Effect of exercise training on the ability of the rat heart to tolerate hydrogen peroxide

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Received 3 October 2002; accepted 4 February 2003

Abstract

Objective: The purpose of this study was to determine whether exercise training could precondition the myocardium against hydrogen peroxide (H\textsubscript{2}O\textsubscript{2})-induced damage.

Methods: Male Fischer 344 rats ran on a treadmill for 9 weeks (60 min/day, 22 m/min, 6° grade, 5 days/week). Isolated perfused working hearts from exercise trained (ET, n=8) and sedentary (SED, n=10) animals were perfused with 150 μM H\textsubscript{2}O\textsubscript{2}. Results: Pre-H\textsubscript{2}O\textsubscript{2} baseline values for cardiac external work (COxSP), coronary flow (CF), and lactate dehydrogenase (LDH) release were similar between groups. At 5 min of H\textsubscript{2}O\textsubscript{2}, COxSP was unchanged from baseline but CF was increased 30% in SED and 46% in ET (P<0.05 vs. SED). COxSP began to decline similarly thereafter in both groups, dropping to 20% of baseline at 20 min. CF in ET remained higher than SED throughout (P<0.05). LDH leakage remained near baseline during the first 15 min of H\textsubscript{2}O\textsubscript{2} exposure, but was elevated (P<0.05) 72% in SED and 40% in ET after 20 min, and was 2.2-fold greater in SED than ET (P<0.05) after 25 min. Heat shock protein 70 was 2.1-fold greater in ET than SED (P<0.05), but ET did not change catalase and glutathione peroxidase.

Conclusions: The results of this study indicate that chronic moderate exercise will enhance coronary flow and attenuate the development of myocardial injury when exposed to H\textsubscript{2}O\textsubscript{2}, but will not affect H\textsubscript{2}O\textsubscript{2}-induced decrease in pump function.

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Keywords: Ventricular function; Vasodilation; Free radicals; Preconditioning; Hemodynamics

1. Introduction

Reactive oxygen species (ROS) have been implicated in myocardial dysfunction associated with several events, including ischemia-reperfusion injury [1], congestive heart failure [2–4], and normal aging [5,6]. While it is known that exercise can generate ROS [7], it is not known whether chronic exercise training affects the heart’s ability to tolerate a direct pro-oxidant challenge. The well-documented finding that exercise training can protect the heart against injury caused by ischemia and subsequent reperfusion [8–12] provides indirect evidence for increased tolerance to ROS. However, it is also possible that exercise may be cardioprotective by a mechanism unrelated to ROS. A recent study by Radak et al. [13] reported that chronic exercise prevented the accumulation of oxidatively modified proteins in the myocardium after 3 weeks of in vivo IP administration of the pro-oxidant t-butyl hydroperoxide. Although this is an interesting finding, it is not clear if adaptations within the heart or other loci within the body could be responsible for the observed protection. Furthermore, t-butyl hydroperoxide is not produced in vivo and its toxic effects are irreversible [14], which may also complicate interpretation of the results. There is no data available concerning the effects of exercise on the ability of the heart to tolerate direct exposure to oxidative stress.

The purpose of the present investigation was to study the effect of a chronic exercise program upon the ability of the heart to tolerate direct exposure to hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}), a reactive oxygen species. Rats ran on a motorized treadmill following a moderate protocol that previously has been found to provide cardioprotection against...
ischemia-reperfusion injury [8,10]. The 9-week exercise protocol has also been found to increase the cardioprotective protein heat shock protein 70 (HSP 70), but not change left ventricle antioxidant enzyme activities [10]. After the training protocol, isolated perfused working hearts of exercised and sedentary animals were compared for changes in mechanical function and lactate dehydrogenase (LDH) release during the H$_2$O$_2$ exposure. Hydrogen peroxide was selected as the pro-oxidant because it diffuses readily throughout the heart and is a well-known experimental model of cardiac dysfunction caused by reactive oxygen species [15].

2. Methods

2.1. Animals and training protocols

Male 6-month-old Fischer 344 rats were obtained from Harlan Sprague–Dawley and kept at the University of Texas Animal Resource Center. The animals were kept on a 12-h/12-h light/dark cycle and fed ad libitum. Rats were randomly assigned to two treatment groups: sedentary control (SED) n=10, and exercise trained (ET) n=8. ET rats were run on a motorized treadmill, at room temperature, for 9 weeks, 5 days/week, 60 min/day at a speed of 22 m/min up a 6° grade. All of the animals were sacrificed 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).

2.2. Isolated heart perfusions

Myocardial function was evaluated using an isolated, working heart preparation [16]. Hearts were perfused at 37 °C with a modified Krebs–Henseleit buffer containing (in mM): 10 glucose, 1.75 CaCl$_2$, 118.5 NaCl, 4.7 KCl, 1.2 MgSO$_4$, 24.7 NaHCO$_3$, and 0.5 EDTA gassed with 95% O$_2$–5% CO$_2$. Animals were anesthetized with an intraperitoneal injection of 40 mg/kg body weight of sodium pentobarbital, and 100 IU of heparin was injected into the inferior vena cava. Hearts were rapidly excised, weighed, and mounted on the perfusion apparatus as described previously [12]. All values were normalized for heart wet weight. Hearts were initially perfused for 30 min in a non-recirculating retrograde, or Langendorff, mode at a perfusion pressure of 80 cm H$_2$O. Working heart function was evaluated at an atrial filling pressure of 12.5 mmHg and an afterload set by an 80 cm high aortic column (ID 3.18 mm). Coronary flow (CF) and aortic flow (AF) were determined by timed collection of the effluent dripping off the heart and aortic column overflow, respectively. Cardiac output (CO) was determined as the sum of CF and AF, and cardiac external work (COxSP) was defined as the product of CO and peak systolic pressure (SP). After switching from Langendorff to working mode, hearts were perfused for 10 min with H$_2$O$_2$-free buffer, then for 20 min with buffer containing 150 μM H$_2$O$_2$. After 20 min, the pump function of the hearts was insufficient to maintain adequate perfusion pressure, so they were returned to the Langendorff mode for 5 min. The final 5 min was important for comparisons of LDH release, which increased dramatically during this time period. Hearts were perfused with H$_2$O$_2$-free buffer during the final 5 min for two reasons: (1) preliminary perfusions indicated that the amount of LDH release during the final 5 min was the same with or without H$_2$O$_2$ in the buffer; and (2) washing out the H$_2$O$_2$ before freeze-clamping and storage minimizes the chance of protein oxidation during storage. In the absence of H$_2$O$_2$, no decline in function occurs during the 25-min evaluation period [8]. At the end of the perfusion period, the beating hearts were then freeze-clamped and stored at −80 °C until analysis.

2.3. Heat shock protein determinations

A piece of left ventricle (130–160 mg) was homogenized (1:20 wt/vol) in N-(2-hydroxyethyl)piperazine-N’-(2-ethanesulfonic acid) (HEPES) buffer containing 5 mM HEPES, 1 mM EDTA, pH 7.4 using a Teflon-glass Potter-Elvehjem homogenizer. Samples were then diluted 1:1 with Laemmli [17] sample buffer and subjected to SDS-PAGE and blotted with HSP 70 monoclonal IgG (No. sc-024, Santa Cruz Biotechnology) as previously described [12]. The HSP 70 content of the myocardium was then reported as a percentage of HSP 70 standard loaded on each gel.

2.4. Cytochrome oxidase, catalase, and glutathione peroxidase determinations

For cytochrome oxidase, whole gastrocnemius muscles were homogenized (1:20 wt/vol) with a Teflon-glass homogenizer in phosphate buffer (50 mM K$_2$HPO$_4$, 0.1 mM EDTA, pH 7.4). An aliquot was treated with 1.7% Triton X-100 (v/v, f.c.), centrifuged at 1500×g for 10 min, and the supernatant used to determine cytochrome c oxidase content spectrophotometrically as described by Balaban et al. [18]. The reduced (2 mM cyanide)–oxidized spectrum at 605–630 nm, with an extinction coefficient of 10.8/mM/cm, was used to calculate the concentration. All assays were performed in triplicate and the mean value used. For antioxidant enzyme assays, a portion of the left ventricle was homogenized (1:20 wt/vol) with a Teflon-glass homogenizer in 50 mM K$_2$HPO$_4$, 0.1 mM EDTA, 0.1% (v/v) Triton X-100, pH 7.4. After centrifugation at 1500×g for 10 min, the supernatant was analyzed for catalase activity polarographically using a Clark-type oxygen electrode according to Del Rio et al. [19] and glutathione peroxidase was measured by spectrophoto-
tests, a probability level of $<0.05$ was used as the decision rule for significance testing.

3. Results

3.1. Animal characteristics

Body weights, heart weights, and gastrocnemius cytochrome oxidase activity are presented in Table 1. The decreased body weight accounted for the higher ($P<0.05$) heart weight-to-body weight ratio of ET as compared to SED. Cytochrome oxidase in gastrocnemius muscle was measured as a marker of mitochondrial changes in active skeletal muscles with training. The magnitude of the exercise-related increase reflects the moderate intensity of the exercise program and is similar to that reported by Harris and Starnes [10], who used a similar exercise protocol and found a significant preconditioning effect against ischemia reperfusion injury.

3.2. Cardiac function and lactate dehydrogenase release

Prior to H$_2$O$_2$ exposure there were no differences ($P>0.05$) between groups for CO, heart rate, or SP (Table 2). Pre-H$_2$O$_2$ COxSP (summarized in Fig. 1A) was $4993\pm147$ ml/min/g×mmHg in SED and $5098\pm199$ in ET ($P>0.05$). In both groups, COxSP did not decrease during the initial 5 min of H$_2$O$_2$ exposure, but ultimately dropped to 20% of pre-H$_2$O$_2$ baseline values by 20 min. CO followed the same pattern as COxSP. As illustrated in Fig. 1B, at 5 min of H$_2$O$_2$ exposure, CF increased 30% above baseline in SED ($P<0.05$) and 46% in ET ($P<0.05$ vs. SED) even though myocardial mechanical function was unchanged in both groups. CF then gradually declined as mechanical function decreased in both groups, but remained higher in ET compared to SED throughout ($P<0.05$). Myocardial LDH release (summarized in Fig. 1C)
3.3. Heat shock protein and antioxidant enzymes

The concentration of HSP 70 in the left ventricle of ET was found to be greater ($P<0.05$) than that of SED (Fig. 2). The results for left ventricular catalase and glutathione peroxidase activities are displayed in Table 3. Neither of these H$_2$O$_2$ scavenging enzymes was increased at the 95% level of confidence after the 9-week exercise program. These findings are also in agreement with those reported by Harris and Starnes [10] after a similar 9-week exercise protocol. The addition of H$_2$O$_2$ to the perfusion buffer increased myocardial GSSG release approximately two-fold within 5 min in both groups. However, consistent with the lack of change in antioxidant enzymes, the amount of GSSG released during the H$_2$O$_2$ exposure was similar in both groups at all time points (data not shown).

4. Discussion

Hydrogen peroxide is a widely used and well-characterized model for studying oxidative stress and is known to

<table>
<thead>
<tr>
<th>Content of ventricular antioxidant enzymes</th>
<th>ET</th>
<th>SED</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutathione peroxidase (U/mg protein)</td>
<td>3.63±0.16</td>
<td>3.36±0.10</td>
</tr>
<tr>
<td>Catalase (U/mg protein)</td>
<td>7.98±0.71</td>
<td>6.47±0.19</td>
</tr>
</tbody>
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Values are means±S.E. for $n=7$. ET, exercise trained group; SED, sedentary group.
play a prominent role in ischemia-reperfusion injury [15]. It is produced at several locations in the heart when SOD dismutates superoxide, then is either converted to H₂O₂ and O₂ by catalase and glutathione peroxidase or to the highly reactive hydroxyl radical via the Fenton reaction [1]. Depending on the dose and length of exposure, H₂O₂ is known to cause contractile dysfunction, disturbances in calcium homeostasis, ATP depletion, vasodilatation or vascular dysfunction, necrosis, and apoptosis [1,15,24–26]. In the present study, we found that chronic moderate exercise will precondition the myocardium by attenuating membrane damage (Fig. 1C) and enhancing vasodilatation (Fig. 1B) in response to a H₂O₂ (150 μM) challenge, but will not prevent H₂O₂-induced left ventricular dysfunction (Fig. 1A).

The observation that the decline in myocardial function preceded an increase of LDH release indicates that the H₂O₂ is directly regulating the activity of specific proteins prior to the onset of myocardial injury. This temporal relationship is consistent with the recent finding of Zeitz et al. [27], who reported that the hydroxyl radical causes myocardial dysfunction in the absence of necrosis by specifically activating the Na⁺/H⁺ exchanger. Another indication that the decline in function observed herein is not related to irreversible necrosis comes from the study of Skjelbakken et al. [15], who demonstrated that cardiac dysfunction associated with exposure to 180 μM H₂O₂ for 10 min is reversible. Skjelbakken et al. also found that the H₂O₂-induced cardiac dysfunction could be attenuated by catalase. Thus, a possible reason that ET was not protected any better than SED during the initial stage of ROS exposure is that the H₂O₂ metabolizing enzymes, catalase and glutathione peroxidase, were not observed to be upregulated in the heart after adaptation to chronic exercise training (Table 3). Lack of changes in these antioxidant enzymes is consistent with other studies with exercise programs of similar and greater intensity than the one used herein [10,11,28].

Evidence is accumulating in support of a Na⁺/H⁺ exchanger hypothesis for the initial cardiac dysfunction associated with physiological concentrations of H₂O₂ (<200 μM without added iron). Hoque and Karmazyn [29] reported that pharmacological inhibition of the sarcolemmal Na⁺/H⁺ exchanger significantly attenuated H₂O₂-induced contractile dysfunction in isolated perfused rat hearts. Direct evidence that H₂O₂ increases the activity of the exchanger, and the mechanism for the increase, was recently reported in two independent studies using adult rat ventricular myocytes [24,30]. These studies found that 50–100 μM H₂O₂ activated the sarcolemmal Na⁺/H⁺ exchanger in less than 10 min via activation of extracellular signal-stimulated kinase (ERK) and protein kinase C. Furthermore, the study by Wei et al. [24] also reported a rapid decrease in contractility. The cardiac dysfunction that occurs by activation of the Na⁺/H⁺ exchanger is thought to be due to a significant elevation of intercellular Na⁺, which will indirectly lead to Ca²⁺ overload via the Na⁺/Ca²⁺ exchanger [31]. Since exercise training is reported to enhance the activation of ERK [32] and increase Na⁺/H⁺ exchanger mRNA in the heart [33], the Na⁺/H⁺ exchanger hypothesis would predict that exercise training will actually result in greater mechanical dysfunction in response to H₂O₂. It should be noted that the Na⁺/H⁺ exchanger hypothesis is in conflict with those who support cellular energy depletion as the underlying cause of ionic dysfunction. Yanagida et al. [26] carried out a very elegant NMR-based study using isolated perfused hearts exposed to a relatively high concentration of H₂O₂ (2 mM) plus Fe³⁺. They observed that Ca²⁺ overload, with severe functional and energetic depletion, occurred before substantial accumulation of intracellular Na⁺.

Although exercise training did not appear to protect pump function during the initial H₂O₂ exposure, it does appear to attenuate myocardial injury as indicated by lower LDH release in ET. The cardioprotective effects of exercise against ischemia-reperfusion injury have been attributed to an increase in myocardial HSP 70 [10,11,34,35] and this chaperone may also be at least partially responsible for the protection against H₂O₂-induced necrosis. It has been reported that heat-shocked cells are resistant to the toxic effects of H₂O₂, and that both the inducible and the constitutive form of the protein can confer this resistance [36,37]. Su et al. [38] have also shown that cells over-expressing the constitutive form of the protein have an increased resistance to H₂O₂ exposure, and that the protective effects of the protein extend to attenuating lipid peroxidation. H₂O₂ exposure is reported to increase the MAP kinase, c-Jun N-terminal kinase (JNK), in isolated perfused rat hearts in less than 30 min [39]. Park et al. [40] and Clerk et al. [39] characterized the function of JNK as being involved in pathways leading to cell death, thus it may have a role in the cellular damage associated with both ischemia-reperfusion injury and H₂O₂-mediated injury. It has been demonstrated that HSP 70 can inhibit activation of JNK in cells [40] and in isolated perfused mouse hearts [41], thereby protecting against its potentially damaging effects. Further research into the role of MAP kinases in short-term injury is necessary to elucidate their mechanism of action.

The coronary flow responses to exposure to H₂O₂ observed in SED are consistent with previously reported findings [15]. The enhanced elevation observed in ET has not been previously reported and may be due to an exercise-induced increase in nitric oxide synthase (NOS) [42]. H₂O₂ induces a nitric oxide-dependent vasodilatation [43], thus an elevation of NOS would be expected to further enhance the flow response. Another plausible explanation for the enhanced flow in ET is that exercise caused an increase in the endothelial Na⁺/Ca²⁺ exchanger, which has been implicated in the H₂O₂-induced increase in coronary flow [25]. This exercise-induced adaptation could potentially have a protective effect.
against H₂O₂ and ischemia-reperfusion injury by assuring adequate perfusion during these stresses.

The potential protective effects of nitric oxide extend beyond the vasodilatory response described, for it has also been shown to block the Ca²⁺ overload associated with H₂O₂ and ischemia-reperfusion injury [44]. However, nitric oxide has been described as having negative inotropic effects [45]. Therefore, if NOS were upregulated in the hearts of ET, an increased production of nitric oxide in response to H₂O₂ could be partially responsible for the lack of protection against the ventricular dysfunction observed herein.

In conclusion, this is the first study to investigate the effects of exercise on the ability of the heart to tolerate direct exposure to H₂O₂. We found that chronic moderate exercise can induce protective intrinsic adaptations in the myocardium that specifically attenuate myocardial injury and enhance coronary flow when challenged with H₂O₂. Although exercise did not appear to attenuate the decline in pump function during the imposed oxidative stress, the observed changes in myocardial injury and coronary flow should be beneficial to the recovery of pump function after the stress. The reported activation of JNK by H₂O₂ exposure [39], and HSP 70’s role in inhibition of JNK [40,41], suggest a potential interrelationship of these two phenomena in exercise-induced cardioprotection.

Acknowledgements

This work was supported by a grant from the American Heart Association, Texas Affiliate. The authors would like to thank Yoon Jung Park for assistance with the cytochrome oxidase assay and B. Jason Mathis for assistance with the GSSG assay.

References


