uncover mechanisms of exercise-mediated cardioprotection revealed an essential role for the endogenous antioxidant manganese superoxide dismutase (MnSOD) against lethal and nonlethal arrhythmias generated during IR insults (25). That elevated MnSOD enzyme activity plays an essential protective role in exercised hearts is logical in light of the fact that oxidative stress is a cornerstone of IR-mediated arrhythmia generation. Findings to implicate MnSOD as a protective mediator, however, do not completely account for the IR-resistant phenotype observed in exercise hearts (22, 25). Indeed, the notion that multiple protective factors are at work in exercise preconditioning is consistent with previous research on other forms of cardiac preconditioning, where myriad mechanisms exert cellular protection, both independently and in synergistic fashion (7, 24).

The ATP-sensitive potassium ($K_{ATP}$) channel is a potential protective mechanism at work in exercised hearts. The functional link between exercise and ischemic protection likely pertains to the energy-sensing capacity of this ion channel, which is closed in the presence of abundant ATP and opened by stress-related signals and falling cellular ATP levels (reviewed in Ref. 31). While the protection afforded by the $K_{ATP}$ channel opening is not fully understood, recent evidence indicates that $K_{ATP}$ channel subtypes on the inner mitochondrial membrane (mito $K_{ATP}$ channel) and the sarcolemma (sarc $K_{ATP}$ channel) provide significant protection through independent and interrelated means (35, 40). Preemptive opening of $K_{ATP}$ channels with pharmacological or physiological stimuli elicits protection against IR-generated arrhythmias (35, 45). In accordance, we undertook the present investigation to test the hypothesis that the mito $K_{ATP}$ channel is essential for exercise-mediated protection against IR-generated arrhythmias. Results from previous investigations of exercised hearts indicate the sarc $K_{ATP}$ channel is essential for myocardial infarct sparing effects of exercise (12, 15, 17). Thus we also tested the rival hypothesis that the sarc $K_{ATP}$ channel was responsible for the anti-arrhythmic protection provided by exercise. These hypotheses were carried out using a rat model of surgically induced IR following a short-duration (days) exercise preconditioning stimulus that was previously shown to be cardioprotective (22, 25, 43). Pharmacological inhibitors selective for both the mito and sarc $K_{ATP}$ channels were used on an individual basis to test the contribution of the respective mito and sarc $K_{ATP}$ channel mediation of anti-arrhythmic protection in exercised hearts. Finally, we explored whether mito and sarc $K_{ATP}$ channel-mediated protection in exercised hearts was associated with preservation of endogenous antioxidant enzymes and attenuation of oxidative stress in the myocardium during IR.

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METHODS

Animals and experimental design. The experimental protocol was approved by the Appalachian State University Animal Care and Use committee and followed guidelines established by the American Physiological Society for the use of animals in research. Adult male Sprague-Dawley rats (4 mo old) were randomly assigned to cardioprotective exercise or sedentary (Sed) treatments. During the experimental period, all animals were housed on a 12:12-h light-dark cycle and provided rat chow (AIN93) and water ad libitum.

Cardioprotective exercise protocol. Rats assigned to the exercise treatment received, over 10 days, an exercise regimen previously shown to elicit a cardioprotected phenotype against IR injury (22, 25, 43). The exercise regimen began with a habituation period of 5 consecutive days of treadmill exercise. Treadmill habituation involved a gradual increase in running time begun on day 1 with 10 min of exercise. Successive days introduced time increases of 10 min/day, concluding with 50 min of total exercise on the 5th and final day of treadmill habituation. At the conclusion of the treadmill habituation period, animals received 2 days of rest, followed by 3 consecutive days of 60-min exercise bouts. Treadmill speed and grade were fixed at 30 m/min and a 0% grade, respectively, for both treadmill habituation and exercise training periods.

Exercise and Sed group study treatments. Rats assigned to the exercise treatment were further randomized into groups to receive one of three treatments; either saline placebo (Ex), 5-hydroxydecanoate (5HD, 10 mg/kg), a selective pharmacological inhibitor to the mitochondrial KATP channel (Km, 5HD, 10 mg/kg), or HMR1098, exercised, sarcolemmal KATP channel inhibitor. Placebo and pharmacological doses were administered via intraperitoneal injection 45 min before anesthesia for IR experimentation in vivo, based on previous work (23). Rats assigned to the Sed treatment were further randomized into either sham (no ischemia) or Sed treatments.

In vivo IR protocol. Twenty-four hours following the final day of the exercise protocol, rats were exposed to a nonsurvival IR protocol in vivo, involving coronary artery ligation, as described previously (25, 43). Before surgery, rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (65 mg/kg). After a surgical plane of anesthesia was reached, a tracheotomy was performed, and animals were mechanically ventilated (Kent Scientific, Torrington, CT) with room air. A catheter was placed in the jugular vein for supplemental delivery of pentobarbital sodium (10 mg/kg) as needed to maintain the surgical plane of anesthesia. An additional catheter attached to a pressure transducer was placed in the carotid artery for real-time assessment of arterial blood pressure. Simultaneous ECG recordings were monitored from limb leads. ECG and blood pressure data were interfaced to a personal computer using a physiological data-acquisition system (Biopac, Santa Barbara, CA). Following a left thoracotomy, a ligature was placed around the left anterior descending coronary artery close to its origin. The ligature was threaded through a small flexible piece of tubing, which was pressed against the coronary artery with a small surgical clamp to induce ischemia. The heart was observed to have both cyanotic and perfused regions. The appearance of ventricular ectopy was used to confirm induction of ischemia. Ligation of the coronary was maintained for 20 min, after which the surgical clamp was removed for a 30-min reperfusion period. Sed rats received an IR challenge identical to Ex rats. Sham rats received an identical surgery, save for induction of ischemia, for a 50-min time period. At the end of IR and sham protocols, ligatures were tightened, and a small volume of 1% Evans blue dye was infused into the arterial blood for differentiation of perfused and nonperfused cardiac regions. Hearts were rapidly excised and dissected into perfused and nonperfused regions for quantification of the respective tissue masses. Ventricular tissue was rinsed in cold buffer, flash frozen, and stored at -80°C for subsequent biochemical analysis.

Arrhythmia scoring. Electrocardiographic tracings were analyzed for ventricular arrhythmias to assess the magnitude of ventricular ectopy for the various treatments. Digital ECG files were read in a treatment-blinded fashion by three trained reviewers, who coded for preventricular contractions (PVCs), ventricular tachycardia (VT), and ventricular fibrillation (VF). An established arrhythmia scoring system was applied to digital arrhythmia tracings of ischemia and reperfusion, in accordance with Lambeth Convention guidelines previously used to assess clinical ECG responses to an ischemic insult (18–19, 46).

Analysis of antioxidant enzyme activity. To assess the effects of exercise training and IR challenge on myocardial antioxidant enzyme capacity, perfused and nonperfused sections of left ventricular sections were homogenized in ice-cold 100 mM phosphate buffer (1:20 wt/vol, pH 7.4). Homogenates were centrifuged at 400 g for 10 min at 4°C. The supernatant was assayed for total protein content (11), along with the activities of SOD and its respective CuZn (CuZnSOD) and Mn (MnSOD) isoforms (36), glutathione peroxidase (GPx) (21), and catalase (CAT) (1) activities. All analyses were performed, at minimum, in quadruplicate at 25°C and were assayed on the same day to avoid interassay variation. The coefficients of variation for SOD, GPx, and CAT assays ranged between 2 and 5%.

Analysis of tissue oxidative stress. To determine the effects of exercise training and IR on tissue oxidative stress, two oxidative stress biomarkers and the reduced and oxidized forms of glutathione, GSH and GSSG, respectively, were assessed in both perfused and nonperfused tissue from all treatments. Myocardial protein carbonyl formation was assessed per manufacturer’s instructions, using commercially available ELISA kits (Northwest Lifesciences, Vancouver, WA). Myocardial 4-hydroxynonenal content was evaluated per manufacturer’s instructions via commercially available ELISA kits (Cell Biolaboratories, San Diego, CA). Myocardial GSH and GSSG content were assayed per manufacturer’s instructions using commercially available assay kits (Cayman Chemical, Ann Arbor, MI).

Data analysis. One-way ANOVA was used to evaluate perfused and nonperfused tissue areas, arrhythmia scoring data, and ventricular ectopy data. Two-way ANOVA was used to evaluate antioxidant enzyme data and oxidative stress measures. Significant group differences were determined via Tukey post hoc analysis. Kruskal-Wallis nonparametric analyses were used to assess differences in the incidence of ventricular ectopy. Significance was established a priori at P < 0.05.

RESULTS

Animal characteristics. For this investigation, 52 animals were used. Animal attrition due to complications during the experimental surgery, such that the experimental protocol was incomplete, occurred once or twice for each of the respective IR treatments. Data from the remaining 45 animals, including animal numbers, body weights, and heart weights, are presented in Table 1. Statistical analysis revealed that body

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Values are means ± SE; n, no. of animals. Sham, sham surgery without ischemia where nonperfused tissue equates to the ischemic region in all other treatments; Sed, sedentary; Ex, exercised placebo; Ex 5HD, exercised, 5-hydroxydecanoate mitochondrial ATP-sensitive potassium (K_{ATP}) channel inhibitor; Ex HMR1098, exercised, sarcolemmal K_{ATP} channel inhibitor.
weights and heart weights were similar for all treatments. Analysis of the nonperfused tissue weight-to-total heart weight ratio (Fig. 1) indicates a similar involvement of ischemic tissue for all treatments receiving IR. Furthermore, the nonperfused tissue weight-to-total heart weight ratio from the four IR treatments was statistically similar to the nonperfused (nonischemic) tissue weight-to-total heart weight ratio from sham hearts.

Electrocardiographic activity during IR and sham treatments. Ventricular arrhythmias were observed within minutes of surgically induced ischemia in all animals, with the majority of ectopic events occurring during ischemia (Fig. 2B). Episodes of VT and VF were observed in animals from all treatments receiving IR, although not all Ex animals experienced episodes of VT or VF. Notably, the majority of ventricular ectopy was observed during ischemia rather than reperfusion (Table 2). Statistical analyses of PVCs and episodes of VT and VF (data not shown) experienced during ischemia and reperfusion failed to produce a significant treatment by time effect (PVC, \(P = 0.240\); VT, \(P = 0.216\); VF, \(P = 0.156\)). Only three animals from the sham group experienced PVCs, presumably in response to passing the surgical ligature through excitable ventricular tissue. No sham animals experienced VT or VF.

Analysis of arrhythmia scoring-system-derived values of the collective ventricular ectopy experienced during IR reveal that, compared with sham, IR elicited a significant rise in ventricular arrhythmias (Fig. 2B). In contrast, exercise resulted in significant, albeit partial, attenuation of IR-induced arrhythmias. In contrast, the mean arrhythmia scores for Ex 5HD animals were statistically identical to those for Sed, indicating that the mito K\(_{\text{ATP}}\) channel contributes to exercise-mediated protection against arrhythmias during IR. Ex HMR1098 arrhythmia scores, alternately, were similar to those of Ex, indicating that the sarc K\(_{\text{ATP}}\) channel is not essential for anti-arrhythmic protection against IR in exercised hearts. Individual evaluation of PVCs between the five treatments (Fig. 3A) revealed mean response, which was similar to the arrhythmia scoring data. Due to large variations in PVCs identified between animals, however, statistical significance was only present between sham animals and Sed, Ex 5HD, and Ex HMR1098 treatments. Similarly, findings for VT (Fig. 3B) and VF (Fig. 3C) revealed nonsignificant differences between the four IR treatments, with the exception of a significant difference in the number of VF episodes between Ex 5HD and Ex HMR1098 treatments.

Myocardial antioxidant enzyme activity in perfused and ischemic tissue. To determine the impact of antioxidant enzyme activity on the preservation of myocardial redox status, several important endogenous antioxidant enzymes were evaluated in both ischemic and perfused tissue exposed to IR. Evaluation of SOD activities demonstrated that total SOD activities (Fig. 4A) were not impacted significantly between ischemic and perfused tissue for the four IR treatments. Similar findings were present for CuZnSOD (Fig. 4B). In contrast, MnSOD enzyme activity levels (Fig. 4C) were elevated significantly in both perfused and ischemic tissues of all three exercised treatments undergoing IR compared with sham and Sed IR treatments. Elevated MnSOD activity was consistent between ischemic and perfused tissue for all three exercised treatments. Evaluation of myocardial CAT activity (Fig. 5A) indicated no differences between treatments exposed to IR for either perfused or ischemic tissue. Similarly, perfused and ischemic myocardial GPx enzyme activities (Fig. 5B) were similar between sham and the four IR treatments.

Myocardial oxidative stress following IR. Several biomarkers of myocardial redox status were evaluated to determine the magnitude of oxidative stress imposed by the experimental IR protocol. Analysis of total myocardial protein carbonyl content (Fig. 6A) revealed that IR resulted in a significant rise in the ischemic tissue of Sed and Ex 5HD hearts. In contrast, myocardial 4-hydroxynonenal content (Fig. 6B) was not impacted by IR in either perfused or ischemic tissue from any treatment group. Finally, the short-duration IR employed in this investigation did not impact the GSH/GSSG levels in the perfused and ischemic myocardium (Fig. 7).

DISCUSSION

Summary of principal findings. This is the first study to investigate whether the mito and sarc K\(_{\text{ATP}}\) channels are essential for exercise-mediated attenuation of arrhythmia generation during IR. Results confirm previous study findings, which indicate that exercised hearts exposed to experimental IR suffer fewer and less severe arrhythmias compared with Sed counterparts (6, 25, 27). The current finding that the exercised heart is 5HD sensitive extends on existing knowledge, demonstrating that the mito K\(_{\text{ATP}}\) channel may promote anti-arrhythmic protection during IR, while the sarc K\(_{\text{ATP}}\) channel does not. Findings from the current investigation suggest that the anti-arrhythmic effects of mito K\(_{\text{ATP}}\) channels in exercised hearts may be associated with improved myocardial redox status.

Exercise and cardiac preconditioning against IR-induced arrhythmias. Hull et al. (27) demonstrated that a 6-wk exercise regimen completely abolished the incidence of VF in dogs identified to be at high risk for ventricular ectopy. Exercise elicited numerous anti-arrhythmic cardiovascular adaptations, including improvements in heart rate variability and vagal tone (27). A more recent study in dogs demonstrated that a single bout of treadmill exercise completely prevented VF during IR and raised survival by 7.5-fold over Sed controls (6). The first mechanistic examination of exercise and arrhythmia prevention was performed by Hamilton et al. (25), where 3 days of
exercise before IR resulted in significantly lower incidence and severity of ventricular arrhythmias. The endogenous antioxidant enzyme MnSOD was partially responsible for this protection, as demonstrated by the fact that anti-arrhythmic protection was mitigated in Ex animals that received antisense oligonucleotides directed against MnSOD transcripts (25). Data from the present study, which utilized identical species, sex, exercise preconditioning, and IR protocols, builds on the existing knowledge by demonstrating that the mito K<sub>ATP</sub> channels in exercised myocardium may also contribute to anti-arrhythmic protection against IR. That both MnSOD and the mito K<sub>ATP</sub> channel protect against IR-generated arrhythmias suggests myriad protection occurs in exercised hearts. In contrast, the sarc K<sub>ATP</sub> channels were not essential for exercise-induced protection against IR-generated arrhythmias. This latter finding, although not universally supported, may indicate that preemptive opening of the sarc K<sub>ATP</sub> channel could promote arrhythmias through heterogeneous repolarization of the myocardium (41, 47).

**Fig. 2.** A: representative images of digital ECG tracings from sham and ischemic conditions. B: ventricular arrhythmia scores. Values are means ± SE. Significantly different from *sham and #Ex, *P < 0.05.
K$_{\text{ATP}}$ channels and arrhythmia prevention during IR. The constitutively expressed, energy-sensing K$_{\text{ATP}}$ channels support metabolic function by opening in response to a host of stimuli, including a drop in cytosolic ATP levels or elevation in bioenergetic metabolites and stress-related signals (31). While IR resistance afforded by the K$_{\text{ATP}}$ channel opening is not fully understood, recent evidence indicates that channel subtypes on the inner mitochondrial membrane and on the sarcolemma provide significant protection through independent and interrelated means (35, 40). Although some debate persists regarding the anti-arrhythmic potential of K$_{\text{ATP}}$ channel opening, premitochondrial and sarc K$_{\text{ATP}}$ channel activation for improved cardiac health and performance in the face of acute IR is established (34). Protection is partially attributed to prevention of reentrant arrhythmias by mitigating phase 3 of the cardiac action potential during early ischemia and low-flow perfusion (39). The precise roles of these ion channels across biochemically diverse physiological scenarios related to IR, however, are not fully understood. Early studies in myocytes from exercised hearts revealed that cardiac K$_{\text{ATP}}$ channel opening elicited resistance to anoxic stress, while the nonselective K$_{\text{ATP}}$ channel inhibitor, glibenclamide, abolished this protection (28). Follow-up study indicated that exercise stimulated overexpression of the sulfonylurea receptor portion of the sarc K$_{\text{ATP}}$ channel promoted protection against IR injury (15). Selective pharmacological blockade of the sarc K$_{\text{ATP}}$ channel demonstrated an essential role for this channel against long-duration ischemic insults, although protection against IR-generated arrhythmias was not evaluated (12). Collectively, previous and present findings may indicate the sarc K$_{\text{ATP}}$ channel mediates tissue preservation during IR, while the mito K$_{\text{ATP}}$ channel protects against arrhythmias (20). The protective contribution of the mito and sarc K$_{\text{ATP}}$ channels does not, however, appear to be consistent across all experimental paradigms and requires further investigation (12–13, 17). Accordingly, we employed a relatively short-duration ischemia (20 min) and proportionate reperfusion period (30 min) to evaluate the impact of these ion channels as potential mechanisms of anti-arrhythmic protection in the exercised heart and investigated biochemical outcomes in perfused and ischemic myocardium before the overt onset of tissue death occurred.

Exercise, K$_{\text{ATP}}$ channels, and myocardial redox balance. How mito K$_{\text{ATP}}$ channels cardioprotect against short-duration IR in exercised hearts is not currently known. Since adaptations to exercise include cellular ATP preservation during IR, it is plausible that ATP-independent stimuli are responsible for mito K$_{\text{ATP}}$ channel opening in the exercised heart (9–10, 28–30). Mito K$_{\text{ATP}}$ channels, for instance, can open in response to a variety of stimuli, independent of a drop in cellular ATP, including PKCε (33, 38). In support, application of a short-term exercise regimen similar to the current training protocol resulted in PKCε overexpression in the heart (16). Once opened, a prevailing rationale supporting anti-arrhythmic effects of the mito K$_{\text{ATP}}$ channels during IR postulates that preconditioned hearts maintain a favorable redox status due to preserved ATP availability. This notion is supported by the finding that cytosolic ATP levels are maintained at 40% of baseline after 30 min of ischemia (2). This rationale may explain why exercise prevented an IR-induced rise in myocardial protein carbonyls, while pre-IR administration of the mito K$_{\text{ATP}}$ channel blocker 5HD resulted in protein carbonyl elevations similar to that of Sed animals. Although cause and effect are not confirmed, it is plausible that exercise-mediated mito K$_{\text{ATP}}$ channel opening maintained the mitochondrial inner membrane potential. As such, preserved state 3 respiration would be associated with attenuated mitochondrial superoxide release and prevention of ventricular arrhythmias due to regional irregularities in early after-depolarization (4, 44).

A novel aspect of this investigation was evaluation of important endogenous antioxidant enzymes in both perfused and ischemic tissue. Current findings from perfused exercised heart tissue exhibited a significant rise in MnSOD enzyme activity and agree with previously observed elevations in MnSOD activity from untrained “exercise sham” ventricular tissue (25, 32). The present data extend on this understanding by demonstrating that elevated MnSOD enzyme activity measured in post-IR ischemic tissue of exercised heart was unaffected by short-duration IR (25). The IR challenge of this study also did not influence CAT, GPx, total SOD, or CuZnSOD enzyme activities for any treatment group. This outcome may reflect the tissue condition at the time of sampling, as it is plausible that MnSOD enzyme activity was diminished at the
unobserved conclusion of ischemia, but recovered to basal levels during the relatively short reperfusion period. The enzyme activities from sedentary ischemic and perfused tissue were statistically similar and support this rationale. In continuation with this rationale, the IR duration from the present study was not sufficient to produce elevations in lipid peroxidation, as measured by 4-hydroxynonenal concentrations, to inactivate endogenous antioxidant enzymes or to impact myocardial glutathione levels. Unlike many previous investigations that document antioxidant and enzymatic activities from un-stressed tissues, cardiac GSH/GSSG levels in the present study were examined in the ischemic and perfused myocardium. Moreover, the extended experimental duration in vivo may have facilitated glutathione fluctuations not typically observed in isolated cell preparations. Collectively, the present findings emphasize that redox alterations to physiological stressors, such as ischemia, are dynamic and can be complex.

**Study limitations.** Short-duration exercise as a model of cardioprotection has several important advantages, including the fact that the cellular mechanisms responsible for acute IR resistance are not confounded by effects related to cardiac remodeling. It should be noted that short-duration exercise exposure may impose a stress response not directly attributable to exercise (14). Whether long-duration exercise preconditioning would have altered anti-arrhythmic protection mediated by either the mito or sarc $K_{ATP}$ channels cannot be determined currently.

Fig. 3. A: preventricular contractions/ischemia-reperfusion experiment. B: ventricular tachycardia episodes/ischemia-reperfusion experiment. C: ventricular fibrillation episodes/ischemia-reperfusion experiment. Values are means ± SE. Significantly different from *sham and *Ex 5HD, $P < 0.05$.

Fig. 4. A: total myocardial superoxide dismutase activity. B: myocardial CuZn superoxide dismutase activity. C: myocardial Mn superoxide dismutase activity. Values are means ± SE. Open bars represent perfused tissue. Solid bars represent ischemic or nonperfused (sham) tissue. Significantly different from *sham and $\Psi$Sed, $P < 0.05$. 

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To investigate ventricular arrhythmias following IR, we employed an established scoring system for collective analysis of PVCs, VT, and VF. These clinically derived scoring systems have been applied more recently to medically relevant research paradigms like the present investigation (18–19). Collective analysis of ventricular arrhythmias is advantageous for identifying physiologically meaningful outcomes, which may be masked statistically due to high variability in PVC, VT, or VF alone. Case in point, the ECG arrhythmia scoring system utilized in the present study yielded robust group-dependent outcomes compared with categorical analysis, as demonstrated by the nonsignificant “trends” observed between Sed and Ex animals (Table 2, Fig. 3). In this regard, retrospective power analyses indicate fourfold increases in sample size would be needed to delineate treatment differences for arrhythmias experienced during ischemia or reperfusion alone. Whether KATP channels contribute more protection to exercised hearts against arrhythmias generated during ischemia or reperfusion is uncertain at this time.

The popular pharmacological inhibitors 5HD and HMR-1098 were used to inhibit the respective mito KATP and sarc KATP channels. It is now generally held that pharmacological blockade of the mito KATP channel in the absence of a preconditioning stimulus does not impact post-IR outcomes (reviewed in Ref. 37). Characteristic of experimentation with pharmacological inhibitors, however, we cannot rule out the possibility of unknown pharmacological effects in the present study, including 5HD-specific effects on substrate availability (5, 26) and sex specific effects (17). Despite the potential role of 5HD metabolism within the heart, the resulting implications for outcomes related to preconditioning remain unknown. Finally, we cannot reconcile in the present study whether simultaneous pharmacological inhibition of the mito and sarc KATP channels of exercised hearts would have altered arrhythmia generation and oxidative stress.

Conclusions. Data collected for this investigation indicate that the mito KATP channel plays an important role in preventing short-duration IR-mediated arrhythmias in the exercised heart, while the sarc KATP channel does not. These findings highlight the complex, multifaceted nature of both IR injury and exercise-induced cardiac preconditioning. The emerging data from this line of study further highlight the unique nature of exercise preconditioning compared with ischemic and pharmacological stimuli (reviewed in Ref. 42). In light of the sustainable and cost-effective nature of exercise in clinical populations, exercise-based research into mediators against IR

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damage may play a growing role in the search for therapeutic treatments against IR injury (8). Future investigations may further elucidate the role of both the mito and sarc KATP channels as mediators of exercise-generated cardioprotection against other forms of IR injury, including myocardial stunning and apoptotic cell death.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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