Glibenclamide improves postischemic recovery of myocardial contractile function in trained and sedentary rats

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Jew, Korinne N., and Russell L. Moore. Glibenclamide improves postischemic recovery of myocardial contractile function in trained and sedentary rats. J Appl Physiol 91: 1545–1554, 2001.—In this study, we sought to determine whether there was any evidence for the idea that cardiac ATP-sensitive K⁺ (K<sub>ATP</sub>) channels play a role in the training-induced increase in the resistance of the heart to ischemia-reperfusion (I/R) injury. To do so, the effects of training and an K<sub>ATP</sub> channel blocker, glibenclamide (Glib), on the recovery of left ventricular (LV) contractile function after 45 min of ischemia and 45 min of reperfusion were examined. Female Sprague-Dawley rats were sedentary (Sed; n = 18) or were trained (Tr; n = 17) for >20 wk by treadmill running, and the hearts from these animals used in a Langendorff-perfused isovolumic LV preparation to assess contractile function. A significant increase in the amount of 72-kDa class of heat shock protein was observed in hearts isolated from Tr rats. The I/R protocol elicited significant and substantial decrements in LV developed pressure (LVDP), minimum pressure (MP), rate of pressure development, and rate of pressure decline and elevations in myocardial Ca<sup>2+</sup> content in both Sed and Tr hearts. In addition, I/R elicited a significant increase in LV diastolic stiffness in Sed, but not Tr, hearts. When administered in the perfusate, Glib (1 µM) elicited a normalization of all indexes of LV contractile function and reductions in myocardial Ca<sup>2+</sup> content in both Sed and Tr hearts. Training increased the functional sensitivity of the heart to Glib because LVDP and MP values normalized more quickly with Glib treatment in the Tr than the Sed group. The increased sensitivity of Tr hearts to Glib is a novel finding that may implicate a role for cardiac K<sub>ATP</sub> channels in the training-induced protection of the heart from I/R injury.

The influence of exercise training on myocardial resistance to ischemia and reperfusion (I/R) injury has been studied using a variety of species, training modalities, I/R protocols, and heart preparations. A majority of studies in this area are supportive of the idea that training confers a protective effect to the heart against I/R injury (for review see Ref. 46). In general, hearts from animals that have been trained at a sufficient intensity demonstrate improved postischemic hemodynamics, faster relaxation, increased high-energy phosphate content, and decreased myocardial stiffness, lipid peroxidation, and incidence of ventricular fibrillation compared with hearts isolated from sedentary controls (5, 6, 20, 24, 27, 31, 41). There is some speculation that enhancements in tissue antioxidant capacity (11) and an increased presence of the 70/72-kDa class of heat shock proteins (HSP72) (41) underlie this cardioprotective phenomenon. Increased myocardial HSP72 content has been associated with decreased infarct size and cell damage and with improved contractility, contractile force, rate of relaxation, sarcolemmal reticulum Ca<sup>2+</sup> uptake, and metabolic status after various protocols of I/R (8, 9, 13, 22, 38, 54). Elevated HSP72 protein content in the heart has been observed after a few days or several weeks of treadmill running (30, 37, 48), and at least two studies have shown enhanced postischemic myocardial recovery after acute exercise-induction of HSP72 (30, 48). Exercise training has also been found to elicit enhancements in myocardial antioxidant defense mechanisms (21, 24), and a correlative link exists between these types of cellular adaptations and training-induced increase in the resistance of the heart to I/R injury (11).

The cellular mechanisms by which training-induced increases in HSP72 and/or antioxidant enzyme activities protect the myocardium against I/R injury are unknown. However, it is interesting to note that there is now evidence that HSP72-induced cardioprotection is mediated via the operation of a glibenclamide (Glib)-sensitive, myocardial ATP-sensitive K⁺ (K<sub>ATP</sub>) channel (18, 23, 26). Additionally, the functional status of sarcolemmal K<sub>ATP</sub> channels (cardiac sulfonylurea receptor (SUR2A)/inward rectifier potassium (K⁺) channel 6.2 (Kir6.2)) (1) that carry the K<sub>ATP</sub> current is known to be strongly influenced by oxygen free radicals (49, 50). It has been proposed that K<sub>ATP</sub> current-mediated cardioprotection occurs when an ischemia-induced reduction in intracellular ATP concentration elicits cellular K⁺ efflux and a subsequent decrease in action potential duration (4, 36, 52). This, in turn, should shorten the time for Ca<sup>2+</sup> entry into the cell (7, 32) and lead to a reduction in cardiac contractile force and, therefore, ATP consumption (12).

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Because training-induced increases in HSP72 expression and myocardial antioxidant activities have been well documented, and because there is evidence that HSP72 and reactive oxygen species modulate the operation of KATP channels, it seems reasonable to speculate that exercise training may alter KATP current characteristics in a way that protects the heart from I/R injury. In this study, we hypothesized that, relative to hearts isolated from sedentary control animals, administration of a KATP channel blocker would have a greater influence on myocardial recovery from a bout of ischemia in hearts from trained animals.

**METHODS**

**Animal Model**

Female Sprague-Dawley rats aged 3–4 mo were randomly assigned to a sedentary (Sed) group (n = 33) and a run-trained (Tr) group (n = 33). The rats were used in a short-duration I/R protocol (15 Sed and 16 Tr) or a long-duration I/R protocol (18 Sed and 17 Tr). Both protocols are described later in this section. All animals were housed in a 12:12-h light-dark cycle and given standard rat chow and water ad libitum. Animals in the Tr group underwent at least 20 wk of treadmill running. During the first 6 wk, daily running duration began at 10 min and was prolonged biweekly in 10-min intervals, running grade was 5%, and treadmill speed ranged from 20 to 28 m/min. During the next 6 wk, running grade was increased to 10%, and treadmill speed ranged from 20 to 35 m/min. The final training protocol consisted of treadmill running 5 days/wk up a 10% grade for 1 h/day at 20 m/min for 15 min, 28 m/min for 30 min, and 35 m/min for 15 min. All animals were 9–11 mo of age when killed for whole heart functional analysis, at which time the plantaris muscle was dissected, homogenized, and assayed for citrate synthase activity (45). This study was conducted under the guidelines accepted by the American Physiological Society and received prior approval from the Institutional Animal Care and Use Committee at the University of Colorado, Boulder campus.

**Whole Heart Preparation**

Animals were anesthetized with intraperitoneal pentobarbital sodium (35 mg/kg) at least 15 min after injection with heparin (250 U). After thoracotomy, hearts were excised and immediately placed in ice-cold buffer. Within 2 min of excision, the aorta was cannulated and Langendorff perfusion established with a modified Krebs-Henseleit (KH) buffer containing (in mM) 117.4 NaCl, 4.7 KCl, 1.9 CaCl2, 1.2 MgSO4, 1.2 KH2PO4, 11 glucose, 5 pyruvate, 25 NaHCO3, and 0.5 EDTA and equilibrated with 95% O2-5% CO2. After a drain was created (with a 45° beveled segment 0.65-mm-OD and 0.45-mm-ID polyethylene tubing) in the left ventricle (LV) apex to prevent accumulation of Thebesian flow, a compliant water-filled latex balloon was inserted into the LV via the mitral valve. Pressure development was recorded with a pressure transducer (Millar), located inside the balloon, which was connected to a personal computer running data acquisition software (Axoscope, Axon Instruments). Balloon volume was adjusted so that minimum pressure (MP; i.e., LV end-diastolic pressure in an isovolumic preparation in which the heart does not actually fill) was 5 mmHg, and hearts were paced at 300 beats/min via the stainless steel aortic cannula and a platinum wire placed on the right atrium. Hearts were perfused at 37°C at a constant pressure of 110 cmH2O. Estimates of diastolic stiffness were made at the beginning and end of each experimental protocol by calculating the change in MP resulting from a 30-μl increase in balloon volume. Small, precise balloon volume changes were accomplished using a 50-μl Hamilton syringe that was connected to the fluid-filled pressure transduction circuit.

**Experimental Protocols**

Both short-duration (30 min) and long-duration (45 min) I/R experiments were conducted in this study. The short-duration I/R protocol did not produce irreversible LV contractile dysfunction, whereas the long-duration I/R protocol did. Because the central focus of this paper is on the data derived from the long-duration I/R experiment, we will describe that one first.

**Long-duration protocol.** After balloon inflation and initiation of pacing, hearts were subjected to one of four experimental protocols. These are described in the text that follows and summarized in the schematic depicted in Fig. 1.

**PROTOCOL 1: 15-MIN CONTROL**. Tr (n = 3) and Sed (n = 3) hearts were paced for 15 min during which contractile function was recorded every 5 min. Contractile function was assessed as systolic pressure (SP), MP, LV developed pressure (LVDP; calculated as SP – MP), rate of pressure development (+dP/dt), and rate of pressure decline (–dP/dt). In addition, measurements of myocardial flow, by timed collection of perfusate, and stiffness were made. This protocol was used to assess the functional baseline that existed in all experimental groups.

**PROTOCOL 2: 108-MIN CONTROL**. Tr (n = 3) and Sed (n = 3) hearts were paced for 108 min during which functional re-

| 15 min pacing | * 3 min substrate-free KH perfusion | # 1 min 0 Ca2+ 0°C perfusion |
| 108 min pacing | # |
| I/R | 45 min ischemia | 45 min reperfusion |
| I/NG | 45 min ischemia | 45 min reperfusion W/ Glibenclamide |

Fig. 1. Schematic representation of the time lines and respective interventions for each of the 4 experimental protocols used in the reported studies. I/R, ischemia-reperfusion; I/RG, ischemia-glibenclamide reperfusion; KH, Krebs-Henseleit.
cordings were taken every 5 min, myocardial flow measured intermittently, and stiffness assessed at 15 and 108 min. This protocol was used to assess the intrinsic stability of normally perfused preparation over time and to account for any effects of perfusion time that might otherwise be misinterpreted as being due to one of our experimental interventions.

**Protocol 3:** I/R. After 15 min of pacing, Tr (n = 5) and Sed (n = 6) hearts were perfused with a substrate-free (no glucose or pyruvate) KH buffer for 3 min, after which global ischemia was induced for 45 min and hearts were not paced. Pacing was restored during 45 min of reperfusion with substrate-containing KH buffer. Contractile function, coronary flow, and myocardial stiffness were assessed throughout reperfusion as in protocol 2. Protocol 3 was used to assess the effects of I/R on hearts from Tr and Sed animals.

**Protocol 4:** Ischemia-GLIB reperfusion (I/RG). Tr (n = 6) and Sed (n = 6) hearts in this group were treated identically to hearts in the I/R group, except for the presence of the KATP channel blocker GLIB (1 μM) in the reperfusion buffer. This protocol was used to determine whether or not GLIB administration during reperfusion differentially affected the recovery of Sed and Tr hearts from a 45-min bout of ischemia.

**Short-duration protocol.** A shorter duration I/R protocol was also used in this study. This experiment employed protocols that were analogous to the I/R and I/RG groups described above, except that the ischemia and reperfusion periods were 30 min in duration. More detail is provided in the legend of Fig. 2.

**Biochemical Analyses**

At the end of each of the four protocols, hearts were perfused for 1 min with a 0°C 0 Na+-0 Ca2+-buffer containing (in mM) 350 sucrose and 5 histidine to halt metabolism and wash out extracellular Ca2+. This nominal-Ca2+-containing washout solution was previously pretreated with DOWEX 50 to facilitate the removal of contaminant Ca2+ (35). The heart was then cut into four pieces, the first of which was immediately freeze clamped for later analysis of ATP, ADP, and phosphocreatine (PCr), using methods described previously (47). Two snippets were used for determination of Ca2+ content by atomic absorption spectroscopy using methods described by Nayler et al. (35). Finally, HSP72 content was measured using a commercially available enzyme immunosay (Stressgen Biotechnologies). A 2-ml sample of perfusate was taken in the first minute of reperfusion (or the equivalent time in the 108-min control group) for the measurement of lactate dehydrogenase (LDH) content using a LDH diagnostic kit (SIGMA Diagnostics) to provide a qualitative metric of cell damage.

**Data Analyses**

Analysis of contractile function data was performed using custom-made JLDL 4.0 software (Research Systems, Boulder, CO). Statistical analyses were performed using SPSS 10.0 software (SPSS, Chicago, IL). Simple preischemia, between-group (Sed vs. Tr) analyses were conducted using a Student's t-test. Between-group comparisons across time were made using a repeated-measures analysis of variance and post hoc analyses (Duncan's multiple-range test) conducted when significant F ratios were obtained. Differences in biochemical markers were detected using one-way analysis of variance. All data are presented as means ± SE. To reduce the possibility of committing a type II interpretive error, i.e., a false negative, significance was reported at both the P < 0.05 and P < 0.10 levels (53).

**RESULTS**

**Animal Model**

Training did not significantly affect body weight, dry heart weight, or tibial length. The training protocol employed in this study has previously been found to elicit a small but significant increase (~7–10%) in ventricular weight (34, 39). Although a statistically significant training-induced increase in heart weight was not observed in this study, it should be noted that mean dry heart weight from Tr rats was ~7% greater than that assessed in Sed rats. The lack of statistical significance in this metric may have been related to a slight increase in heart weight variability that resulted from the derivation of heart weights from at least four pieces of tissue (used for metabolite, HSP72, and/or tissue Ca2+ measurements) from each heart. Similar to earlier work using this training model, our training protocol did elicit marked increases in plantaris muscle citrate synthase activity (Table 1) (34, 39) and myocardial HSP72 content (37). It should be noted that elevated HSP72 does not by itself represent a true training adaptation because a wide variety of stressors can also produce HSP72 elevation in the heart. However, in the context of our training model, the citrate synthase and cardiac HSP72 data certainly support the idea that
Table 1. Baseline characterization of the isovolumic LV preparation and plantaris muscle citrate synthase activity across all Sed and Tr animals

<table>
<thead>
<tr>
<th></th>
<th>Sed (n = 18)</th>
<th>Tr (n = 17)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum pressure, mmHg</td>
<td>5.2 ± 0.2</td>
<td>5.3 ± 0.2</td>
<td>0.54</td>
</tr>
<tr>
<td>LVSP, mmHg</td>
<td>114 ± 3</td>
<td>110 ± 4</td>
<td>0.46</td>
</tr>
<tr>
<td>LVDP, mmHg</td>
<td>105 ± 3</td>
<td>105 ± 4</td>
<td>0.45</td>
</tr>
<tr>
<td>+dP/dt, mmHg/s</td>
<td>2,525 ± 70</td>
<td>2,397 ± 86</td>
<td>0.26</td>
</tr>
<tr>
<td>−dP/dt, mmHg/s</td>
<td>−1,827 ± 58</td>
<td>−1,768 ± 57</td>
<td>0.48</td>
</tr>
<tr>
<td>Diastolic stiffness, mmHg</td>
<td>131.1 ± 1.7</td>
<td>103.3 ± 1.2</td>
<td>0.18</td>
</tr>
<tr>
<td>30 μl</td>
<td>131.1 ± 1.7</td>
<td>103.3 ± 1.2</td>
<td>0.18</td>
</tr>
<tr>
<td>Myocardial flow, ml·min⁻¹·g⁻¹ dry tissue⁻¹</td>
<td>74 ± 3</td>
<td>77 ± 5</td>
<td>0.56</td>
</tr>
<tr>
<td>Citrate synthase activity, μmol·min⁻¹·g⁻¹ wet tissue⁻¹</td>
<td>16 ± 1</td>
<td>24 ± 1</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats. Myocardial measurements were taken before ischemia after 15 min of normal perfusion. Sed, sedentary; Tr, trained; LV, left ventricle; LVSP, left ventricular systolic pressure; LVDP, left ventricular developed pressure; +dP/dt, rate of pressure development; −dP/dt, rate of pressure decline.

Both peripheral and central compensatory adaptations were elicited by our training paradigm.

LV Functional Adaptations to Training

Before ischemia there were no training-induced differences in ventricular functional parameters, including MP, LVSP, LVDP, +dP/dt, and −dP/dt (Table 1). There were also no differences in myocardial stiffness or myocardial flow between the Tr and Sed groups.

Evidence of training adaptation was apparent in the 108-min nonischemic control group where the hearts from the Tr group maintained steady MP values over time, whereas MP values from the Sed hearts increased toward the end of the perfusion period (ANOVA “training × time” interaction, P < 0.05; see Fig. 3, inset).

Postischemia Contractile Function

I/R caused a significant increase in MP in early reperfusion in both the I/R and I/RG groups compared with the 108-min controls (I/R effect, P < 0.01). Whereas MP values for hearts in the I/R groups remained elevated throughout the reperfusion period, those in the I/RG groups decreased over this time and did not differ from the nonischemic controls after 10 min of reperfusion (drug effect, P < 0.01; Fig. 3). Training elicited faster normalization of MP in the Tr I/RG group in early reperfusion (P = 0.06; Fig. 3, inset), suggesting a training-induced enhancement in the sensitivity of the heart to Glib. However, this between-group MP difference was absent by the end of the 45-min reperfusion period.

Results for LVDP revealed a similar pattern as that for MP. Whereas the I/R and I/RG groups had depressed LVDP in early reperfusion (I/R effect, P < 0.01), LVDP values for hearts in the I/RG groups increased more quickly and to a greater extent than those in the I/R groups. Relative to the I/R groups, LVDP in I/R hearts remained depressed during most of the reperfusion period (group × I/R interaction, P < 0.05; Fig. 4). With respect to LVDP, training did not increase the resistance of heart to I/R. However, as was found with MP, there was a trend for LVDP in the Tr group treated with Glib to normalize more quickly than in the Sed group (P = 0.09; Fig. 4, inset). These Tr vs. Sed LVDP differences were absent by the end of reperfusion.

Similar response patterns were evident for LVSP, +dP/dt, and −dP/dt. Values for these variables from hearts subjected to I/R reflected contractile dysfunction in early reperfusion, but those treated with Glib normalized over time whereas values for hearts not receiving the drug displayed sustained impairment. No training differences were observed in either the I/R or I/RG groups, although there was a trend for hearts in the Tr I/RG group to respond more quickly (i.e., normalize faster) than their Sed cohorts.

LV Stiffness

All groups had similar LV stiffness values before induction of ischemia. Post-I/R, LV stiffness was lower in Tr hearts compared with Sed hearts (P = 0.085; see Fig. 5). Additionally, compared with time-matched nonischemic control values, I/R elicited an increase in myocardial stiffness only in the Sed I/R group (P = 0.05; see Fig. 5). Treatment with Glib did affect stiffness in the Sed animals because hearts in the Sed I/RG group had decreased stiffness values compared with those in the Sed I/R condition (P = 0.075). Post-I/R LV stiffness was lower in I/RG animals (P = 0.001). LV stiffness values for the I/RG-Tr group were more similar to those in the nonischemic control group than in the I/R group (P = 0.05).
stiffness values in the Tr I/R hearts did not differ significantly from nonischemic controls or the I/RG group.

Heat Shock Protein

Training had a dramatic effect on HSP72 content (Fig. 6). Hearts from Tr animals had significantly greater HSP72 (32.3 ± 4.2 ng HSP72/mg homogenate protein) than did hearts from Sed animals (7.3 ± 0.5 ng HSP72/mg homogenate protein) (training effect, \( P < 0.001 \)).

LV \( \text{Ca}^{2+} \) Content and Metabolites

Total LV \( \text{Ca}^{2+} \) content decreased over perfusion time in both Tr and Sed groups (time effect, \( P < 0.05 \)), although follow-up t-tests revealed that only the Sed group experienced a statistically significant decline (\( P < 0.05 \); see Table 2). I/R caused an increase in LV \( \text{Ca}^{2+} \) content in both Sed and Tr groups (I/R effect, \( P = 0.01 \)), with a trend for the Tr I/R group to take up more \( \text{Ca}^{2+} \) than the Sed (\( P = 0.056 \)). Application of the \( \text{K}_{\text{ATP}} \) channel blocker resulted in a decrease in \( \text{Ca}^{2+} \) content in both Tr and Sed animals (drug effect, \( P = 0.085 \)). With respect to LV \( \text{Ca}^{2+} \) content, there was no apparent training-induced alteration in sensitivity to Glib.

Across three experimental groups, hearts from Tr animals had lower ATP contents than did Sed hearts (training effect, \( P = 0.05 \)). However, the Tr I/R hearts did have a greater mean ATP content than their Sed counterparts. I/R resulted in a significant decrease in myocardial ATP levels (I/R effect, \( P < 0.001 \)), whereas the \( \text{K}_{\text{ATP}} \) channel blocker had no effect. Training had no effect on PCr content in any of the experimental conditions.

I/R elicited an increase in the LDH content of the coronary effluent in all groups (I/R effect, \( P < 0.05 \); Fig. 5). Postischemia myocardial stiffness measurements for 108-min control (108), I/R, and I/RG groups. After I/R, stiffness was greater in Sed (open bars, \( n = 6 \)) than in Tr (solid bars, \( n = 5 \)) hearts (\( *P = 0.085 \)). [Viewed in another way, relative to the appropriate 108-min nonischemic control values (Tr, \( n = 3 \); Sed, \( n = 3 \)), stiffness increased significantly after I/R but only in the I/R-Sed group (\( P = 0.05 \)).] Glibenclamide elicited a reduction in stiffness only in the I/RG-Sed group (\( P = 0.075 \); Sed, \( n = 6 \); Tr, \( n = 6 \)). Stiffness values were similar for all Tr groups at the end of the reperfusion period. Values are means ± SE.
More detailed statistical comparisons.

From time-matched I/R value, minutes 3–4 between phases of reperfusion.

Table 3. Postischemic reperfusion of hearts with the KATP channel blocker, Glib, resulted in the general improvement of postischemic LV contractile function, LV stiffness, and myocardial Ca\(^{2+}\) content.

Specifically, by the end of the recovery reperfusion period, values for LVDP, MP, LVSP, \(\Delta P/\Delta t\), and myocardial stiffness in both Sed and Tr I/RG groups were strikingly similar to the time-matched nonischemic controls. In addition, post-I/R myocardial Ca\(^{2+}\) contents were lower in both Sed and Tr I/RG groups compared with their time-matched I/R counterparts. Our findings are consistent with other investigations that suggest that KATP channel blockade is beneficial to postischemic recovery (40, 51). Both studies (40, 51) reported that, after ischemic insult, Glib produced a reduction in reperfusion-induced arrhythmias. In addition to the decrease in arrhythmias, Tosaki and Hellegouarch (51) report improved myocardial contractility and tissue ATP content during reperfusion in hearts treated with a KATP channel blocker compared with those exposed to a channel opener.

It seems likely that the transient Glib-induced augmentation in coronary flow during early reperfusion contributed to the improved functional recovery of hearts in both the Sed and Tr I/RG group. The Glib-induced early hyperreperfusion cannot, however, explain the observation that Tr hearts recovered more quickly than Sed hearts because the augmentation in coronary flow during early reperfusion was identical between the groups. The Glib-induced early hyperreperfusion cannot, however, explain the observation that Tr hearts recovered more quickly than Sed hearts because the augmentation in coronary flow during early reperfusion was identical between the groups.

Table 3. Postischemic and time-matched control (nonischemic) coronary flows occurring in the early and late phases of reperfusion.

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Table 2. Neither training nor KATP channel blockade effects on effluent LDH content were observed.

### Myocardial Flow

Preischemia baseline myocardial flow (coronary flow) rates were between Tr and Sed groups (Table 1). Hearts reperfused with Glib had greater coronary flows during the early phase of reperfusion than those from the normally reperfused hearts (Table 3). The Glib-induced enhancement in early reperfusion coronary flow was transient insofar as it was observable only through the first 6 min of reperfusion. By the 16th min of reperfusion with Glib, coronary flows had fallen to values similar to those observed in the I/R group. At the end of the 45-min reperfusion period, hearts from both I/R and I/RG groups were strikingly similar to the time-matched nonischemic controls. In addition, post-I/R myocardial Ca\(^{2+}\) contents were lower in both Sed and Tr I/RG groups compared with their time-matched I/R counterparts.

DISCUSSION

Glib Treatment and Functional Recovery From I/R Challenge

A major finding of this investigation was that the postischemic reperfusion of hearts with the KATP channel blocker, Glib, resulted in the general improvement and/or normalization of postischemic LV contractile function, LV stiffness, and myocardial Ca\(^{2+}\) content.

Table 2. Myocardial Ca\(^{2+}\) content and metabolite concentrations

![Table 2. Myocardial Ca\(^{2+}\) content and metabolite concentrations](image-url)

Values are means ± SE. Measurements of myocardial Ca\(^{2+}\), ATP, ADP, and phosphocreatine (PCr) contents were made from tissue taken at the end of each protocol. Lactate dehydrogenase (LDH) activity was measured in coronary effluent collected 30 s after perfusion was reestablished in the ischemia-reperfusion (I/R) groups or at the equivalent time in controls. I/RG, ischemia-glibenclamide reperfusion; ND, not detected. *I/R effect, \(P < 0.05\). †Drug effect, \(P < 0.1\). ‡Training effect, \(P < 0.05\). §Training × I/R interaction, \(P < 0.05\). See text for more detailed statistical comparisons.
are blocked during exercise. Therefore, it is possible that the accumulation of myocardial adenosine during prolonged ischemia overrides the inhibitory effect of Glib on the smooth muscle sarcolemmal KATP channels, resulting in vascular hyperpolarization and subsequent vasodilation and increased coronary flow. However, this type of mechanism would require that the adenosine response be potentiated by Glib in some fashion. We know of no information to support or refute the existence of such a potentiating mechanism. Alternatively, Glib might act at the level of coronary vascular endothelial sulfonylurea receptors (55). In a posts ischemic setting, it is possible that Glib could stimulate the release of one or more endothelially derived vasodilatory substances that could override any vasoconstrictive effect that Glib might have at the level of the coronary vascular smooth muscle. We hasten to point out that these are purely speculative mechanisms and that this is an area in need of further investigation.

One issue that must be considered when interpreting the results of the present study is the timing of Glib application. The effects of the drug appear to vary depending on when it is administered. Schultz et al. (43) report that a dose of Glib given 30 min before a brief ischemic preconditioning bout abolishes the protective preconditioning effect, whereas one given 5 min before does not. We acknowledge that treating the hearts with Glib before or during ischemia may have produced very different results than those observed in this study. Indeed, these are the types of experimental designs appearing in much of the cardiac preconditioning literature that conclude that KATP blockers negatively influence recovery after ischemic insult (7, 15, 25, 33, 43). Our finding that blocking the KATP channels during reperfusion had a beneficial effect on posts ischemic recovery is noteworthy. It may be that KATP blockers administered before or during ischemic challenge exacerbate the severity of the ischemic insult, whereas administration of KATP blockers during the recovery reperfusion period contributes to a normalization of contractile function secondary to the elimination of a suppressive, hyperpolarizing current. This timing issue may be clinically relevant in situations where interventions are undertaken to normalize or reestablish acutely disrupted coronary flow (i.e., primary angioplasty for an acute myocardial infarction).

**Training Alters the Responsiveness of the Heart to Glib After Severe I/R Challenge**

The most significant finding in the present study was that Glib differentially affected the recovery of Sed and Tr heart after ischemia. Values for LVDP, MP, LVSP, +dP/dt, and −dP/dt were normalized for hearts in the Tr and Sed I/RG groups by the end of reperfusion. In addition, Ca2+ content was similarly decreased in both of these groups. However, there are two noteworthy differences in the response to KATP channel blockade between hearts from Tr and Sed animals. First, contractile function in early reperfusion, including values for LVDP and MP, was normalized more quickly in the Tr group, suggesting an increased sensitivity to Glib. Second, Glib produced a dramatic decrease in myocardial stiffness in the Sed I/RG but not in the Tr I/RG hearts. At first glance, these findings might seem contradictory, the first suggesting an enhanced and the second a reduced functional sensitivity to Glib with exercise training. It is important to note that stiffness was not significantly increased after ischemia in the Tr group as it was in the Sed. Although stiffness was slightly decreased after Glib application in the Tr group, mean myocardial stiffness for the Tr I/RG group was not different from that in the nonischemic control or the I/R group. It is possible that the degree of stiffness or, alternatively, compliance was optimal under these conditions (i.e., 108 min of simulated perfusion) in the Tr hearts and that any further decrease in stiffness would impair the normal heterometric regulation of force development.

The apparent heightened sensitivity to KATP channel blockade observed with exercise training, although only apparent in early reperfusion, is intriguing. We can only offer very speculative explanations for this observation. First, our data are consistent with a mechanism where training has the effect of suppressing the opening of KATP channels and/or rendering KATP channels more sensitive to closure by Glib. It may be that training induced an increase in the binding affinity of Glib to SUR2A or on the effect of SUR2A occupancy on the KATP channel (Kir6.2) closure. In either case, it may be that, during early reperfusion, K+ efflux was suppressed in Tr hearts relative to Sed hearts. From the mechanical data, one might surmise that this resulted from an earlier normalization of ventricular action potential configuration, and this could explain why the Tr hearts resumed normal contractile function sooner than did Sed hearts. Second, the physiological consequences of the more rapid normalization of contractile function in Tr hearts are severalfold and can be viewed in the context of a balance that must be achieved between myocardial oxygen supply and demand. In a physiologically relevant setting where the perfusion of the coronary vasculature is directly dependent on the mechanical activity of the LV, the more rapid normalization of contractile function in Tr hearts would be viewed in a positive light because this would improve oxygen delivery to myocardium. However, the earlier resumption of normal contractile activity would be accompanied by an increase in tissue metabolic demand that could impose additional metabolic stress on the heart. This latter consequence may explain why myocardial ATP content tended to be lower in the Tr I/RG hearts and at the end of the 45-min reperfusion period. We hasten to point out, however, that at the end of the reperfusion period, LV contractile function in Tr I/RG hearts was similar to that observed in Sed I/RG hearts and the nonischemic time-matched controls.

Finally, it is interesting to note that, with respect to the effect of Glib on posts ischemic LV contractile function, there were several key differences between the
results of our 30-min (Fig. 2) and 45-min I/R protocols. In the former studies (see Fig. 2), Glib did not significantly influence the magnitude or temporal characteristics of recovery of LV contractile function whereas the effects were quite profound in the latter studies. It appears that the restorative effects of Glib on LV contractile function and the differential functional sensitivity of Tr and Sed hearts to Glib treatment were evident only under conditions where the severity of ischemic insult was sufficient to elicit irreversible LV contractile function deficits in the absence of Glib (contrast Fig. 2 with Figs. 3 and 4). Clearly, this further underscores the importance of considering the timing and experimental setting in which Glib is applied when addressing the issue of whether or not blocking the KATP channels hinders postischemic myocardial recovery.

Training and Functional Recovery From I/R Challenge

Our finding that chronic treadmill running produced a marked increase in HSP72 expression corroborates our laboratory’s previous work using an identical training paradigm (37) and the work of others (29, 48). Numerous reports exist in the literature demonstrating improved cardiac functional recovery after ischemia and/or hypoxia and reperfusion in hearts from trained animals (3, 5, 6, 30, 41, 42, 44). In our study, the only mechanical measure that was “protected” by training was LV stiffness. This corroborates the earlier findings of Bowles et al. (6) and provides mechanical evidence of a trained state. Finally, our observation that the absolute decline in tissue ATP concentration after ischemia and reperfusion was greater in hearts from the Sed than in the Tr rats was indicative of a training-induced enhancement in the ability of the heart to defend ATP stores after I/R challenge. This finding is further evidence of a cardioprotective training effect and is consistent with the work of others using a variety of different training models (3, 5, 6).

In our study, training did not improve or protect any postischemic metric of LV contractile function (i.e., LVDP, MP, +dP/dt, and −dP/dt) during reperfusion with a normal KH buffer. We speculate that this apparent discrepancy can be explained by differences between our experimental design and those used by most others. To our knowledge, our study and one other (27) are the only ones to address the training-I/R issue using an isovolumic left heart preparation exposed to global ischemia and reperfusion. The isovolumic left heart preparation permits the assessment of homoeometric changes in LV contractile function, whereas it is not suitable for the assessment of heterometric, working LV function (i.e., where the heart goes through filling and ejection phases). Our finding of lower postischemic myocardial stiffness with training corroborates the earlier work of Bowles et al. (6) in which a working heart model was employed, and this finding should not be underemphasized for the following reasons. In a heterometric heart preparation (i.e., working or simple Langendorff-perfused preparations), disproportionate increases in myocardial stiffness in hearts from sedentary animals would be expected to compromise LV filling during diastole, which would in turn produce deficits in the length-dependent regulation of LV function. Such length-dependent functional deficits would (should) not be observable in an isovolumic, homeometric LV preparation. It is likely that the training-induced reduction in post-I/R myocardial stiffness seen in the present study and in that of Bowles et al. (6) would be functionally most apparent in situations where Frank-Starling mechanisms were operational.

Paradoxically, the lower LV stiffness observed in the Tr group occurred in conjunction with a greater post-I/R myocardial Ca2+ content than was observed in myocardium isolated from the Sed I/R group. One might predict that greater cellular Ca2+ would cause increased activation of the contractile element and impaired relaxation, which would be reflected in higher myocardial stiffness. Interestingly, whereas both the present study and Bowles et al. (6) reported decreased post-I/R myocardial stiffness with training, Bowles et al. found that the training-induced reduction in post-I/R stiffness was associated with a decrease in myocardial 45Ca2+ uptake. The only purely hypothetical explanation that we can offer for this discrepancy is that our assessment of myocardial Ca2+ content was based on a physical measurement of total tissue Ca2+, whereas Bowles et al. based their myocardial Ca2+ content estimates on tissue 45Ca2+ uptake measurements. The latter method is subject to the assumption that intracellular and extracellular Ca2+ pools were in equilibrium at the time of assessment. Nonetheless, a potential explanation for our surprising result is that the total Ca2+ buffering and/or internal Ca2+ sequestration capacity of the myocardium was improved in the hearts from Tr animals. This is an intriguing possibility that deserves further experimental investigation.

Potential Limitations

There are two important considerations to be addressed in interpreting our data. First, Glib has been reported to have vasoconstrictor properties that could influence coronary reflow after ischemia and, therefore, recovery during reperfusion (10). It is unlikely that the vasoactive effects of Glib are responsible for the findings in this investigation, especially because the observed drug effects were positive, whereas the effects of vasoconstriction would, mostly likely, be negative. Myocardial flow was not different during the course of reperfusion between groups treated and not treated with the drug, except in early reperfusion when Glib administration was associated with an increase in flow (Table 3). Together, these observations diminish concern about the vasoconstrictor effects of the drug influencing our results. Second, cardiac mitochondrial KATP channels have been implicated in the defense of the heart from I/R injury (16, 17, 28), and it is possible
that our results were influenced by the action of Glib on mitochondrial rather than sarcolemmal KATP channels. Again, we believe that this is unlikely for two reasons. The blockade of mitochondrial KATP channels suppresses oxidative phosphorylation (19, 28) and would be expected to have a deleterious effect on the recovery of contractile function after ischemic challenge. The latter was not the case in our study. In addition, in our study we used 1 μM Glib, a concentration that is sufficient to completely block sarcolemmal KATP channels (2) but lower than that required to completely block mitochondrial KATP channels (dissociation constant = ~4 μM; Ref. 28).

In summary, treatment of hearts with a KATP channel blocker during reperfusion was associated with an improved recovery of myocardial contractile function after a severe ischemic challenge. Exercise training conferred cardioprotection to the heart against I/R insult and appeared to enhance the sensitivity of the heart to KATP channel blockade. More work will be required to elucidate the cellular adaptations that underlie the cardioprotective effects of exercise training, including more detailed examination of the KATP current or channel properties that might play a role in this phenomenon.

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