Exercise-induced HSP-72 elevation and cardioprotection against infarct and apoptosis

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Exercise-induced HSP-72 elevation and cardioprotection against infarct and apoptosis. J Appl Physiol 103: 1056–1062, 2007. First published June 14, 2007; doi:10.1152/japplphysiol.00263.2007.—Successive bouts of endurance exercise are associated with both increased cardiac levels of heat shock protein-72 (HSP-72) and improved cardioprotection against ischemia-reperfusion (I/R)-induced cardiac cell death. Although overexpression of HSP-72 has been shown to be cardioprotective in transgenic animals, it is unclear whether increased levels of HSP-72 are essential for exercise-induced cardioprotection against I/R-mediated cell death. We tested the hypothesis that exercise-induced increases in myocardial levels of HSP-72 are required to achieve exercise-mediated protection against I/R-induced cardiac cell death. To test this postulate, we investigated the effect of preventing the exercise-induced increase in cardiac HSP-72 on myocardial infarction and apoptosis after 50 min of in vivo ischemia and 120 min of reperfusion. Adult male rats remained sedentary or performed successive bouts of endurance exercise in cold (8°C) or warm (22°C) environments. We found that, compared with sedentary control animals, exercise in a warm environment significantly increased myocardial HSP-72 content. In contrast, exercise in the cold environment prevented the exercise-induced increase in myocardial HSP-72 levels. After in vivo myocardial I/R, infarct size was reduced in both exercised groups compared with sedentary animals. Furthermore, compared with sedentary rats, I/R-induced myocardial apoptosis (as indicated by terminal deoxynucleotidyl transferase dUTP-mediated nick-end labeling-positive nuclei and caspase-3 activity) was attenuated in both groups of exercised animals. Therefore, although HSP-72 has cardioprotective properties, our results reveal that increased myocardial levels of HSP-72 (above control) are not essential for exercise-induced protection against I/R-induced myocardial infarction and apoptosis.

cell death; heat shock; ischemia-reperfusion; stress protein

DISEASES OF THE CARDIOVASCULAR system are the leading cause of mortality in both men and women in most regions of the world. In this regard, ischemic heart disease accounts for the majority of cardiovascular disease morbidity and mortality (1). Hence, the development of countermeasures to prevent heart disease and protect the heart against ischemia-reperfusion (I/R)-induced cardiac injury continues to be a major research goal (5). To this end, ongoing research is aimed at elucidating the mechanisms of endogenous cardioprotection. Promising cardioprotective candidates include overexpression of heat shock protein-72 (HSP-72) in the myocardium (21, 22). HSP-72 overexpression protects many cell types against a wide variety of stresses, including hypoxia and reoxygenation injury (16, 21, 23, 31). More specifically, previous work using pharmacological preconditioning (24), ischemic preconditioning (11), and transgenic means (31, 32) of HSP-72 overexpression elicit similar I/R-resistant phenotypes. Specifically, in hearts exposed to an I/R challenge, HSP-72 overexpression is associated with myocardial preservation against necrotic and apoptotic cell death (10, 31, 32, 37, 39).

Previous research links HSP-72 to cardioprotection via chaperone activity that stabilizes the myocardium through mitochondrial preservation (33) and membrane stabilization (34). Furthermore, a wealth of recent data highlights the essential role of HSP-72 in preventing I/R-induced apoptosis (3, 36, 39). For example, after I/R, HSP-72 overexpression retards I/R-induced apoptosis through caspase-dependent and -independent pathways (32). Specifically, HSP-72 binds with apoptosis protease-inhibiting factor, thereby inhibiting subsequent activation of both caspase-9 and caspase-3 (32). Furthermore, caspase-independent inhibition by HSP-72 occurs through interference with the apoptotic effector molecule apoptosis-inducing factor (2, 3). Collectively, these findings demonstrated that increased cardiac HSP-72 levels are essential for protection against I/R-mediated cell death (16, 23, 31, 36). Ongoing research with these and other experimental models continue to reveal the unique cardioprotective roles of HSP-72.

In recent years, endurance-based exercise has also been explored as an experimental means of achieving a myocardium resistant to I/R damage (29). There is reason to suspect that mechanisms of exercise-induced cardioprotection may be unique. The value of exercise-induced cardioprotection research cannot be understated due to the well-established link between chronic exercise exposure and improved heart health (4). Indeed, recent findings demonstrate that regular endurance exercise protects the heart against I/R-induced apoptosis (30) and infarction (13, 14). To date, however, the mechanisms responsible for exercise-induced cardioprotection remain largely undefined. As with other physiological models of stress, exercise produces an elevation in myocardial HSP-72 by 50% or more (15). This has led to speculation that HSP-72 may be responsible for exercise-induced cardioprotection (21, 23).

Early cardioprotection studies of HSP-72 in exercised animals, exercise in the cold environment significantly increased myocardial HSP-72 content. In contrast, exercise in the cold environment prevented the exercise-induced increase in myocardial HSP-72 levels. After in vivo myocardial I/R, infarct size was reduced in both exercised groups compared with sedentary animals. Furthermore, compared with sedentary rats, I/R-induced myocardial apoptosis (as indicated by terminal deoxynucleotidyl transferase dUTP-mediated nick-end labeling-positive nuclei and caspase-3 activity) was attenuated in both groups of exercised animals. Therefore, although HSP-72 has cardioprotective properties, our results reveal that increased myocardial levels of HSP-72 (above control) are not essential for exercise-induced protection against I/R-induced myocardial infarction and apoptosis.

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hearts have been equivocal, demonstrating both a protective effect (15, 27) and no influence of elevated HSP-72 against I/R-induced myocardial stunning following a relatively short duration ischemic challenge (20, 38). Previous work using nonexercise experimental models, however, suggests that HSP-72 is a potent mediator of cell survival as a result of longer duration I/R insults, in particular through the prevention of apoptosis (36). Nonetheless, it is currently unknown whether elevated cardiac levels of HSP-72 are essential to achieve exercise-induced cardioprotection against I/R-induced cell death, including apoptosis. This forms the rationale for the present study.

Because of the cellular protective properties of HSP-72 and the fact that exercise predictably elevates cardiac levels of HSP-72, we hypothesized that elevated cardiac levels of HSP-72 are required to achieve exercise-induced cardioprotection against an I/R insult of sufficient duration to induce apoptosis and infarction. We tested this hypothesis by preventing the exercise-induced increase in cardiac levels of HSP-72. Our results did not support this postulate and reveal that elevated myocardial levels of HSP-72 are not required to achieve exercise-induced cardioprotection against I/R-induced cardiac death.

MATERIALS AND METHODS

Animals and experimental design. The experimental protocol was approved by the University of Florida Animal Care and Use Committee and followed guidelines established by the American Physiological Society for the use of animals in research. Adult male Sprague-Dawley rats (~6 mo old) were randomly assigned to an exercise treatment or to a sedentary protocol (control). Rats assigned to exercise were further randomized into groups exposed to exercise in either a warm (22°C) or cold (8°C) environment. From previous experiments by our group and others, exercise at 24°C has been shown to elicit elevations in myocardial HSP-72, whereas exercise at 8°C prevents a significant rise in body core temperature during exercise, thereby preventing the subsequent rise in myocardial HSP-72 (12, 14, 38). Importantly, to prevent confounding effects of cold stress on cardioprotection, cold exposure of exercised animal was limited to the duration required to complete the treadmill exercise. Animals from sedentary, warm exercise, and cold exercise treatments were further randomized into two groups: in vivo myocardial I/R surgery or sham surgery (no I/R). Therefore, our collective experimental design included the following six experimental groups: 1) sedentary sham (Sed; n = 10), 2) warm exercised sham (W Ex; n = 10), 3) cold exercised sham (C Ex; n = 10), 4) sedentary I/R (Sed I/R; n = 24), 5) warm exercised I/R (W Ex I/R; n = 24), and 6) cold exercised I/R (C Ex I/R; n = 24).

After a surgical plane of anesthesia was reached, hearts from sham animals were rapidly removed and used for biochemical analyses of HSPs. Hearts from animals exposed to an I/R insult were used for biochemical detection of myocardial necrosis or apoptosis. During the experimental period, all animals were housed on a 12:12-h light-dark cycle and provided food (AIN93 diet) and water ad libitum. Exercise protocol. Animals assigned to the exercise groups were habituated to treadmill exercise on a daily basis for 5 consecutive days. This habituation period involved a gradual increase in running time beginning with 10 min/day and ending with 50 min/day. After 2 days of rest, the exercise-habituated animals then performed 3 consecutive days of treadmill exercise for 60 min/day at 30 m/min, 0% grade. On the basis of previous experiments by our group (8, 19), this work rate represents an estimated 70% of maximum oxygen consumption. Colonic temperatures were recorded before exercise and immediately after each exercise session to verify the efficacy of warm and cold exercise ambient temperatures for generating either hyperthermic or normothermic responses in the warm and cold exercise groups, respectively. Importantly, to eliminate the confounding effects of handling stress on HSP expression, sedentary animals were treated identically to the W Ex group, except for treadmill running.

In vivo I/R and sham protocols. The in vivo I/R model of coronary ligation has been used successfully by our laboratory for the induction of regional myocardial ischemia and has been described in detail in previous publications (12–14). Briefly, this model elicits an ischemic area of ~60% of the left ventricular free wall. Sham and I/R protocols were performed 24 h after the final exercise bout. Rats were anesthetized (80 mg/kg ip pentobarbital sodium) and ventilated with room air via a tracheostomy tube. A saline-filled catheter attached to a pressure transducer was placed in the carotid artery and interfaced with a computerized heart performance analyzer for continuous monitoring of heart rate and arterial blood pressure (Digi-Med, Louisville, KY). Another catheter was placed in the jugular vein for delivery of pentobarbital sodium (10 mg/kg) as needed. After a left thoracotomy, a ligature was placed around the left anterior descending artery close to its origin. For sham surgery, the heart was removed without coronary artery occlusion. For the I/R surgery, a soft piece of tubing was threaded through the ligature and pressed on the surface of the heart to impinge flow to the ligated coronary artery. The ligature-tubing apparatus was secured to the heart with a small hemostat. Coronary occlusion was maintained for 50 min followed by 120 min of reperfusion. Electrical activity of the heart (continuous ECG monitoring) and visualization of cyanotic heart tissue were used to confirm the presence of ischemia. Gentle heart massage and electrical cardioversion were used to alleviate ventricular fibrillation persisting longer than ~30 s without spontaneous conversion. In these experiments, needs for massage and cardioversion were rare, occurring in three sedentary animals and once for the respective warm and cold exercised animals. Note that, in the animals not exposed to I/R (i.e., sham surgery), the hearts were removed from the animal without coronary artery occlusion.

Tissue preparation and infarct staining. Within each experimental group exposed to I/R, a subgroup of animals was given an arterial infusion of Evans blue dye (4%), following the I/R protocol to delineate the area at risk (AAR). To identify the infarcted tissue area, hearts were excised, rinsed in cold saline, and sliced into 1- to 2-mm sections for incubation in 1% triphenyl tetrazolium chloride at 37°C for 10 min. Digital photographs of the heart slices were taken and analyzed for percent AAR and percent infarction with a Kodak image analysis system.

Hearts were excised from a second group of animals from each treatment after reperfusion, rinsed in cold antioxidant buffer (50 mM NaHPO₄, 0.1 mM butylated hydroxy toluene, 0.1 mM diethylenetriaminepentaacetic acid, pH 7.4), and stored for subsequent analysis. One portion of the left ventricular free wall was placed in a storage vial, rapidly frozen in liquid nitrogen, and stored at ~80°C until further biochemical analyses were performed. A separate section of left ventricular free wall and septum was stored in Tissue-Tek embedding medium (Sakura Finetek, Torrance, CA) for subsequent cryosectioning and histochemical analysis. Finally, hearts from sham animals received no arterial occlusion and were removed and rapidly frozen as previously described.

Biochemical analysis of myocardial HSP content. To determine the effects of exercise training on induction of myocardial HSP content, we performed PAGE and immunoblotting techniques. Briefly, left ventricular free wall samples from sham hearts were homogenized 1:10 in 100 mM potassium phosphate buffer (pH = 7.4) supplemented with a protease inhibitor cocktail (Sigma, St. Louis, MO) and centrifuged at 4,000 g (4°C). The protein concentration of the supernatant was assessed by the method of Bradford (Sigma) (6). Proteins (25–30 µg) were then separated on 10% or 12% polyacrylamide gels containing 0.1% SDS. After electrophoresis at 120 V, the proteins were transferred to nitrocellulose membranes (250 mA for 1.5 h). Membranes were stained with Ponceau to ensure equal protein loading and...
Animal body weights and colonic temperatures

<table>
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<tr>
<th>Group</th>
<th>Number</th>
<th>Body Weight, g</th>
<th>Preexercise Temperature, °C</th>
<th>Postexercise Temperature, °C</th>
<th>Heart Weight, g</th>
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<tr>
<td>Sed</td>
<td>10</td>
<td>290±2.4</td>
<td>37.8±0.07</td>
<td>39.6±0.14†</td>
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<td>W Ex</td>
<td>10</td>
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<tr>
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<td>38.1±0.05</td>
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<td>1.18±0.02</td>
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Values are means ± SE. *Significantly different from sedentary sham animals (Sed), P < 0.05. †Significantly different from warm exercised sham animals (W Ex) preexercise temperature and cold exercised sham animals (C Ex) postexercise temperature, P < 0.05.

RESULTS

Animal characteristics. Animal body weights, colonic temperatures, and heart weights for sedentary, warm exercised, and cold exercised treatments are presented in Table 1. As expected, W Ex animals had significantly increased core temperature of ~1.7°C, as determined by colonic temperature. In contrast, C Ex animals experienced a nonsignificant 0.1°C rise in core temperature. Preexercise colonic temperatures in W Ex and C Ex animals did not differ from the resting temperatures measured in Sed animals. Finally, body weights of C Ex animals were significantly higher than those of Sed animals.

Myocardial HSP content in warm and cold exercised hearts. To determine the effects of exercise on HSP content, myocardial HSP content was evaluated in unstressed hearts from sedentary and exercised animals and expressed as a percentage of Sed results. Compared with Sed animals, exercise in the warm environment resulted in an increase (+50%) in HSP-72 in the left ventricle (Fig. 1). Exercise in the cold environment did not result in significant increases in myocardial HSP-72 levels. Furthermore, evaluations of other potentially protective HSPs in the left ventricle were also performed by Western blot techniques. Our results indicate that neither warm nor cold exercise resulted in significant elevations in HSP-27, HSP-32, HSP-40, HSP-60, or HSP-90 compared with hearts from the sedentary group (Table 2).

Myocardial infarction after I/R. The in vivo I/R insult resulted in comparable risk areas for sedentary and exercised groups (%AAR/total area = 34% Sed I/R, 27% W Ex I/R, and 29% C Ex I/R; P = 0.149). I/R resulted in ~67% (%infarction/

![Image](http://jap.physiology.org/)
AAR) in hearts from Sed animals (Fig. 2). As expected, W Ex I/R animals demonstrated a marked cardioprotective response (22% infarction/AAR) against in vivo I/R infarction relative to their sedentary counterparts. Similarly, compared with Sed animals, C Ex I/R animals demonstrated cardioprotective resistance against I/R-induced infarction (30% infarction/AAR). Importantly, no differences existed in the level of cardioprotection between the two exercise groups (i.e., cold and warm environments). Hence, these results indicate that cardioprotection against I/R-induced infarction occurred independent of myocardial HSP-72 content.

**Analysis of myocardial apoptosis.** Tissue sections from the ischemic zone of the left ventricle were analyzed for indexes of apoptosis. Representative images of ventricular cross sections stained for TUNEL-positive nuclei are presented in Fig. 3A. After 50 min of in vivo ischemia and 120 min of reperfusion, numerous TUNEL-positive nuclei were present in hearts from Sed animals. In contrast, hearts from both W Ex I/R and C Ex I/R animals exhibited significantly fewer TUNEL-positive nuclei in response to an identical I/R challenge (Fig. 3B). Moreover, a fluorescent assay was performed to detect caspase-3 activity in left ventricular homogenates from sedentary and exercised hearts exposed to in vivo I/R (Fig. 4). Compared with hearts from sham animals not exposed to I/R, the I/R insult resulted in a significant rise in caspase-3 activity in the left ventricles of Sed animals. In contrast, caspase-3 activity in hearts from both warm and cold exercised animals was not elevated by I/R.

<table>
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<tr>
<th></th>
<th>Sed</th>
<th>W Ex</th>
<th>C Ex</th>
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<tr>
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<td>100±4.0</td>
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<tr>
<td>HSP-90</td>
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<td>101±5.3</td>
<td>105±8.7</td>
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Values are a densitometric means ± SE expressed as a percentage of Sed results; n = 9 animals/group. HSP, heat shock protein.

**Fig. 2.** Analysis of myocardial infarction after 50 min of in vivo ischemia and 120 min of reperfusion. Sed IR, sedentary ischemia-reperfusion (I/R) group; W Ex IR, warm exercised IR group; C Ex IR, cold exercised IR group. A: representative cross-sectional images of %infarction/area at risk. Blue areas represent perfused myocardium during in vivo ischemia, red areas indicate viable myocardium within the area at risk for infarction based on triphenyl tetrazolium chloride reactivity, and white areas indicate infarcted tissue. B: computerized image analysis of infarction area. Values are means ± SE of myocardial infarction expressed as %area at risk; n = 7 animals/group. *Significantly different from Sed IR, P < 0.05.

**Fig. 3.** Terminal deoxynucleotidyl transferase dUTP-mediated nick-end labeling (TUNEL) staining of nuclei within myocardial tissue sections following I/R. A: representative images of cryosections from hearts obtained from W Ex IR, C Ex IR, and Sed IR hearts. Hearts were exposed to 50 min of in vivo ischemia and 120 min of reperfusion. Sections were stained for nuclei (blue), laminin (red), and TUNEL-positive nuclei (green). Images represent 0.3-mm² tissue sections. B: analysis of TUNEL-positive nuclei within hearts from sedentary and exercised animals. Results reveal that warm exercise training and cold exercise training provide equal levels of protection against I/R-induced myocardial apoptosis. Values are mean TUNEL-positive nuclei/mm² ± SE; n = 10, 9, and 10 for W Ex IR, C Ex IR, and Sed IR, respectively. *Significantly different from Sed IR, P < 0.05.

**Fig. 4.** Analysis of caspase-3 activity in the left ventricles of animals from all groups after 50 min of in vivo ischemia and 120 min of reperfusion. Values are mean relative fluorescent units (RFU) ± SE; n = 9, 11, 9, and 10 for Sed, Sed IR, W Ex IR, and C Ex IR, respectively. *Significantly different from Sed, W Ex IR, and C Ex IR, P < 0.05.
DISCUSSION

Summary of principal findings. This is the first study to investigate whether elevated myocardial HSP-72 content is an essential mediator of exercise-induced cardioprotection against in vivo I/R-induced cell death. Our results confirm previous studies that indicate endurance exercise confers cardioprotection against I/R-induced cell death (14, 30). Nonetheless, our data reveal that, although HSP-72 is a molecule with cytoprotective properties, elevated cardiac levels of HSP-72 are not required to achieve exercise-induced protection against I/R-induced cell death. This finding is quite interesting in light of an overwhelming body of literature indicating that HSP-72 is essential in preventing I/R-induced apoptosis in nonexercise experimental models (2, 3, 36, 39). Resolving the fundamental difference between cardioprotection mediated by exercise and the various preconditioning methods may prove useful in elucidating clinical solutions against I/R injury.

Exercise-induced cardioprotection against I/R-induced apoptosis and necrosis. Epidemiological data have long supported the notion that exercise provides protection against diseases of the heart, including ischemic heart disease (4). Moreover, a wealth of published data confirms that exercise protects the myocardium against I/R-induced arrhythmias, myocardial stunning, and cell death (13, 14, 20, 30). Interestingly, as few as three to five consecutive exercise sessions provide the same level of cardioprotection as weeks to months of exercise training (9). Although it is clear that regular exercise is cardioprotective, identification of the required cardioprotective mediators in the exercised heart has been largely elusive. This gap in the understanding of the mechanism(s) responsible for exercise-induced cardioprotection formed the basis for the present study.

The search for essential cardioprotective mediators. The pursuit of the mechanism(s) responsible for exercise-induced cardioprotection has been ongoing for over a decade. Historically, it has been postulated that at least three primary mechanisms could explain the cardioprotective effects of exercise: 1) anatomic changes in the coronary arteries (i.e., collateral circulation), 2) improved myocardial antioxidant capacity, and/or 3) induction of myocardial heat shock and other stress proteins (HSPs) (reviewed in Refs. 28, 29).

Evidence indicates that the development of collateral coronary arteries during only 1–5 days of exercise is not a strong candidate to explain exercise-induced cardioprotection (28). Indeed, 1–5 days of exercise training does not alter the collateral coronary circulation in healthy adult animals (e.g., dogs, pigs, or rats) (18). Furthermore, a recent study has demonstrated that the beneficial effect of short-term exercise in prevention of myocardial I/R injury in the rat is not due to coronary vascular adaptations (40).

Given the important role that radicals and other reactive oxygen species play in I/R-induced cardiac injury, an increase in myocardial antioxidant capacity is a potential contributory mechanism to cardioprotection against I/R-induced cardiac injury (29, 30). In this regard, recent evidence indicates that exercise-induced increases in endogenous myocardial antioxidants are significant contributors to exercise-induced cardioprotection against I/R injury. For example, Yamashita et al. (40) reported that abolishment of exercise-induced increases in manganese superoxide dismutase prevents the cardioprotection associated with exercise. Similarly, a recent study by our group further supported the concept that manganese superoxide dismutase is an important cardioprotective mediator against I/R-induced arrhythmias (13). These findings do not fully explain exercise-induced cardioprotection against I/R, however, suggesting that other cardioprotective mediators must be in effect.

Other potential mediators in the exercised heart may include several stress proteins found to be overexpressed in the exercised myocardium. For example, Brown et al. (7) concluded that exercise-induced expression of sarcolemmal KATP channel is required to achieve the cardioprotection associated with exercise. Furthermore, a recent study by Marini et al. (25) found that the I/R resistance afforded by exercise was associated with elevations in myocardial levels of heme oxygenase-1, cyclooxygenase-2, glucose-regulated protein 75, and HSP-70/72. Clearly, more research is needed to systematically confirm or eliminate these potential cardioprotective mediators in the exercised heart. Accordingly, the present experiment tested the hypothesis that increased cardiac levels of HSP-72 are essential to achieve exercise-induced cardioprotection against I/R-induced cardiac cell death. We focused our experiments on HSP-72 as a cardioprotective mediator for several reasons.

The cellular response to many physiological stressors, including exercise stress, promotes an elevation in a variety of HSPs (21). The HSP stress adaptation is a well-described defense mechanism thought to help maintain cellular homeostasis (29, 30). The contributions of elevated HSP content in the myocardium as a beneficial defense against I/R are widely documented and have been reviewed previously (21, 32). Potentially protective HSP species include HSP-27, HSP-32, HSP-40, HSP-60, HSP-70, and HSP-90. Among the various HSPs, HSP-72 has received special focus as a cardioprotective mediator. For instance, elevations in myocardial HSP-72 content limit I/R-induced contractile dysfunction (33), necrosis (16), and apoptosis (36). Importantly, the magnitude of the myocardial HSP-72 increase induced by hyperthermia in rat models has been variable, but consistent cardioprotection against I/R injury has been demonstrated with cardiac HSP-72 overexpression ranging between 1.5 and 5 times the constitutive levels (26, 33).

Acute exercise normally results in hyperthermia and subsequent elevation of myocardial HSP-72 content. In the present experiments, we took advantage of the fact that an increase in body temperature is required to achieve exercise-induced increases of cardiac levels of HSP-72 (35). That is, separate experimental groups of animals were exercised in warm and cold environments to respectively induce and prevent the myocardial HSP-72 accumulation associated with exercise. Inhibition of an exercise-induced increase in body temperature (via exercise in a cold environment) successfully prevented the exercise-induced increase in myocardial HSP-72 (Fig. 1). In contrast, compared with both sedentary and cold exercised animals, exercise in a warm environment promoted a significant increase in cardiac HSP-72 levels. Nonetheless, animals trained in the cold environment exhibited cardioprotection against I/R-induced myocardial infarction (Fig. 3) and apoptosis (Fig. 4) similar to animals exercised in the warm environment. Collectively, these findings indicate that exercise-induced cardioprotection against I/R-induced cell death can be achieved without an increase in myocardial HSP-72 content. Therefore, although overexpression of HSP-72 has the potential to protect cells against I/R.
injury, our results do not support the hypothesis that elevated cardiac levels of HSP-72 are essential to fully achieve exercise-induced cardioprotection against I/R-induced cell death.

Furthermore, because HSPs other than HSP-72 have also been reported to possess cellular protective qualities (21), we also measured cardiac levels of five different HSPs possessing protective properties. Our results revealed that myocardial levels of HSP-27, HSP-32, HSP-40, HSP-60, and HSP-90 were not increased during short-term endurance exercise training. Hence, on the basis of these results, we also conclude that these stress proteins are not essential components of cardioprotection in the exercised heart.

Previous research has demonstrated that HSP-72 expression is proportional to heat stress (16). In the present study, 60 min of exercise in the warm environment resulted in a mean colonic temperature of 39.6°C. In comparison, in a rat model exercise study by Hamilton et al. (12), 60 min of endurance exercise resulted in a mean postexercise colonic temperature of 41.1°C. Hence, the magnitude of myocardial HSP-72 elevation in our animals exercised in a warm environment was less marked than that shown by Hamilton et al. and probably explains why we did not observe a rise in myocardial HSP-40 and HSP-90 levels (12). Importantly, our finding that exercise in a warm environment elevated myocardial levels of HSP-72 but did not increase other potentially cardioprotective HSPs provides a clear means for independent evaluation of HSP-72 as a cardioprotective mediator against I/R injury.

Critique of experimental model. Adult male Sprague-Dawley rats were chosen as the experimental model for this investigation because this rat strain does not display large interanimal variation with respect to coronary anatomy. Finally, male rats were chosen to avoid potentially confounding effects of menstruation on HSP-72 expression on cardioprotection (27).

We chose to use an in vivo model of regional myocardial ischemia because of the physiological relevance of this animal model to human myocardial I/R injury. The I/R time periods used in this study were selected because durations for I/R induce an identifiable infarction and apoptosis in the hearts of sedentary rats (10, 30). Notably, warm and cold exercised hearts exhibited a limited infarct and apoptotic response to I/R, while in these hearts caspase-3 levels were not significantly elevated above those in sedentary control hearts. This observation could be the result of several possibilities. For instance, apoptotic cell death can be mediated independent of caspase-3, the primary effector caspase (17). In addition, this finding in the present study may indicate variance in the respective assay sensitivity for necrosis, apoptosis, and caspase-3. Accordingly, we cannot rule out the possibility that heart tissue deemed necrotic may have included myocardial cells undergoing apoptosis. Future examination of the exercised heart is needed to better reveal the mechanisms of cell survival following an ischemic challenge.

Finally, to inhibit the exercise-induced rise in HSP-72, we exercised a group of animals in a cold environment and monitored body temperatures to ensure that constant core temperature was maintained during exercise. This experimental approach reduces the risk of side effects associated with various pharmacological approaches to the inhibition of HSP-72 gene expression. Importantly, by exercising animals in a cold environment, we were successful in attenuating the exercise-induced rise in HSP-72 in the cold exercised animals. Conclusions. Exercise has been repeatedly shown to evoke cardioprotection against all levels of I/R-induced cellular injury and death (13, 14, 20, 30). Despite the well-defined role of HSP-72 as a cytoprotective molecule and as a constituent of the exercise-trained heart (28), elevated levels of HSP-72 are not essential to achieve exercise-induced cardioprotection against I/R-induced myocardial infarction or apoptotic cell death following I/R in the rat. Although the possibility remains that HSP-72 serves an unknown function against I/R injury in the exercised heart, the present data reveal that the exercise-induced cardioprotective phenotype is not dependent on increased myocardial levels of HSP-72.

GRANTS

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