Acute exercise can improve cardioprotection without increasing heat shock protein content

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Taylor, Ryan P., M. Brennan Harris, and Joseph W. Starnes. Acute exercise can improve cardioprotection without increasing heat shock protein content. Am. J. Physiol. 276 (Heart Circ. Physiol. 45): H1098–H1102, 1999.—The aim of this study was to determine the effects of acute bouts of exercise on myocardial recovery after ischemia and heat shock protein expression. Adult female Sprague-Dawley rats were divided into five groups: 1) 1-day run (1DR; n = 6) and 2) 3-day run (3DR; n = 7), in which rats ran for 100 min at a speed of 20 m/min up a 6° grade for 1 or 3 consecutive days; 3) 1-day cold run (1CR), in which rats ran the same as 1DR but with wet fur at 8°C, which prevented an elevation of core temperature (n = 8); 4) heat shock sedentary (HS), in which rats had their core temperatures raised to 42°C one time for 15 min (n = 5); and 5) sedentary control (n = 15). Cardiac function was analyzed 24 h after the last treatment using an isolated, working heart model. Nonpaced hearts were initially perfused under normoxic conditions, then underwent 17 min of global, normothermic (37°C) ischemia, and, finally, were allowed to recover for 30 min under normoxic conditions. The concentration of the 72-kDa heat shock protein (HSP 72) was measured in each left ventricle. Compared with that in the sedentary group, recovery of cardiac output systolic pressure (CO × SP) was enhanced (P < 0.05) in all treatment groups when the postischemic value was covaried with the preischemic value. No differences in CO × SP were found (P > 0.05) between the following groups: 1DR vs. 3DR, 1DR vs. HS, and 1DR vs. 1CR. Heat shock protein concentration was significantly greater (P < 0.05) than that in the sedentary controls in HS, 1DR, and 3DR groups, but not for 1CR. The concentration of HSP 72 was not significantly correlated with postischemic CO × SP (R² = 0.197, P > 0.05). We conclude that acute bouts of exercise can produce cardioprotective effects without an elevation of HSP 72.

myocardium; ischemia-reperfusion; rat; perfused heart; hemodynamics

SEVERAL STUDIES have reported that chronic exercise training improves the ability of the heart to recover contractile pump function after a bout of ischemia (1–4, 10). Studies on this topic from our laboratory (1, 2) have shown that isolated perfused working hearts of rats trained on a motorized treadmill had improved cardiac output and developed pressure, increased coronary flow, increased high-energy phosphate content, and attenuated calcium overload. Locke et al. (11) have recently reported that rats exercising for only three consecutive days also had improved postischemic cardiac function. Using the retrograde perfused Langendorff heart preparation, these investigators found that recovery of postischemic developed pressure was better after three running bouts, but coronary flow recovery was not improved, and just a single bout did not improve any measure of postischemic cardiac function. Locke et al. (11) found a correlation between the improvement in postischemic pressure development and the concentration of the 72-kDa heat shock protein (HSP 72), which led to the suggestion that HSP 72 is the molecular adaptation responsible for the cardioprotection. The discovery that an intrinsic cardioprotective response can be achieved quickly has important ramifications in understanding the underlying mechanism and the exercise “dosage” required for optimum protection.

The purpose of this experiment was to gain further insight into the acute exercise-induced cardioprotective response. Specifically, the first purpose was to determine whether the response is observed when a working heart model is utilized that more closely resembles the in vivo function of the heart. Second, because strenuous exercise significantly elevates temperature and HSP 72 production is strongly stimulated by elevated temperature (5, 7), we sought to determine whether exercise without an increase in body temperature would produce cardioprotection and increase expression of HSP 72.

METHODS

Animals and training protocols. Female 5- to 7-mo-old retired-breeder Sprague-Dawley rats, weighing 256.8 ± 4.8 g, were obtained from the breeding colony maintained by the University of Texas Animal Resource Center. The animals were kept on a 12:12-h light-dark cycle and fed ad libitum. Rats were randomly divided into five treatment groups: 1) sedentary control (n = 15); 2) 1-day run (1DR) rats exercised for 100 min/day at a speed of 20 m/min up a 6° grade on a motorized treadmill in a 23–24°C room (n = 6); 3) 3-day run (3DR) rats exercised the same as 1DR for three consecutive days (n = 7); 4) 1-day cold run (1CR) rats with wet fur exercised the same as 1DR in an 8°C room (n = 8); and 5) sedentary heat-shocked (HS) rats with their core temperatures raised once to 42°C for 15 min (n = 5). All of the animals...
in 1DR, 3DR, 1CR, and HS groups were rested for 24 h after the last treatment before subsequent analyses were made. Control animals were evaluated throughout the study to ensure consistency in the perfusion preparation.

Isolated heart perfusions. Myocardial function was evaluated by utilizing an isolated, working heart preparation (13). Hearts were perfused at 37°C with a modified Krebs-Henseleit buffer containing (in mM) 110 glucose, 1.75 CaCl₂, 118.5 NaCl, 4.7 KCl, 1.0 MgSO₄, 24.7 NaHCO₃, and 0.5 EDTA gassed with 95% O₂-5% CO₂. Animals were anesthetized with an intraperitoneal injection of 0.3 ml of rodent anesthesia cocktail consisting of acepromazine (10 mg/ml), ketamine (100 mg/ml), and xylazine (20 mg/ml) and given 100 IU heparin injected into the inferior vena cava. Hearts were rapidly excised and placed in iced-cold saline on a tared electronic balance for determination of gross weight. The aortas were secured on a stainless steel cannula of the perfusion apparatus and initially perfused in a nonrecirculating retrograde, or Langendorff, mode at a perfusion pressure of 80 cmH₂O. During this time, extraneous tissue was trimmed from the hearts and weighed, its weight was subtracted from the gross weight to obtain the final wet weight, and the left atrium was cannulated. All subsequent values were normalized for heart weight and expressed per gram of wet heart weight. After 15 min of Langendorff perfusion, hearts were switched to the working heart mode, and preischemic heart weight and expressed per gram of wet heart weight. After ischemia, hearts were initially reperfused in the Langendorff mode for 10 min at a perfusion pressure of 80 cmH₂O. During this time, extraneous tissue was trimmed from the hearts and weighed, its weight was subtracted from the gross weight to obtain the final wet weight, and the left atrium was cannulated. All subsequent values were normalized for heart weight and expressed per gram of wet heart weight. After 15 min of Langendorff perfusion, hearts were switched to the working heart mode, and preischemic heart function was evaluated at 13 cmH₂O (atrial filling pressure) with an 80-cm-high aortic column (ID 3.18 mm). Coronary (CF) and aortic (AF) flows 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. Cardiac external work in the working heart mode is defined as the product of CO and peak aortic systolic pressure (CO × SP). Left ventricular pressure and heart rate were monitored with a Gould DTX pressure transducer interfaced with a Gould 2200S recorder.

Global, normothermic ischemia was induced by simultaneously cross-clamping the atrial inflow and the aortic outflow lines for 17 min. During ischemia, hearts were encased in a sealed, water-jacketed chamber maintained at 37°C. After ischemia, hearts were initially reperfused in the Langendorff mode at a perfusion pressure of 80 cmH₂O and then switched to the working heart mode. Hearts were allowed to recover for 30 min after ischemia. After the final postischemic aortic pressure was obtained, a 20-gauge needle was inserted into the left ventricle for the determination of intraventricular pressures to check for the possibility of elevated end-diastolic pressure. Because intraventricular diastolic pressure did not differ among groups and did not exceed 8 mmHg in any heart, peak aortic SP was used in the comparisons of cardiac function. The heart was removed, and the left ventricle was stored at −100°C until analyzed for heat shock protein content.

Heat shock protein determinations. A piece of left ventricle (130–160 mg) was homogenized (1:20 wt/vol) in HEPES buffer containing (in mM) 5 HEPES and 1 EDTA (pH 7.4) using a Teflon-glass Potter-Elvejhem homogenizer. The protein concentration of each sample was determined by the Biuret method (see Ref. 6). Samples were then diluted 1:1 with Laemmli (9) sample buffer, and duplicate aliquots of 80 µg of protein were subjected to SDS-PAGE on 10% resolving gels using the Mini-Protean II system (Bio-Rad, Richmond, CA). The proteins were then transferred to a polyvinylidene difluoride (PVDF) sheet (Bio-Rad) by the method of Towbin et al. (18), with a Bio-Rad SD semidry transfer unit utilizing the buffer system of Kyhse-Anderson (8). The PVDF membranes were initially blotted with HSP 72 mouse monoclonal IgG (no. sc-024, Santa Cruz Biotechnology). The membranes were then blotted with anti-mouse IgHRP (horseradish peroxidase-linked whole antibody from sheep; no. NXA 931, Amersham Life Science) and detected with Super Signal chemiluminescent substrate luminol/enhancer (Pierce, Rockford, IL). The resulting labeled bands were quantified by scanning the images on a Macintosh IIxs computer (Apple Computer, Cupertino, CA). The scans were created by using an image scanner (600-dpi Transparency Module, Mirror Technologies) connected to the computer. The scans were subsequently digitized and imported into an image-analysis software program (NIH Image 1.66, National Institutes of Health, Bethesda, MD), and the density of each individual sample was calculated. On each gel, a standard sample, created from the myocardium of a heat-shocked rat, was loaded along with the samples of the treatment groups. The HSP 72 content of each sample was reported as a percentage of the standard loaded on each gel and was adjusted for the concentration of protein loaded for the respective sample.

Statistical analysis. Postischemic differences in cardiac functional parameters between treatment and control groups as well as differences among the various treatment groups were determined by comparing the postischemic values using an analysis of covariance (ANCOVA) with the preischemic values as the covariant. HSP 72 content and core temperature were compared by one-way ANOVA. The correlation between the HSP content and the postischemic values for function was made with a bivariate correlation, and the R² value was reported.

RESULTS

Core temperature. The 100-min, moderate-intensity exercise bout carried out at room temperature increased core temperature 3°C, from 38.4 ± 0.3 to 41.1 ± 0.1°C (P < 0.05). No increase in temperature occurred when the same exercise was carried out in an 8°C room in rats whose fur was wetted to provide for evaporative cooling. Under these conditions, core temperature dropped to 36.3 ± 0.3°C (P < 0.05 vs. control), making it unlikely that temperature within the active myocardium was elevated.

Cardiac function. Absolute values for both preischemia and postischemia for several functional parameters are reported in Table 1. ANCOVA with preischemic values revealed that postischemic coronary flow was significantly (P < 0.05) greater in 3DR and 1DR groups than in the control group and that cardiac output was significantly (P < 0.05) greater in all treatment groups than in the control group. Heart rate was not significantly (P > 0.05) different from that in the control group for any treatment, and only the 3DR group had greater (P < 0.05) systolic pressure development than the control group when covaried with the preischemic values. Finally, only in the heat-shocked group was the product of heart rate and SP (rate-pressure product) found to be significantly (P < 0.05) greater than that in the control group.

Cardiac external work (CO × SP) during ischemia and subsequent reperfusion relative to normoxic baseline values is displayed in Fig. 1. ANOVA revealed that normoxic baseline values were similar among all groups. At the end of the perfusion period, values in all treatment groups were found to be significantly (P <
0.05) greater than those in the control group when covaried with preischemic values. In addition, no differences (P > 0.05) were found among any of the treatment groups after reperfusion.

Heat shock protein concentrations. Left ventricular heat shock protein content from all groups is displayed in Fig. 2. The HS, 3DR, and 1DR groups all had similar levels of HSP 72 (P > 0.05) that were 11.7-fold greater than the level in the control group (P < 0.05). No difference was found between the control group and the 1CR group (P > 0.05). The correlation for HSP 72 content and postischemic CO was not found to be significant (R² = 0.197, P > 0.05).

DISCUSSION

The results of this study confirm the important findings of Locke et al. (11) that a rapid exercise-induced response can occur within the heart, rendering it more resistant to ischemia-reperfusion injury. Using the Langendorff preparation with a constant retrograde perfusion pressure of only 50 mmHg, Locke et al. found that hearts from rats that ran for three consecutive days were better able to recover pressure development after ischemia, but there was not a corresponding improvement in recovery of coronary flow. This left open the possibility that there was significant damage to the vasculature that could limit delivery of blood to the myocardium and thus would limit its performance at higher levels of work or pressure development. In the present study, we used the more physiologically relevant working heart preparation and found that post-

Table 1. Functional characteristics of isolated perfused hearts

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>HS</th>
<th>3DR</th>
<th>1DR</th>
<th>1CR</th>
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<tbody>
<tr>
<td>n</td>
<td>15</td>
<td>5</td>
<td>7</td>
<td>6</td>
<td>8</td>
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<td>Preischemia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CF</td>
<td>16.3 ± 0.6</td>
<td>16.7 ± 1.3</td>
<td>17.8 ± 0.8</td>
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<td>16.9 ± 1.3</td>
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<tr>
<td>CO</td>
<td>57.4 ± 1.9</td>
<td>58.2 ± 4.4</td>
<td>54.6 ± 2.4</td>
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<td>55.2 ± 2.9</td>
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<tr>
<td>SP</td>
<td>99 ± 2</td>
<td>106 ± 5</td>
<td>97 ± 2</td>
<td>106 ± 2</td>
<td>92 ± 1</td>
</tr>
<tr>
<td>HR</td>
<td>333 ± 7</td>
<td>318 ± 24</td>
<td>366 ± 21</td>
<td>331 ± 10</td>
<td>341 ± 14</td>
</tr>
<tr>
<td>RPP</td>
<td>32,759 ± 425</td>
<td>33,096 ± 1,260</td>
<td>35,179 ± 1,267</td>
<td>35,015 ± 1,179</td>
<td>31,301 ± 1,259</td>
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<tr>
<td></td>
<td>30 min Postischemia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CF</td>
<td>11.2 ± 0.4</td>
<td>14.9 ± 1.9*</td>
<td>14.1 ± 1.7</td>
<td>12.8 ± 0.5</td>
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</tr>
<tr>
<td>CO</td>
<td>27.1 ± 2.1</td>
<td>41.6 ± 2.9*</td>
<td>34.2 ± 3.8*</td>
<td>35.8 ± 1.9*</td>
<td>32.7 ± 1.5*</td>
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<tr>
<td>SP</td>
<td>82 ± 2</td>
<td>93 ± 6</td>
<td>87 ± 2*</td>
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<td>HR</td>
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<td>323 ± 33</td>
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</tr>
<tr>
<td>RPP</td>
<td>25,921 ± 715</td>
<td>29,195 ± 1,507*</td>
<td>28,194 ± 920</td>
<td>28,584 ± 948</td>
<td>26,875 ± 1,284</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of hearts. HS, heat shock; 3DR, 3-day runners; 1DR, 1-day runners; 1CR, 1-day cold runners; CF, coronary flow (ml·min⁻¹·g wet wt⁻¹); CO, cardiac output (ml·min⁻¹·g wet wt⁻¹); SP, systolic pressure (mmHg); HR, heart rate (beats/min); RPP, rate-pressure product (mmHg·beats·min⁻¹). *Significantly different from control (P < 0.05).
ischemic recovery of CO and cardiac external work (CO × SP) can be significantly improved after acute exercise (Table 1 and Fig. 1).

The improvement in postischemic recovery was apparent 24 h after just one exercise bout in the present study, whereas three consecutive days were required to reveal improved recovery in the study by Locke et al. (11). There are several possible reasons for the different time courses observed in the two studies. As already discussed, Locke et al. used a Langendorff preparation, and they used the measurement of developed pressure as an indicator of cardiac function. This is not as sensitive as CO measurements in the working heart, and, in fact, we did not observe differences in rate-pressure product between exercised and sedentary groups when there were differences in CO measurements (Table 1). Differences in the animals and/or exercise bouts between the two studies also may have played a role. Locke et al. ran 250- to 300-g male Sprague-Dawley rats at a speed of 30 m/min at 0% grade for 60 min, whereas we ran 257 ± 5-g female Sprague-Dawley rats at a speed of 20 m/min up a 6° grade for 100 min. The longer exercise bout with the associated longer elevation of temperature and cardiac workload may have been responsible for the increase in HSP 72 after one bout observed by us but not by Locke et al.

The improved cardioprotection observed herein after the exercise bouts at room temperature could be at least partially due to the severalfold increases in HSP 72 (Fig. 2). It is now well established that this protein can be rapidly increased by heat alone (5, 7) or by exercise (11, 16) and that an increase in HSP 72 is strongly correlated with improved cardioprotection from ischemia-reperfusion injury (12). In fact, we observed that heat shock without exercise consistently resulted in postischemic functional recovery that was as good as or better than that in the exercised groups (Table 1 and Fig. 1). However, when we prevented the rise in core temperature during exercise, HSP 72 was not increased in the myocardium above control levels 24 h postexercise, yet an improvement in postischemic functional recovery was still observed. This observation suggests that 1) an exercise-related increase in temperature is more important than the exercise per se in stimulating an increase in HSP 72 at 24 h postexercise, and 2) cardioprotective factors other than HSP 72 can be increased by exercise. The former point appears to be in conflict with Skidmore et al. (17), who reported an increase in HSP 72 in the left ventricle immediately after exercise in rats whose core temperatures were clamped at resting temperature. However, the level of HSP 72 increase was small and the concentration was only about one-third of that in rats whose temperatures increased during the run. Furthermore, the concentration of the protein 24 h postexercise was not determined, and Neufer et al. (14) have recently reported that elevation of HSP 72 mRNA after contractile activity in temperature-clamped muscles is transient, returning to precontraction values within 8 h. Finally, Qian et al. (15) measured myocardial heat shock protein level and postischemic recovery at 2, 4, 12, 24, and 30 h after heat shock treatment and found no correlation between HSP 72 and cardioprotection. Specifically, they reported that HSP 72 levels can rise to within 80% of peak values within 1 h after heat shock but that no cardioprotective effects are present until 24 h after heat shock (15).

Although it does not appear that HSP 72 is the factor directly responsible for the improved exercise-related cardioprotection in the rats exercised without increased core temperature, the responsible factor remains unknown. It is possible that other temperature-independent factors are also stimulated during exercise and that these play a larger role when HSP 72 is low. Redundant mechanisms are common in biological systems, and there are many potential factors that have been reported to confer protection against ischemia-reperfusion injury; thus one should not be surprised if there is more than one cardioprotective factor stimulated by exercise. Our results, along with the wealth of data supporting the protective role of HSP 72, lead us to conclude that the improved postischemic function after acute exercise is due, in part, to a rise in HSP 72 concentration. However, this rise in HSP 72 is primarily due to the exercise-related elevation in temperature, and it appears that other mechanisms work in concert with HSP 72 to provide the exercise-induced cardioprotection.

This work was supported by grants from the American College of Sports Medicine Foundation and the American Heart Association, Texas Affiliate.

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Received 2 September 1998; accepted in final form 4 December 1998.

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