Improved postischemic function following acute exercise is not mediated by nitric oxide synthase in the rat heart

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First published September 1, 2006; doi:10.1152/ajpheart.00094.2006.—The mediators of acute exercise-induced preconditioning against ischemia-reperfusion injury are not understood. This study assesses the role of nitric oxide synthase (NOS), a reported mediator of other forms of preconditioning. Male Fischer 344 rats were divided into five groups (n = 6–7): sedentary (Sed); exercised 2 days on a treadmill at 20 m/min, 6° grade, for 60 min (Run); sedentary, perfused with 100 μM Nω-nitro-L-arginine methyl ester hydrochloride (L-NAME) to inhibit NOS (Sed/L-N); exercised, perfused with L-NAME (Run/L-N); and exercised in a 4°C environment, perfused with L-NAME (CRun/L-N). Twenty-four hours following exercise, isolated, perfused working hearts were subjected to 22.5 min of global ischemia plus 30 min of normoxic reperfusion. Left ventricle contents of several putative preconditioning mediators were determined. Postischemic recovery of cardiac output times systolic pressure was better in Run than Sed (78.4 vs. 50.2% of preischemia, P < 0.05). Inhibition of NOS did not abrogate the improved recovery in the exercise groups or alter recovery in Sed. All exercise groups also displayed improved myocardial efficiency (cardiac output times systolic pressure/oxgen consumption) postischemia and less lactate dehydrogenase release (P < 0.05). L-NAME appeared to lower lactate dehydrogenase release independent of exercise. The only change in antioxidant enzyme activity was a decrease in manganese superoxide dismutase in CRun/L-N (P < 0.05). Heat shock protein 72 expression increased only in Run and Run/L-N and endothelial NOS only in CRun/L-N (P < 0.05). Acute exercise-induced preconditioning of the Fischer 344 rat heart is not mediated by NOS and does not require increases in heat shock protein 72 or antioxidant enzymes.

reactive oxygen species; reperfusion injury; oxygen consumption; stunning; contractile function

IT IS NOW WELL ESTABLISHED that exercise can result in late-phase preconditioning against ischemia-reperfusion (I/R) injury (13–15, 18, 22, 25, 33, 47, 50, 53); however, the mechanisms that provide this protection are not fully understood. Unraveling the mechanisms for how exercise induces myocardial self-protection has enormous health care implications, including reducing health care costs and providing the conceptual framework for developing therapeutic strategies aimed at mimicking the cardioprotective benefits of exercise. Locke et al. (35) initially suggested that the late-phase preconditioning observed after 3 days of exercise was due to an increase in inducible heat shock protein 72 (HSP72) concentration, because it was positively correlated to functional tolerance to I/R. To determine whether increased HSP72 is required for exercise-induced cardioprotection, we exercised Sprague-Dawley rats in the cold to prevent a rise in HSP72 concentration and found improved I/R tolerance 24 h after a single exercise bout (50). Subsequently, these findings were corroborated in an in vivo model of I/R injury (22). Yamashita et al. (53) carried out studies demonstrating a correlation between increased mitochondrial manganese superoxide dismutase (MnSOD) expression after acute exercise and protection against I/R-induced infarct development. However, other studies have subsequently determined that elevated MnSOD is not required for exercise-induced cardioprotection against stunning or infarction (15, 33, 47).

An interesting observation from our earlier study was that coronary flow (CF) following I/R was greater in the group exercised in the cold than in the sedentary group (50). We have also observed a positive correlation between CF during early reperfusion and eventual recovery of function in chronically exercised Fischer 344 rats (13). A potential mechanism by which acute exercise provided cardioprotection, independent of HSP72, could be related to a reactive oxygen species (ROS)-induced increase in CF through the action of nitric oxide (NO) produced by an increased concentration of NO synthase (NOS). Many studies report that elevated NO protects against myocardial I/R injury (5, 6, 9, 30). Importantly, inhibition of NOS has been reported to block late-phase preconditioning following ischemic preconditioning (11, 44), heat stress (1, 28), and various pharmacological treatments (6, 8, 19, 20, 26). The precise mechanism by which NO protects is not known, but possibilities include inhibition of calcium overload, activation of mitochondrial ATP-sensitive K+ (KATP) channels, or antioxidant actions (see Ref. 9 for review). Thus the purpose of this study is to explore the role of NOS in mediating cardioprotection against I/R associated with 2 consecutive days of exercise. Response to I/R will be evaluated using an isolated, perfused working heart model, which provides a very sensitive and specific measure of myocardial function and injury.

MATERIALS AND METHODS

Animals and training protocols. Male 4- to 6-mo-old Fischer 344 rats were obtained from Harlan Sprague Dawley (Indianapolis, IN) and kept at the University of Texas Animal Resource Center. The animals were maintained on a 12:12-h light-dark cycle and fed ad libitum with Harlan Teklad 7013, NIH-31 diet. Rats were randomly assigned to five different treatment groups: sedentary (Sed); exercised by treadmill running (Run); Sed and Nω-nitro-L-arginine methyl ester hydrochloride (L-NAME) (a competitive inhibitor of NOS) added to the perfusion buffer (Sed/L-N); Run and L-NAME added to the perfusion buffer (Run/L-N); and running in a cold room and L-NAME

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added to the perfusion buffer (CRun/L-N). Groups Run and Run/L-N were run on a motorized treadmill (Collins, Braintree, MA), at room temperature, for 2 consecutive days, 60 min/day, at a speed of 20 m/min, up a 6° grade. CRun animals were run identically, but in a 4°C room to prevent a rise in core temperature, as described previously (50). Rectal temperature was monitored with a Bannantype J Thermocoupler, as described previously, to ensure that core temperature did not increase (50). All exercised animals were killed 24 h after their last exercise bout. This investigation, approved by the University’s Animal Care and Use Committee, conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996).

Isolated heart perfusions. Animals were anesthetized with an intraperitoneal injection of 40 mg/kg body wt of pentobarbital sodium. Hearts were weighed, and myocardial function and oxygen consumption were evaluated at 37°C using an isolated, working heart preparation, as previously described (46, 47, 50). The perfusion buffer contained (in mM) 10 glucose, 1.75 CaCl2, 118.5 NaCl, 4.7 KCl, 1.2 MgSO4, 24.7 NaHCO3, 0.5 EDTA, 12 mU/ml insulin, and was gassed with 95% O2-5% CO2. In some groups, all NOS isoforms were inhibited by including 100 μM L-NAME (no. N-5751, Sigma Aldrich, St. Louis, MO) in the perfusate throughout the entire perfusion procedure. This concentration of L-NAME was selected, because it has been reported to inhibit nearly 100% of all NOS isoforms in the perfused rat heart (21, 43), and several investigations have employed L-NAME at doses of 100 μM or less to successfully block I/R protection from various forms of late preconditioning that rely on NO (1, 8, 11, 19, 20, 28, 44).

Atrial filling pressure was maintained at 12.5 mmHg, and afterload was set by an 80-cm-high aortic column (inner diameter 3.18 mm). Hearts were allowed to beat at their own intrinsic heart rate (HR); however, if this rate was lower than 270 beats/min before ischemia, they were electronically paced at 295 beats/min. The purpose of this was to make sure that potential variations in HR in our model were not attributable to differences in cardiac function. Hearts not paced during preischemia were also not paced during reperfusion to keep pre- and postischemic HRs similar. During global, no-flow ischemia for 22.5 min, hearts were enclosed in a sealed water jacketed chamber maintained at 37°C. An ischemia length of 22.5 min was selected, because it resulted in a 50% recovery of function at the end of the reperfusion period in the Sed group. Upon reperfusion, hearts were initially perfused for 10 min in a retrograde, or Langendorff, mode at a perfusion pressure of 80 mmHg, then returned to the working mode for the final 20 min of reperfusion. At the end of the reperfusion period, the beating hearts were freeze-clamped and stored at −80°C until further analysis.

Preliminary studies were carried out to verify the functional stability of the working heart preparation in the absence of I/R. As displayed in Fig. 1, hearts perfused in the working mode do not decline in function for at least 60 min. As part of another study currently underway, we also verified that the use of L-NAME, as described above, would abolish improved cardioprotection, if it were mediated by NOS. Several investigators have reported that treatment with statin drugs induces late-phase preconditioning against I/R injury and that the improved protection is abolished by L-NAME (8, 19, 20).

In agreement with these studies, we found that daily administration of simvastatin attenuated I/R injury in rat hearts, and that perfusing the hearts with 100 μM L-NAME abolished the protective effect (data not shown).

Lactate dehydrogenase assay. Coronary effluent samples were collected for 30 s at various time points throughout the experiment (see Fig. 3) and stored on ice until being analyzed for lactate dehydrogenase (LDH) activity. LDH was analyzed using a standard kinetics assay of the rate of decline in NADH absorbance at 340 nm, as lactate is generated from pyruvate under nonlimiting substrate conditions (7). Elevated LDH release indicates that the sarcolemma has become damaged and is one of the most widely used markers of tissue injury. It should be noted that, although necrosis will certainly result in cytosolic enzyme leakage (54), initial release may precede actual irreversible cell death (49).

Catalase and superoxide dismutase assays. A piece of left ventricle (130–160 mg) was homogenized (1:20 wt/vol) in phosphate buffer (50 mM K2HPO4, 0.1 mM EDTA, 0.1% Triton X-100, pH 7.4) using a Teflon-glass Potter-Elvehjem homogenizer. After centrifugation at 1,500 g for 10 min, the supernatant was analyzed for catalase activity (1 unit = 1 μmol H2O2/min) polarographically using a Clark-type electrode, according to Del Rio et al. (17), and MnSOD activity (1 unit = 50% inhibition of baseline) was measured spectrophotometrically according to McCord and Fridovich (37). All tissue preparation steps were carried out at ice-cold temperatures with enzyme assays run at 25°C.

Protein blotting. An aliquot of the above homogenate was further diluted 1:1 with Laemmli (32) sample buffer, and 80 μg of protein subjected to SDS-PAGE and blotted for HSP72 and endothelial NOS (eNOS). Protein concentration of the homogenate was determined by the method of Lowry et al. (36). The concentration of inducible NOS (iNOS) in the whole tissue homogenate was too low to detect when directly loaded onto a gel; therefore, 1 ml of 1:20 tissue homogenate was purified through the use of 2.5′-ADP Sepharose, as described by Harris et al. (24). Positive controls for each respective protein were also loaded onto the gels. Following electrophoresis, samples were transferred to polyvinylidene difluoride membranes (Bio-Rad) and immunoblotted with one of the following antibodies: HSP72 monoclonal IgG (no. sc-024, Santa Cruz Biotechnology), eNOS monoclonal IgG (no. 610297, BD Biosciences, San Diego, CA), or iNOS monoclonal IgG (no. 610329, BD Biosciences). Membranes were then blotted either with anti-mouse Ig (NA931V) or anti-rabbit Ig (NA934V) horseradish peroxidase-linked whole antibody (Amersham Pharmacia Biotech) and detected with SuperSignal West Pico chemiluminescent substrate on Kodak Biomax ML imaging film. The densities of resulting bands were quantified using NIH image software on a Macintosh Power PC.

Statistical analysis. Descriptive data (means ± SE) were calculated for each dependent variable. Overall group differences were analyzed using a one-way ANOVA. When appropriate, post hoc analyses were made using Tukey’s honestly significant difference test. In all tests, a probability level of <0.05 was used as the decision rule for significance testing.
NITRIC OXIDE SYNTHASE IN EXERCISE PRECONDITIONING

Table 1. Animal characteristics

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Body Weight, g</th>
<th>Heart Weight, mg</th>
<th>Ratio, mg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sed</td>
<td>7</td>
<td>336.6±9.3</td>
<td>921±25</td>
<td>2.74±0.04</td>
</tr>
<tr>
<td>Run</td>
<td>7</td>
<td>328±9.6</td>
<td>957±9</td>
<td>2.92±0.06</td>
</tr>
<tr>
<td>Sed/L-N</td>
<td>6</td>
<td>345±8.3</td>
<td>933±14</td>
<td>2.71±0.06</td>
</tr>
<tr>
<td>Run/L-N</td>
<td>6</td>
<td>306.7±3.5</td>
<td>905±11</td>
<td>2.95±0.02</td>
</tr>
<tr>
<td>CRUn/L-N</td>
<td>7</td>
<td>323.1±10.2</td>
<td>990±34</td>
<td>3.06±0.04*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of rats. Sed, sedentary group; Run, group that exercised 2 days on a treadmill at 20 m/min, 6° grade, for 60 min; Sed/L-N, sedentary group perfused with 100 μM Nω-nitro-l-arginine methyl ester hydrochloride (l-NAME) to inhibit nitric oxide synthase; Run/L-N, group exercised and perfused with l-NAME; CRUn/L-N, group exercised in a 4°C environment and perfused with l-NAME. *P < 0.05 vs. Sed.

RESULTS

Animal characteristics. Body weights, heart weights, and their ratio are presented in Table 1. The only variation found within these variables was in CRun/L-N, which had a slightly higher heart weight-to-body weight ratio than Sed (P < 0.05).

Cardiac function. Hemodynamic parameters before and 30 min following ischemia are displayed in Table 2. During the preconditioning period, all parameters except for CF were similar among all groups. Perfusion with l-NAME decreased CF by approximately 20% in both Sed and Run groups (P < 0.05). Even when eNOS is totally inhibited, redundant vasodilatory factors prevent flow from decreasing to a level that would adversely affect aerobic metabolism (51). Cardiac external work, an indicator of pump function, was also similar among all groups before ischemia, but was considerably higher in the runners compared with the nonrunners after I/R (Fig. 1). Thirty minutes following ischemia, Run recovered 78.4% of preischemic function compared with only 50.2% by Sed (P < 0.05). The nonspecific NOS inhibitor l-NAME did not affect recovery in the Sed animals (P > 0.05), nor did it block the preconditioning effects of exercise, regardless of whether it was carried out in ambient temperature or in the cold. Similarly, no differences in cardiac efficiency were observed before ischemia (P > 0.05), whereas all exercise groups displayed greater efficiency of work (P < 0.05) than the Sed groups following I/R (Fig. 2). Administration of l-NAME did not affect cardiac efficiency under any circumstances.

Release of LDH was similar in all groups before entering ischemia (P > 0.05) and increased (P < 0.05) in all groups, except CRun/L-N, following ischemia (Fig. 3). The magnitude of LDH release was greatest at 10 min postischemia and was significantly attenuated by exercise (Sed vs. Run, P < 0.05). Perfusion with l-NAME also appears to protect against sarcolemmal damage as LDH release was reduced in Sed/L-N vs. Sed (P < 0.05), but this protective effect was not manifested into better functional recovery (Fig. 1). The lack of correlation between LDH release and functional recovery was likely due to the fact that LDH release was rapidly returning toward preischemic values after 10 min of reperfusion and that the total amount released was low. This is consistent with other studies when only a small amount of necrosis, occurred (38, 41). For example, McCully et al. (38) found that 25 min of global ischemia produced a small amount of necrosis and that decreasing it by adding caspase inhibitors did not affect function. Overall the LDH release profile indicates that stunning is the primary form of myocardial injury in the present study (12, 31).

The lowest LDH release tended to be from the exercise groups perfused with l-NAME; however, their release was not different from Run when analyzed by ANOVA. Since both Run/L-N and CRun/L-N displayed similar LDH release at 5 and 10 min of reperfusion, we combined these groups and compared the new, larger group to Run using a two-tailed t-test. By this analysis, exercise plus l-NAME resulted in less LDH release at both 5 and 10 min than exercise without l-NAME (P < 0.01).

Tissue analysis. As displayed in Fig. 4, expression of HSP72 in the left ventricle increased in Run vs. Sed (P < 0.05) in a manner similar to that previously observed (50). No significant changes in the expression of iNOS were observed (P > 0.05);

Table 2. Cardiac functional parameters

<table>
<thead>
<tr>
<th>Group</th>
<th>CF, ml·min⁻¹·g⁻¹</th>
<th>AF, ml·min⁻¹·g⁻¹</th>
<th>CO, ml·min⁻¹·g⁻¹</th>
<th>SP, mmHg</th>
<th>HR, beats/min</th>
<th>Oxygen consumption, μmol·min⁻¹·g⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Preischemia</td>
</tr>
<tr>
<td>Sed</td>
<td>13.5±0.5</td>
<td>41.3±2.6</td>
<td>54.8±2.9</td>
<td>114.1±2.4</td>
<td>277±9</td>
<td>8.92±0.38</td>
</tr>
<tr>
<td>Run</td>
<td>14.2±0.6</td>
<td>45.6±1.9</td>
<td>59.8±2.4</td>
<td>111.0±1.4</td>
<td>300±5</td>
<td>9.07±0.31</td>
</tr>
<tr>
<td>Sed/L-N</td>
<td>10.9±0.6†</td>
<td>44.1±2.0</td>
<td>55.1±2.4</td>
<td>108.5±3.4</td>
<td>284±6</td>
<td>8.40±0.49</td>
</tr>
<tr>
<td>Run/L-N</td>
<td>11.9±0.4‡</td>
<td>49.2±1.2</td>
<td>61.0±1.5</td>
<td>111.2±1.1</td>
<td>298±4</td>
<td>8.79±0.29</td>
</tr>
<tr>
<td>CRun/L-N</td>
<td>12.4±0.3‡</td>
<td>46.4±1.2</td>
<td>58.8±1.4</td>
<td>114.4±1.8</td>
<td>295±0</td>
<td>9.44±0.29</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>30 min Postischemia</td>
</tr>
<tr>
<td>Sed</td>
<td>11.7±0.7</td>
<td>19.2±2.8†</td>
<td>30.9±2.9†</td>
<td>101.3±2.7†</td>
<td>262±13</td>
<td>7.53±0.26</td>
</tr>
<tr>
<td>Run</td>
<td>14.2±0.8*</td>
<td>33.4±2.7†</td>
<td>47.7±3.4*</td>
<td>109.1±1.4</td>
<td>298±4</td>
<td>8.75±0.15</td>
</tr>
<tr>
<td>Sed/L-N</td>
<td>8.7±0.4‡</td>
<td>19.1±4.7†</td>
<td>27.8±5.0†</td>
<td>95.3±4.5</td>
<td>248±17</td>
<td>6.67±0.29</td>
</tr>
<tr>
<td>Run/L-N</td>
<td>11.4±0.6</td>
<td>37.7±1.8†</td>
<td>49.0±2.2†</td>
<td>106.8±1.5</td>
<td>296±2</td>
<td>8.21±0.15</td>
</tr>
<tr>
<td>CRun/L-N</td>
<td>11.7±0.4</td>
<td>37.5±1.6†</td>
<td>49.2±1.4†</td>
<td>113.7±1.7*</td>
<td>295±0</td>
<td>8.67±0.20</td>
</tr>
</tbody>
</table>

Values are means ± SE for 6–7 hearts. CF, coronary flow; AF, aortic flow; CO, cardiac output; SP, systolic pressure; HR, heart rate. *P < 0.05 vs. Sed at same time point; †P < 0.05 vs. preischemic value; ‡P < 0.05 vs. hearts not receiving l-NAME.
is blocked. Thus our results indicate that NO production is not against I/R, even when NO production from all NOS isoforms is incorrect. We found that exercise induces cardioprotection. Results of the present study indicate that our hypothesis was postischemic CF compared with Sed rats (50). However, the exercising in a cold environment is accompanied by increased previously observed that improved cardioprotection in rats enhances cardioprotection via an increase in iNOS (3), and we 44). One study concluded that a single exercise bout by dogs produced by acute exercise. As will be discussed below, the results of the present study also provide insight into other potential mediators of exercise-induced cardioprotection.

Key evidence that NO is a mediator of enhanced cardioprotection is from studies using NOS inhibitors during I/R. Like many of these studies, we selected L-NAME to inhibit NOS during I/R. The perfusate concentration of L-NAME used (100 μM) is sufficient for nearly 100% inhibition of total NOS (21, 43) and is equivalent to or even higher than the concentrations that successfully blocked preconditioning from ischemic preconditioning (11, 44), heat stress (1, 28), and statin treatment (8, 19, 20). However, unlike these studies on other forms of preconditioning, we found that NOS inhibition did not abrogate preconditioning induced by exercise. Furthermore, Western blot analysis indicated that the myocardial content of iNOS was not affected by exercise, regardless of temperature, and eNOS was increased only in the animals that ran in the cold (Fig. 4). Overall, these results indicate that increased NOS activity is not a required mediator for cardioprotection following acute exercise. However, it is important to point out that NOS may still have a critical role in the process, as increased NO synthesis during exercise may act as the trigger to induce the subsequent adaptations that lead to exercise-induced preconditioning (9).

The findings presented herein appear to conflict with those of Babai et al. (3), who reported that the iNOS inhibitor aminoguanidine (AG) blocked the preconditioning effects 24 h after a single, 21-min bout of exercise in dogs. They also found that the activity of iNOS increased threefold in the myocardium 24 h following exercise. Although we did not find iNOS to be elevated after a considerably greater exercise stress, the difference in NOS response may be due to species differences. Interestingly, Babai et al. also observed that the enhanced protection was lost 48 h after exercise, but iNOS activity at that time point was not reported; thus it is unknown whether the

DISCUSSION

The hypothesis tested in the present study was that an increase in NO production is the mediator of enhanced late-phase cardioprotection following acute exercise in rats. This hypothesis was based on the following observations. NO has been implicated in the enhanced cardioprotection that occurs 24 h after heat stress (1, 28) or ischemic preconditioning (11, 44). One study concluded that a single exercise bout by dogs enhances cardioprotection via an increase in iNOS (3), and we previously observed that improved cardioprotection in rats exercising in a cold environment is accompanied by increased postischemic CF compared with Sed rats (50). However, the results of the present study indicate that our hypothesis was incorrect. We found that exercise induces cardioprotection against I/R, even when NO production from all NOS isoforms is blocked. Thus our results indicate that NO production is not required for the enhanced late phase of cardioprotection induced by acute exercise.
correlation between iNOS activity and cardioprotection was still present. Also, as the authors point out, AG can act on a number of enzymes other than iNOS. The short-lived cardioprotection and uncertainty regarding the action of AG may be among the reasons that Babai et al. claim only that there is a possible involvement of NO in exercise-induced cardioprotection. In addition, there are reports that selective iNOS inhibition protects against I/R-induced dysfunction and enzyme release (23, 52).

Our interest in NOS as a potential mediator of exercise-induced preconditioning was initiated by an observation made while investigating another potential mediator, HSP72 (50). In that study, we prevented any increase in HSP72 expression following exercise by exercising a group of rats in a cold environment and demonstrated that acute exercise-induced cardioprotection against I/R injury (stunning) could be acquired without increased HSP72. We also observed a positive correlation between increased CF and cardioprotection, which contributed to our speculation that elevated eNOS may be responsible for the exercise-induced protection. Thus, in the present study, we extended our previous findings by blocking both HSP72 and eNOS in one group by exercise in the cold combined with administration of L-NAME throughout perfusion. Exercise-induced cardioprotection was not prevented by this combined manipulation (Figs. 1–3), leading us to conclude that changes in proteins other than eNOS or HSP72 can mediate the protection. Furthermore, elevation of CF does not appear necessary for cardioprotection, as decreasing CF by ~20% with L-NAME did not affect posts ischemic recovery. Other investigators have also found that L-NAME administration decreased CF but did not affect I/R injury in non-preconditioned hearts (2, 20, 28).

Perhaps the strongest evidence that NOS is not the mediator of exercise-induced cardioprotection is that LDH release during reperfusion decreased when NOS was inhibited by L-NAME (Fig. 3). NOS inhibition alone and exercise alone appeared to decrease LDH release by similar amounts compared with Sed. When NOS inhibition and exercise were combined, LDH release was decreased even more, suggesting that NOS inhibition and exercise protected by different mechanisms and that the two treatments provide additive protection.

In the absence of NO and HSP72 as protective mediators, some other changes must have been induced by exercise to result in the observed cardioprotection. The antioxidant enzyme MnSOD has received considerable attention as a possible mediator of improved cardioprotection following short-term exercise, but a consensus regarding its importance has not been reached (15, 18, 22, 34, 53). Increased ROS production during I/R appears to be a major contributing factor to myocardial dysfunction and cell death (4, 55), and overexpression of MnSOD is known to reduce I/R injury (16, 27). Yamashita et al. (53) found that acute exercise-induced protection against myocardial infarction coincided with an increase in myocardial MnSOD and that preventing the increase in MnSOD abrogated the exercise-induced protection. Similarly, other investigators have found a correlation between protection against I/R-induced dysfunction and increased MnSOD after exercise (18, 22). However, two more recent studies reported that acute exercise-induced cardioprotection against infarct size or dysfunction following ischemia is not related to MnSOD (15, 34).

The results of our study are consistent with these recent studies as we found that exercise provides protection against posts ischemic dysfunction and LDH release in the absence of any increase in MnSOD. Perhaps the most important observation in the present study is that the improvement in cardioprotection attained by the group that exercised in the cold and received L-NAME was similar to that of the other exercise groups, even though their left ventricular contents of HSP72, NOS, and MnSOD were equal to or less than that of the Sed control group.

Another proposed cardioprotective strategy against I/R injury is the suppression of ROS production at their source (39). One method proposed to attenuate the rise in ROS during I/R and provide protection is an uncoupling of mitochondrial respiration (39, 40). If acute exercise were to induce these changes, cardiac efficiency should be expected to decrease both before and after, or potentially only after, ischemia, if the activity of the uncoupling protein were to be activated by increased production of ROS and/or other stresses. Before the present investigation, efficiency had not been evaluated following acute exercise. In the present study, hearts of exercised animals functioned much more efficiently than those of sedentary counterparts throughout posts ischemic recovery (Fig. 2). If uncoupling of oxygen consumption from ATP production occurred in any animals, it was the Sed groups whose posts ischemic recovery of mechanical function was considerably less than that of the runners. Because of the fact that cardiac efficiency was not decreased, but rather increased, in all exercising groups, it is not likely that uncoupling serves to mediate acute exercise-induced preconditioning.

Clearly, a mechanism other than those considered herein is important in the development of exercise-induced cardioprotection against I/R injury. Some recent studies have provided insight into other potential mediators. Brown et al. (15) found that increased expression of myocardial sarcolemmal KATP channels was associated with improved resistance against I/R injury following short-term exercise of 1–5 days in both male and female rats. In a subsequent study using chronic exercise, Brown et al. (14) confirmed the importance of sarcolemmal KATP channels by finding that pharmacological blockade of the channels abrogated the exercise-induced cardioprotection. Importantly, the infarct sparing effect was not affected by blockade of mitochondrial KATP channels, which are considered to be important in other forms of preconditioning. Judge et al. (29) found that lifelong voluntary wheel running reduced hydrogen peroxide production from mitochondria isolated from rat heart. The decrease was accompanied by a decrease in MnSOD activity and no change in other antioxidant enzymes measured or in reduced glutathione level. These results led the authors to speculate that their exercise program resulted in a reduction of superoxide production during electron transport. The reduction was not due to mitochondrial uncoupling, as measures of oxidative phosphorylation indicated that respiratory rates and respiratory control ratio were similar in both groups. Further research is needed to determine the mechanism underlying the suppression of ROS production and whether it is involved in cardioprotection induced after short-term exercise. In this regard, Judge et al. noted that a study using 1 and 7 days of voluntary wheel running found results remarkably similar to theirs in that myocardial total SOD activity was decreased, whereas glutathione peroxidase and catalase activity remained unchanged (45). Future research should also be directed at clarifying the roles of other putative mediators of late-phase precon-
ditioning, including adolesecent reductase, heme oxygenase-1, and cyclooxygenase-2 (see Ref. 48 for review), in exercise-induced preconditioning. However, it should be noted that at least one study has reported that cyclooxygenase-2 plays no role in delayed exercise-induced cardioprotection against arrhythmias following 1 day of exercise in dogs (42), and another observed that myocardial heme oxygenase-1 expression is not altered by endurance exercise training in rats (25). Overall, the findings to date on potential mediators of exercise-induced preconditioning are consistent with the notion that they may differ from those of other forms of preconditioning.

In conclusion, the results of the present experiments clearly demonstrate that increased NOS content is not required for acute exercise-induced late preconditioning. This finding suggests that important differences exist between exercise-induced cardioprotection and other forms of late preconditioning, including heat stress and ischemic preconditioning. The experimental manipulation of NOS and HSP72 during the course of this study also provides novel insight into acute exercise-induced late preconditioning by showing that it can occur even when these putative mediators are simultaneously prevented from increasing above control level. Elimination of potential targets is a valid objective that is underrepresented in the literature today (10).

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