Voluntary exercise protects against acute doxorubicin cardiotoxicity in the isolated perfused rat heart

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Submitted 17 September 2004; accepted in final form 13 April 2005

Chicco, Adam J., Carole M. Schneider, and Reid Hayward. Voluntary exercise protects against acute doxorubicin cardiotoxicity in the isolated perfused rat heart. Am J Physiol Regul Integr Comp Physiol 289: R424–R431, 2005. First published April 21, 2005; doi:10.1152/ajpregu.00636.2004.—The clinical use of doxorubicin (DOX) is limited by a dose-dependent cardiotoxicity. The purpose of this study was to determine whether voluntary exercise training would confer protection against DOX cardiotoxicity in the isolated perfused rat heart. Female Sprague-Dawley rats were randomly assigned to standard holding cages or cages with running wheels for 8 wk. Twenty-four hours after the sedentary (SED) or voluntary exercise (VEX) running period, rats were anesthetized with pentobarbital sodium, and hearts were isolated and perfused with oxygenated Krebs-Henseleit (KH) buffer at a constant flow of 15 ml/min. After a 20-min stabilization period, hearts were paced at 300 beats per minute and perfused with KH buffer containing 10 μM DOX for 60 min. A set of control hearts from SED and VEX rats were perfused under identical conditions without DOX for the same period. DOX perfusion led to significant decreases in left ventricular developed pressure, +dP/dt, and −dP/dt, and significant increases in LV lipid peroxidation in sedentary rats compared with non-DOX controls (P < 0.05). Prior voluntary exercise training attenuated these DOX-induced effects and was associated with a significant increase (78%, P < 0.05) in heat shock protein (HSP72), but not mitochondrial isoform of SOD (MnSOD) or CuZnSOD protein expression in the hearts of wheel-run animals. These data indicate that chronic physical activity may provide resistance against the cardiac dysfunction and oxidative damage associated with DOX exposure and provide novel evidence of HSP72 induction in the heart after voluntary exercise.

adriamycin; cardioprotection; heart; physical activity; cardiac function

DOXORUBICIN (DOX) is a highly effective antineoplastic antibiotic commonly used to treat a variety of solid tumors and leukemias. Unfortunately, the clinical use of doxorubicin is limited by a dose-dependent cardiotoxicity that may progress to chronic cardiomyopathy and congestive heart failure (CHF) (50). Characterization and prevention of DOX cardiotoxicity (DCT) have been the subjects of intense research for decades, but no single intervention has proven to be completely successful in ameliorating the damaging effects of DOX on the heart, while maintaining its antineoplastic efficacy.

DOX cardiomyopathy and CHF typically develop after multiple intravenous DOX treatments over a period of several months in humans. Incidence of chronic cardiomyopathy appears to be highly dependent on the cumulative DOX dose administered (50) and has been recently reported to be as high as 26% in patients receiving 550 mg/m² (54). Evidence from human (37) and animal (30) studies suggests that acute myocardial dysfunction and/or injury occurring early during the course of DOX treatment is highly predictive of later cardiomyopathy. Furthermore, it appears that the chronic manifestations of DCT may result from myocardial injuries associated with acute DOX exposure (40, 58). This has led to the use of a wide variety of experimental models aimed at exploring the mechanisms and circumvention of acute DCT in vivo and in vitro. Several investigators have used the isolated perfused rat heart model for this purpose, which has provided a reliable and reproducible means of assessing the biochemical and functional aspects of acute DCT (19, 40, 42).

Free radical formation has been consistently reported to play a pivotal role in several of the acute and chronic manifestations of DCT (23, 27, 30, 34). DOX is believed to interact with intracellular redox enzymes such as nicotinamide adenine dinucleotide dehydrogenase to produce superoxide radicals and increase the levels of cytotoxic reactive oxygen species (ROS) in cardiomyocytes (10). The resulting oxidative stress is known to initiate a myriad of destructive events in cardiomyocytes and has been specifically linked to membrane lipid peroxidation (27, 34, 39), impaired Ca²⁺ handling (15), mitochondrial dysfunction (13), apoptosis (23), and DNA injury (30). Superoxide dismutase (SOD) is an endogenous antioxidant enzyme that protects the heart from superoxide by catalyzing its dismutation into hydrogen peroxide (H₂O₂), which is then converted to oxygen and water by catalase (CAT) and the glutathione peroxidase (GPx) system. Acute DCT has been shown to be enhanced when SOD activity is compromised (49) and is attenuated in transgenic mice overexpressing the mitochondrial isoform of SOD (MnSOD) (62). These studies further support the role of superoxide in DCT and suggest that any treatment that increases myocardial SOD activity may protect the heart from DOX-induced injury.

Heat shock proteins of the 70-kDa family (particularly the inducible isoform, HSP72) are believed to protect cardiomyocytes against injury during states of cellular stress (5, 21). HSP72 induction has been specifically associated with the prevention of apoptosis, necrosis, and oxidative injury in the myocardium following exposure to ischemia-reperfusion (I/R) (8, 45, 47), hydrogen peroxide (55), and DOX (17). HSP72 most likely protects cardiomyocytes by preserving protein structure and function during states of stress (21) and may selectively protect mitochondria from the damaging effects of ROS (41).
Exercise training has been reported to increase SOD (2, 44) and HSP72 content (28, 45) in the myocardium. These adaptations have been associated with an exercise-induced preservation of cardiac function and attenuation of myocardial lipid peroxidation observed following I/R (2, 45). Therefore, it is plausible that exercise training may also confer protection against the cardiac dysfunction and myocardial lipid peroxidation induced by DOX exposure. The authors are aware of only one study that has examined the effect of prior exercise training on DCT (20). These investigators reported that mice swim-trained before, during, and after DOX therapy in vivo had fewer myocardial lesions than untrained DOX-treated mice, but no assessment of cardiac function was conducted.

The vast majority of studies that have examined the cardioprotective effects of exercise training have utilized a forced treadmill running or swimming model. Although these protocols permit strict control of the duration and intensity of exercise training, animals appear to experience significant systemic stress, which may have far-reaching physiological implications (32, 48). Therefore, we have employed a voluntary running model in an effort to isolate the effects of habitual physical activity from the additional stress associated with forced-exercise protocols.

The purpose of this study was to determine whether chronic voluntary exercise before DOX exposure protects the heart from the acute manifestations of DCT. We have used an established model of acute DCT (19, 42, 43) to examine the direct effects of DOX exposure on intrinsic cardiac function and lipid peroxidation in isolated perfused hearts from sedentary and physically active rats. Given prior evidence of their role in exercise-induced cardioprotection against oxidative stress, MnSOD, CuZnSOD, and HSP72 content were also examined in hearts of sedentary and physically active animals.

METHODS

Animals and experimental design. All experimental procedures were approved by the University of Northern Colorado Institutional Animal Care and Use Committee and were in compliance with guidelines established by the American Physiological Society. Female Sprague-Dawley rats (200–225 g) were randomly assigned to a sedentary or voluntary exercise group. Female rats were selected because of their greater propensity for voluntary running compared with males (60). All animals were housed in a temperature-controlled facility (19–21°C) maintained on a 12:12-h light-dark cycle with food and water provided ad libitum. Rats in the voluntary exercise group were housed individually in cages with stainless-steel running wheels (1.06 m circumference, Mini-Mitter, Bend, OR) and were allowed free access to the wheel 24 h per day for 8 wk. Sedentary rats were housed in standard holding cages without running wheels for the same period. The voluntary wheel-running model was selected in an effort to isolate the effects of habitual physical activity from the additional stress associated with forced-exercise protocols.

Isolated heart perfusion protocol. All rats were anesthetized with heparinized (1.000 U/kg) pentobarbital sodium (40 mg ip), and hearts were rapidly excised and placed in ice-cold KH buffer consisting of (in mM): 120 NaCl, 5.9 KCl, 2.5 CaCl2, 1.2 MgCl2, 25 NaHCO3, 17 glucose, and 0.5 EDTA, (Sigma, St. Louis, MO). The aorta was then immediately cannulated for retrograde cardiac perfusion at 37°C with oxygenated (95% O2-5% CO2) KH buffer at a constant perfusion rate of 15 ml/min. After a 15-min stabilization period, hearts were electrically paced at 300 beats per minute (bpm; 5 Hz) using a stimulus isolator (AD Instruments, Colorado Springs, CO) and allowed to stabilize for an additional 5 min before being perfused with KH buffer (SED and VEX) or KH buffer containing 10 μM DOX (SED+DOX and VEX+DOX) for an additional 60 min. This DOX perfusion protocol has been previously shown to provide a reproducible model of acute DOX cardiotoxicity in the isolated perfused rat heart (19, 42, 43).

Measurement of LV function. To monitor left ventricular function during the perfusion period, a microtip catheter pressure transducer (Millar Instruments, Houston, TX) was inserted into the left ventricular (LV) cavity through the mitral valve for continuous measurement of LV developed pressure (LVDP) and its maximal and minimal first derivatives (+dP/dt and −dP/dt, respectively). After the 20-min stabilization period, LV pressure data were recorded under paced conditions (300 bpm) at 10-min intervals using a PowerLab/8e data acquisition system (ADInstruments). Immediately after the perfusion period, hearts were trimmed free of surrounding connective tissue and fat, blotted dry, and weighed. The LV was then isolated, frozen in liquid nitrogen, and stored at −80°C for future biochemical analyses.

Lipid peroxidation assay. To examine the effect of DOX perfusion on myocardial lipid peroxidation in sedentary and wheel-running, malondialdehyde and 4-hydroxy-alkenals (MDA + 4-HAE) were analyzed in LV of all hearts after the perfusion experiments described above using a spectrophotometric assay kit (Calbiochem, San Diego, CA), according to the manufacturer’s instructions. Frozen LV tissue was homogenized in ice-cold 20 mM Tris+HCl, pH 7.4, containing 5 mM butylated hydroxytoluene (to prevent lipid peroxidation during homogenization) at a dilution of 1:5 (wt/vol) and centrifuged at 3,000 g for 10 min at 4°C. The supernatant was collected and used for future spectrophotometric analyses. MDA + 4-HAE is expressed relative to the protein content of sample supernatant determined by the method of Bradford (1) using bovine serum albumin as the standard.

Western blotting. The effect of voluntary exercise on potential candidates of exercise-induced cardioprotection, HSP72, CuZnSOD, and MnSOD contents were determined in LV samples obtained from SED and VEX animals following the perfusion protocol described above using Western immunoblotting methods previously described (2, 28). Briefly, LV samples were homogenized in ice-cold buffer containing (in mM) 100 KCl, 50 MOPS, 5 MgCl2, 1 ATP, 1 EGTA (pH 7.4), and protease inhibitor cocktail (Roche Diagnostics, Mannheim, Germany). Homogenates were then diluted 1:3 (vol/vol) in lysis buffer containing 20 mM HEPES, 150 mM NaCl, 1 mM EDTA, 1% NP-40, and 0.1% SDS (pH 7.4); allowed to incubate at room temperature for 10 min; and then centrifuged at 3,000 g for 5 min. The supernatant was then assayed for total protein (1), and a volume of supernatant containing 50 μg (HSP72) or 3 μg (SOD isoforms) of LV protein was electrophoresed on polyacrylamide gels for separation of proteins by molecular weight. Proteins were then transferred to membranes (Amersham, Piscataway, NJ) and incubated with an alkaline phosphatase-conjugated polyclonal antibody specific for rat HSP72, or polyclonal antibodies specific for CuZnSOD or MnSOD (Stressgen, Victoria, British Columbia, Canada). HSP72 blots were then reacted with 5-bromo-4-chloro-3-indolyl phosphate-nitro blue tetrazolium substrate (Sigma Chemical; HSP72) and scanned for band density analysis. SOD isoform blots were incubated with a secondary horseradish peroxidase-conjugated goat anti-rabbit antibody (Santa Cruz Biotechnology, Santa Cruz, CA) followed by a chemiluminescent substrate (Western Lighting, Perkin-Elmer Life Sciences, Boston, MA), and developed on film. Quantification of relative HSP72 and SOD isoforms band densities were performed on computerized scans of...
immunoblots using ImageJ densitometry software (National Institutes of Health, Bethesda, MD). VEX band density is expressed as a percentage of the mean SED band density from the same membrane.

Soleus citrate synthase activity. To examine any training effect resulting from the voluntary running protocol on skeletal muscle, the right soleus muscle was excised from sedentary and wheel-run rats and analyzed for citrate synthase activity at 30°C by the method of Srere (52).

Statistical analyses and protein assay. All data are expressed as group means ± SE. Cardiac function parameters were analyzed using a two-way (VEX × DOX) ANOVA with repeated measures, followed by independent sample t-tests to examine individual group differences. In addition, within-group differences between baseline and time point measurements during perfusion were examined using paired t-tests with the Bonferroni correction for multiple comparisons. The MDA 4-HAE data were analyzed by a two-way ANOVA with independent sample t-tests post hoc. HSP72, SOD isoforms, and morphological data from SED and VEX animals were compared by independent sample t-tests. Significance was established at \( P < 0.05 \) for all statistical analyses.

RESULTS

Voluntary exercise and animal characteristics. Animals in the running-wheel cages voluntarily ran an average of 6,880 ± 348 m/day. Fig. 1 illustrates the average distance run per week over the 8-wk period. The effects of voluntary exercise on animal characteristics are presented in Table 1. No significant differences in body weight were observed between the sedentary and wheel-run rats at the end of the 8-wk study. However, wheel running led to significant cardiac hypertrophy, indicated by greater heart weight and heart-to-body weight ratio in the VEX compared with SED animals (\( P < 0.001 \)). In addition, a skeletal muscle training effect was observed in the VEX animals, indicated by significantly higher soleus weight and citrate synthase activity compared with SED (\( P < 0.05 \)).

Effect of voluntary exercise and DOX perfusion on LV function. At the end of the stabilization period, there were no differences in any of the selected parameters of LV function observed between the sedentary or wheel-run animals, indicating that voluntary exercise did not significantly alter basal cardiac function (Table 2). Fig. 2 illustrates the effect of VEX and DOX perfusion on LV function relative to baseline levels throughout the 60-min perfusion period. Perfusion with KH buffer alone had no significant effect on LV function in the SED or VEX hearts relative to baseline values, and there were no significant functional differences observed between these two groups. DOX perfusion led to a significant decline in all parameters of LV function in the hearts from sedentary animals throughout the perfusion period. LVDP decreased to 84% of baseline values after 30 min of DOX perfusion (\( P < 0.05 \)) and steadily declined thereafter to 38% of baseline by 60 min. Similar decrements were observed in LV \(-dP/dt\) and \(+dP/dt\), which decreased to 46% and 50% of baseline by 60 min, respectively (\( P < 0.05 \)). These DOX-induced decrements in LV function were attenuated in the hearts of wheel-run rats. LVDP remained at baseline levels for 40 min in the hearts of VEX + DOX rats, then steadily declined to 60% of baseline after 60 min (\( P = \text{not significant} \)). Although \(-dP/dt\) and \(+dP/dt\) also declined in hearts of wheel-run animals, after 60 min of DOX perfusion (decreased to 91% and 78% of baseline, respectively), these decreases were not statistically significant. Furthermore, relative values of LVDP, \(-dP/dt\) and \(+dP/dt\) were significantly higher in the VEX + DOX than SED + DOX hearts at several time points throughout the perfusion period (see Fig. 2). These data suggest that VEX before DOX exposure results in an attenuation of DOX-induced LV dysfunction.

Lipid peroxidation. Two-way ANOVA analysis revealed a significant effect of DOX perfusion on myocardial lipid peroxidation indexes. Although there was no effect of VEX alone on MDA + 4-HAE levels, there was a significant interaction effect of VEX and DOX (\( P < 0.05 \)). Compared with SED and VEX hearts, MDA + 4-HAE levels were significantly higher in the SED + DOX hearts (\( P < 0.05 \)), but not the VEX + DOX hearts (Fig. 3). These data suggest that voluntary exercise attenuates myocardial lipid peroxidation induced by DOX exposure.

Left ventricular SOD and HSP72 content. No significant differences in LV MnSOD or CuZnSOD content were observed between SED and VEX animals (Fig. 4). HSP72 content, however, was 78% higher in the hearts of VEX compared with SED (Fig. 5), indicating that voluntary exercise induced HSP72 protein expression in the heart.

DISCUSSION

The results of this study indicate that prior voluntary exercise training attenuates the cardiac dysfunction and lipid peroxidation induced by acute DOX exposure. To our knowledge, these data are the first to provide direct evidence of exercise-induced protection against the acute functional manifestations of DCT. In addition, we provide novel evidence of HSP72 induction in the rat heart by chronic voluntary exercise.

Effect of DOX perfusion on LV function and lipid peroxidation. To determine the effects of DOX exposure on intrinsic cardiac function in previously sedentary or physically active

| Table 1. Effects of voluntary wheel running on animal characteristics |
|-------------------------------|------|------|------|
|                                | SED  | VEX  | \( P \) Value |
| Final body weight, g           | 266±12| 287±4| 0.12 |
| Heart weight, g                | 1.09±0.04 | 1.36±0.04 | <0.001 |
| Heart/body weight ratio, g/kg  | 4.12±0.10 | 4.72±0.11 | <0.001 |
| Soleus weight, mg/kg body wt   | 0.39±0.01 | 0.44±0.02 | 0.03 |
| Soleus citrate synthase activity, µmol/g wet wt \(^{-1}\text{min}^{-1}\) | 25.2±2.3 | 30.2±1.6 | 0.04 |

Values are presented as means ± SE. SED, sedentary (\( n = 15 \)); VEX, voluntary exercise (\( n = 13 \)).
rats, we have monitored LV pressure characteristics of isolated hearts perfused with 10-μM DOX over a 60-min period. This model provides a rapid and reproducible means of assessing the cumulative effects of continuous DOX exposure at a concentration similar to peak levels observed in plasma following single intravenous treatments in humans (7, 12). In accordance with previous studies (19, 40, 42), we observed that perfusion of hearts with 10-μM DOX results in a progressive decline in LVDP, with parallel decrements in dP/dt over a 60-min perfusion period.

The mechanisms by which DOX exposure induces cardiac dysfunction have not been fully elucidated. Prevailing hypotheses suggest that myocardial oxidative stress is a primary event in the etiology of DCT and is believed to initiate several of the deleterious cellular events reported following DOX treatment (6, 63). Oxidative destruction of fatty acids in cell and organelle membranes indicated by increased levels of lipid peroxidation products has been frequently reported following DOX exposure (26, 39, 53) and has long been considered to be a critical event in DCT (34). In accordance with these studies, we observed a significant increase in MDA + 4-HAE in the LV from sedentary animals exposed to DOX, which supports the association between lipid peroxidation and DOX-induced cardiac dysfunction.

Cardioprotective effects of voluntary wheel running. Voluntary exercise elicited significant cardiac hypertrophy and skel-

Table 2. Absolute values of left ventricular function in isolated hearts

<table>
<thead>
<tr>
<th></th>
<th>EDP, mmHg</th>
<th>ESP, mmHg</th>
<th>LVDP, mmHg</th>
<th>+dP/dt, mmHg/s</th>
<th>−dP/dt, mmHg/s</th>
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<tr>
<td>Baseline (0 min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>SED</td>
<td>2.1±0.1</td>
<td>86.6±2.8</td>
<td>84.5±3.2</td>
<td>2507±96</td>
<td>−1610±60</td>
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<td>VEX</td>
<td>1.5±0.1</td>
<td>88.4±3.7</td>
<td>86.9±2.6</td>
<td>2497±73</td>
<td>−1698±42</td>
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<tr>
<td>SED + DOX</td>
<td>2.3±0.2</td>
<td>85.4±2.9</td>
<td>83.1±6.4</td>
<td>2332±58</td>
<td>−1573±40</td>
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<tr>
<td>VEX + DOX</td>
<td>2.0±0.1</td>
<td>86.8±3.1</td>
<td>84.8±4.7</td>
<td>2445±124</td>
<td>−1627±61</td>
</tr>
<tr>
<td>30-min perfusion</td>
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<td></td>
<td></td>
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<tr>
<td>SED</td>
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<td>91.8±2.7</td>
<td>89.6±3.1</td>
<td>2566±100</td>
<td>−1826±169</td>
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<tr>
<td>VEX</td>
<td>1.5±0.1</td>
<td>89.8±5.0</td>
<td>88.3±5.2</td>
<td>2822±351</td>
<td>−1817±48</td>
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<tr>
<td>SED + DOX</td>
<td>14.6±4.1*</td>
<td>84.4±2.3</td>
<td>69.8±2.9*</td>
<td>1909±82*</td>
<td>−1272±75*</td>
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<td>7.9±1.8</td>
<td>93.0±5.0</td>
<td>85.1±4.7</td>
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<td>−1665±93</td>
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<td>60-min perfusion</td>
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<tr>
<td>SED</td>
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<td>93.0±3.6</td>
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<td>3080±518</td>
<td>−1954±62</td>
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<td>51.8±70.2</td>
<td>102.6±17.2</td>
<td>50.1±7.7</td>
<td>1896±179</td>
<td>−1481±196</td>
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Values are means ± SE. EDP, end-diastolic pressure; ESP, end-systolic pressure; LVDP, left ventricular developed pressure (ESP-EDP); +dP/dt, maximum rate of pressure development; −dP/dt, maximum rate of pressure decline; SED, sedentary (n = 6); VEX, voluntary exercise (n = 8); SED + DOX, sedentary perfused with DOX (n = 7); VEX + DOX, VEX perfused with DOX (n = 7). *Significantly different than baseline level in the same group (P < 0.05).

Fig. 2. Response of left ventricular developed pressure (A), maximum rate of left ventricular pressure development (+dP/dt) (B), and maximum rate of left ventricular pressure decline (−dP/dt) (C) during the 60-min perfusion period. SED, sedentary hearts perfused with buffer only (n = 6); VEX, voluntary exercise hearts perfused with buffer only (n = 7); SED + DOX, sedentary hearts perfused with buffer + 10 μM DOX (n = 8); and VEX, voluntary exercise hearts perfused with buffer + 10 μM DOX (n = 7). All data are expressed as mean % of baseline (0 min) values ± SE. *Significantly lower than baseline in the SED + DOX group (P < 0.05). †Significant difference between VEX + DOX and SED + DOX (P < 0.05).

Fig. 3. Left ventricular malondialdehyde and 4-hydroxy-alkenals (MDA + 4-HAE) content after the 60-min perfusion period. Data are means ± SE. Two-way ANOVA revealed a significant VEX×DOX interaction. *Significantly greater than SED and VEX (P < 0.05).
etal muscle adaptations characteristic of wheel-run female rats in previous studies (22, 61). Despite reports of favorable adaptations in cardiac muscle after voluntary exercise training (35, 59, 61), no differences in absolute parameters of LV function were observed between the SED and VEX hearts in the current study when perfused in the absence of DOX (see Table 2). However, this finding is in agreement with previous reports that exercise training does not alter baseline LV function in the isolated perfused rat heart under standard perfusion conditions, despite eliciting significant cardiac hypertrophy and cardioprotection against I/R stress and hydrogen peroxide exposure (3, 45, 55). Similarly, no alteration in myocardial lipid peroxidation was detected in the VEX vs. SED hearts after KH buffer perfusion in the current study. This finding is also in agreement with previous investigations that reported no effect of 8–10 wk of treadmill training on myocardial lipid peroxidation in unchallenged hearts, despite observing a significant training-induced attenuation of lipid peroxidation following I/R or ethanol treatment (16, 45). Collectively, these data suggest that the physiological benefits of these training-induced adaptations may be evident only in the context of myocardial stresses that exceed those encountered under normal resting conditions.

Despite the numerous reports of exercise-induced cardioprotection following treadmill exercise training, few studies have explored the cardioprotective effects of voluntary exercise training. Woodiwiss et al. (59) reported a preservation of diastolic function after 16 wk of voluntary wheel running in rats with streptozocin-induced diabetes mellitus. Friberg et al. (11) provided evidence that chronic wheel running enhances cardiac contractile function in spontaneously hypertensive rats. However, the current study is the first to examine the effects of voluntary running on the deleterious effects of DOX exposure. Our results indicate that hearts isolated from wheel-run animals resist the DOX-induced LV dysfunction and lipid peroxidation observed in the hearts of sedentary animals. Although this is the first report of an exercise-induced cardioprotection against DOX exposure, several previous investigations have demonstrated similar cardioprotection against other forms of oxidative stress after chronic exercise (2, 45, 55). However, the vast majority of these studies used a forced treadmill exercise training model rather than voluntary running. We have used a voluntary running model in an effort to isolate the effects of physical activity from the additional stress associated with forced exercise protocols (32, 48). Therefore, our results provide evidence of exercise-induced cardioprotection in the absence of the coercive stress that may accompany forced-exercise training.

Mechanisms of exercise-induced cardioprotection. Myocardial antioxidant enzymes defend the heart against the damaging effects of ROS and have been hypothesized to play an important role in exercise-induced resistance to oxidative stress (46) and in the attenuation of DOX cardiotoxicity (51). In particular, several studies have indicated that the presence of myocardial SOD is critical for the prevention of DOX cardiotoxicity (18, 49, 62). This is reasonable, as SOD removes superoxide from cells, thereby providing the first line of defense against DOX-induced oxidative stress. The activity and protein expression of myocardial SOD isoforms has been reported to be greater in the male and female rat heart following chronic (≥8 wk) forced treadmill running in some (2, 16, 44, 45), but not all studies (8, 24). To our knowledge, the current study is the first to examine myocardial SOD isoforms in the healthy rat heart after chronic voluntary wheel running. We detected no effect of voluntary exercise on the protein expression of Mn-SOD or CuZnSOD in the LV of rats in the current study, suggesting that other mechanisms must be involved in the observed cardioprotection against DOX exposure. A putative explanation for the lack of SOD increase observed in our study
and the equivocal findings of prior studies that used treadmill training may be differences in the duration and/or intensity of the selected exercise training protocols. Moran et al. (31) recently reported that although 24 wk of treadmill exercise training elicited significant increases in myocardial SOD activity, no changes were detected after 12 wk. Powers et al. (44, 45) and Brown et al. (2) reported increases in myocardial SOD activity and protein expression after high-intensity treadmill running protocols (≥30 m/min, ≥10% grade) lasting 10 to 20 wk, whereas other studies using less intense treadmill running protocols have reported no effect (8, 24). Although female rats have been reported to voluntarily achieve brief periods of high-intensity running in the wheels (>45 m/min), the running is intermittent in nature (38). Therefore, is it plausible that the voluntary wheel-running protocol used in the current study did not provide the sustained high-intensity running that may be required to elicit increases in myocardial SOD protein.

Although no changes in SOD isoforms were observed in this study, earlier investigations have indicated that an increase in myocardial SOD is not required for exercise-induced cardioprotection (8, 14, 25). Although it is possible that increases in other antioxidant enzymes (e.g., CAT and GPx) may have contributed to the cardioprotection observed in wheel-run rats, exercise has been reported to attenuate lipid peroxidation and LV dysfunction after I/R in the absence of any increase in CAT or GPx (8, 14, 25). Therefore, it appears that whereas antioxidant enzymes clearly play a role in defending the heart against ROS, an increase in their expression may not be required for the enhanced cardioprotection conferred by exercise training.

Increasing evidence suggests that myocardial HSP72 induction plays a pivotal role in the exercise-induced cardioprotection against oxidative stress (14, 46, 47, 56). HSP72 induction has been shown to attenuate apoptosis, necrosis, and oxidative injury in the myocardium following I/R (14, 47) and DOX exposure (17). Interestingly, HSP72 induction by exercise training or heat stress has attenuated myocardial lipid peroxidation and preserved cardiac function following I/R without a concomitant increase in cardiac SOD, CAT, or GPx activity (8, 14, 57). Although previous studies have reported 400–500% increases in cardiac HSP72 content after forced treadmill training (36, 47), a similar effect has not been observed after voluntary wheel running. To our knowledge, only two studies have previously examined cardiac HSP72 content after voluntary wheel running, and both reported no effect (4, 36). In contrast, we have observed a 78% higher HSP72 level in the hearts of wheel-run rats compared with the hearts of sedentary rats (P < 0.001). These discrepant findings may be explained by the fact that the two aforementioned studies that reported no effect of wheel running on cardiac HSP72 used male rats. The present study is first to examine the effect of voluntary wheel running on myocardial HSP72 protein in female rats, which were selected because of their greater propensity for voluntary exercise compared with males (60). Indeed, the females in the present study averaged 9- to 15-fold longer running distances per week than the males in the previous studies (4, 36). Therefore, a substantially greater volume of physical activity may account for the observed differences in HSP72 protein expression. However, it has been suggested that intensity, rather than duration of exercise, is the critical factor for cardiac HSP72 induction (29, 36). Milne and Noble (29) reported that cardiac HSP72 induction following a 60-min treadmill running bout occurred only when rats ran at speeds of 24 m/min or greater. The intensity (i.e., speed) and duration of wheel-running bouts were not measured in our study; however, female rats have been reported to voluntarily attain running speeds of 48–68 m/min for up to 5 min with heart rates exceeding 500 bpm (38). It is not known whether brief episodes of high-intensity running occurring intermittently over a period of several weeks provides sufficient stimulus for HSP72 induction in the heart. Further investigation is required to elucidate the mechanisms by which exercise intensity, duration, or their interaction may influence HSP72 induction in the rat heart.

Although our data suggest a potential role of cardiac HSP72 induction in the cardioprotection observed in the wheel-run rats of the present study, evidence from prior studies suggest that other mechanisms may be involved. Joyeux et al. (19) examined the effect of heat stress (42°C for 15 min) on acute DOX cardiotoxicity in the isolated rat heart, similar to the model used in the current study. They reported that although prior heat stress protected the myocardium against electrophysiological injury, it did not attenuate DOX-induced decreases in LVDP to the extent observed in our study. Although cardiac HSP72 was not determined in this study, Demirel et al. (9) reported a nearly sevenfold increase in cardiac HSP72 content after an identical heat stress protocol. Furthermore, recent evidence from Lennon et al. (25) suggests that sustained increases in HSP72 are not essential for exercise-induced protection during myocardial stunning. Therefore, it is plausible that mechanisms other than HSP72 may have contributed to the observed exercise-induced cardioprotection against DOX exposure.

Summary and Conclusions. In summary, our data provide the first direct evidence of an exercise-induced protection against the cardiac dysfunction induced by acute DOX exposure. This functional cardioprotection was associated with an attenuation of DOX-induced lipid peroxidation and higher levels of HSP72 protein in the hearts of wheel-run rats. Although these data suggest a possible role of HSP72 induction in the cardioprotection conferred by voluntary exercise, the precise mechanisms of protection remain to be elucidated. In conclusion, our results indicate that chronic physical activity provides resistance against DOX cardiotoxicity, thereby potentially improving the therapeutic value of DOX in individuals who have been previously active.

Limitations and future study. We have used an established model of acute DCT in the isolated rat heart to examine the direct effects of DOX exposure on intrinsic cardiac function in previously sedentary or physically active rats. Although our data clearly indicate that prior physical activity is associated with a reduction in lipid peroxidation and preservation of cardiac function during acute DOX exposure, the protective effect of exercise training against chronic DOX treatment in vivo cannot be determined by our experiments. As previously mentioned, clinical presentation of DOX cardiomyopathy typically occurs after completion of several intravenous DOX injections over a period of several months. Therefore, further investigation is needed to determine the effect of exercise training before, during, or after chronic DOX treatment in vivo to fully explore the potential benefits of physical activity on the acute and chronic cardiotoxicity of DOX treatment.
ACKNOWLEDGMENTS

We wish to acknowledge Russell Moore, David Brown, Korinne Jew, and Micah Johnson at the University of Colorado, Boulder, for their technical advice and assistance with Western blotting experiments.

GRANTS

This study was supported by grants from the American Cancer Society and the University of Northern Colorado Sponsored Programs and Academic Research Center to R. Hayward.

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