Activation of JNK in rat heart by exercise: effect of training

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Submitted 24 June 2003; accepted in final form 15 August 2003

Boluyt, M. O., A. M. Loyd, M. H. Roth, M. J. Randall, and E. Y. M. Song. Activation of JNK in rat heart by exercise: effect of training. Am J Physiol Heart Circ Physiol 285: H2639–H2647, 2003. First published August 21, 2003; 10.1152/ajpheart.00596.2003.—The purpose of the study was to determine whether exercise would activate JNK in the heart and whether chronic exercise training would alter the response. Untrained rats were familiarized with the treadmill and assigned to one of four groups: low intensity (LI), 10 min, 0%, 15 m/min; medium intensity (MI), 10 min, 0%, 33 m/min; high intensity (HI), 10 min, 25%, 33 m/min; long duration (LD), 30 min, 0%, 15 m/min. Another cohort of rats was subjected to a progressive 6 wk high-intensity training protocol that produced a 12% increase in heart mass. In untrained rats, JNK activity was LI: 1.5 (fold nonrun control), MI: 2.0, HI: 2.5, LD: 1.25 immediately after a single bout of exercise. In trained rats, no activation of JNK above baseline was detected after either a 10-min or 1-h bout of exercise. We concluded that treadmill exercise activates JNK in the rat heart in an intensity-dependent manner and that chronic training abrogates the myocardial JNK response to a bout of exercise.

Dose dependent; treadmill training; exercise intensity; cardiac hypertrophy

Exercise perturbs cardiac homeostasis, producing an immediate enhancement of cardiac function. Adjustments to an acute bout of exercise include increases in heart rate and stroke volume, resulting in augmented cardiac output (19, 20). Chronic exercise training leads to a constellation of long-term positive adaptations in the heart. The beneficial adaptations include a resting and submaximal bradycardia, increased maximal stroke volume, cardiac hypertrophy, resistance to ischemia-reperfusion damage, and improved contractile function (19, 36). Epidemiologic studies have ascribed substantial health benefits to regular physical activity, primarily in decreasing the risk of cardiovascular diseases (24). The short-term and long-term cardiac responses to exercise have been described extensively and comprehensively in humans and in many other mammalian species (19, 23, 26, 30, 31). However, our understanding of the molecular mechanisms that regulate short-term and long-term adaptations of the heart to exercise in the heart is incomplete.

The JNK comprise a family of intracellular signaling molecules that are activated in response to cellular stresses (9, 37). JNK was initially referred to as SAPK reflecting the early impression that JNK and another related family member, p38, functioned primarily in response to cellular stress events, such as DNA damage and membrane disruption. It is now obvious that although JNK and p38 kinases exhibit some overlap, there are also distinctions in their upstream activation pathways and their downstream targets (9, 37). JNK is activated by phosphorylation of threonine and tyrosine residues by the SEK1/MKK4 and MKK7 kinases. Activation of JNK leads to phosphorylation of the DNA binding proteins c-Jun, JunB, JunD, ATF2, and Elk1 (37). These DNA binding proteins bind the upstream regulatory regions of a variety of genes leading to an increase in transcriptional activity. For example, one of the targets of JNK phosphorylation, c-Jun, is itself a target of a heterodimeric complex of c-Jun and ATF2 (37). Thus activation of JNK would be expected to lead to phosphorylation of c-Jun and ATF2 proteins present in the cell, which would subsequently bind to target DNA sequences and increase the levels of c-Jun mRNA and protein.

JNK has been implicated as an important transducer of the mechanical perturbations that lead to alterations in gene expression and phenotype in skeletal muscle. Contractile activity and stretch both activate JNK in skeletal muscle, as well as downstream immediate-early genes (1, 6, 7). Eccentric (lengthening) contractile activity is the most potent stimulus for the activation of JNK in skeletal muscle in vivo (5). The work by Martineau and Gardiner (15, 16) demonstrated that the pronounced effect of eccentric contractile activity on JNK responsiveness in skeletal muscle is due to the greater contractile forces elicited. The strong relationship between JNK activity and contractile force in skeletal muscle coupled with its apparent activation of downstream immediate-early genes, such as c-jun, makes it a very attractive candidate for a causal factor in the long-term adaptations of muscle tissue to exercise.

Cardiac muscle is the ultimate endurance-trained form of striated muscle, featuring an abundance of...
mitochondria that exceeds the most endurance-trained skeletal muscle by approximately threefold (17). Although JNK is activated by exercise in skeletal muscle, it does not necessarily follow that the much more aerobic cardiac muscle would respond to exercise by activating JNK. There are, however, other findings that lend credence to the hypothesis that exercise would likely activate JNK in the heart. JNK is activated in cardiac muscle tissue by angiotensin II infusion (43), and by pressure overload hypertrophy (10). Mechanical stretch of isolated cardiac myocytes was also sufficient to activate JNK (13, 32), but stretch failed to activate JNK in intact isolated hearts (38). These findings suggest that common mechanotransducers may work in skeletal and cardiac muscle to activate JNK but that there may be important differences as well. On the basis of the available information, it was hypothesized that exercise-induced changes in hemodynamics that lead to increased venous return and active stretch of the myocardial wall would activate JNK in a dose-dependent manner. Angiotensin II levels also rise markedly during exercise (41), providing another potential stimulus for exercise-induced activation of JNK.

Although it is clear that JNK is closely related to mechanical aspects of striated muscle, there have been no studies to determine whether the hemodynamic changes that accompany physical exercise are sufficient to activate JNK in the heart. This is an important question because of the links that have been made between JNK activity and apoptosis, cardioprotection, and other cellular functions that bear on cardiovascular performance. The purpose of the present investigation was to first determine whether a single bout of exercise is sufficient to activate JNK in the rat heart. A second aim was to investigate the effects of exercise training on the response of JNK to an acute bout of exercise. Data presented here demonstrate an intensity-dependent activation of JNK in rat heart after a single bout of treadmill running. Chronic exercise training on a treadmill abolished the responsiveness of cardiac JNK to a single acute bout of exercise.

**MATERIALS AND METHODS**

**Materials.** The primary antibody directed against p46 and p54 JNK (C-17) was from Santa Cruz. Glutathione S-transferase (GST)-c-Jun fusion protein substrate and the phospho-specific antibodies directed at JNK (Thr183/Tyr185) and SEK1 (Thr261) were from Cell Signaling. Radiolabeled [32P]-dATP and kits for enhanced chemiluminescence were from Amersham. Polyvinylidene difluoride membrane was from Millipore. Protein A was from Sigma. Bicinchoninic acid and FluoroBlot Peroxidase Substrate reagents were from Pierce.

**Animal care.** Female Wistar rats were obtained from Charles River Laboratories. All animal protocols were approved by the University of Michigan’s Committee on the Use and Care of Animals. Principles embodied in the declaration of Helsinki were adhered to and all animal protocols conformed to the “Guiding Principles for Research Involving Animals.” The rats were housed 2–3 per cage and fed a standard laboratory diet (5001, PMI Nutrition International) and water ad libitum. Rats were maintained on a reverse 12:12-h light-dark cycle, such that training took place during the dark cycle.

**Treadmill running of untrained rats.** Treadmill running was conducted on a 10-lane motorized treadmill equipped with a shock grid at the rear of the belt (Quinton Instruments). Untrained rats were familiarized with the treadmill by running at 15 m/min for 5 min/day for 5 days, followed by at least 3 days of no contact with the treadmill. On the experimental day, rats were run at the assigned intensity and duration and euthanized by decapitation either immediately after exercise or at the designated time after exercise. Rats that were not euthanized immediately after exercise were placed back in their cages until the designated time. Rats were subjected to one of four running protocols that varied in intensity and duration (Table 1). Rats in the sedentary nonrun (NR) group were placed on a stationary treadmill for 10 min and euthanized immediately thereafter.

**Treadmill training.** Rats assigned to the chronic training groups were trained 5 days per wk using a protocol that progressively increased the duration and intensity. The protocol was designed based on previous work (2) that demonstrated cardiac hypertrophy with an interval training protocol that alternated submaximal running with a progressive sprint regimen. After 6 wk of training, the rats were running at least 60 min per day (Table 2). The progressive increments in speed allowed for individual variation among rats, while ensuring that they were training at or near their maximal aerobic capacity. That is, some rats completed all five sprints and achieved 66 m/min while some failed to complete each of the 66 m/min final sprints that occurred six times in the 60-min protocol. Estimates of their relative workloads (see Tables 1 and 2) at each stage in the protocol were made by creating regression equations based on previously published measurements of O2 uptake at various workloads in trained and untrained rats (22, 33, 34, 42).

**JNK activity.** Activation of JNK was assessed by two methods. The first was by immunoblotting with a phospho-specific antibody directed at p46 and p54 JNK. The second was an in vitro assay using immunoprecipitated JNK and a GST-c-Jun fusion protein as substrate. The immunoprecipitated JNK pellets were incubated in a microfuge tube with 10 microliters containing 2 μg of GST-c-Jun and 7.5 μl of a 1:10 mixture of 10 mCi/ml γ-[32P]ATP, 50 mM Mg, and 100 μM cold ATP. The tube was incubated at 30°C for 20 min with brief vortex mixing every 2 min. The reaction was stopped by the addition of a 4× Laemmli sample buffer. Ten microliters of the supernatant were loaded on a 10% polyacrylamide gel and size fractionated. The gel was dried and the dried gel was placed in a cassette with a phosphoscreen. The signals were quantitated with a Personal Phosphoimager FX (Bio-Rad) and QuantityOne software (Bio-Rad).

<table>
<thead>
<tr>
<th>Group</th>
<th>Speed, m/min</th>
<th>Grade, %</th>
<th>Duration, min</th>
<th>Estimated % VO2max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sedentary</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>NA</td>
</tr>
<tr>
<td>Low intensity</td>
<td>15</td>
<td>0</td>
<td>10</td>
<td>61</td>
</tr>
<tr>
<td>Medium intensity</td>
<td>33</td>
<td>0</td>
<td>10</td>
<td>94</td>
</tr>
<tr>
<td>High intensity</td>
<td>33</td>
<td>25</td>
<td>10</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Long duration</td>
<td>15</td>
<td>0</td>
<td>10</td>
<td>61</td>
</tr>
</tbody>
</table>

Estimates of the relative workloads at each stage in the protocol were made by creating regression equations based on previously published measurements of oxygen uptake at various workloads in trained and untrained rats (22, 33, 34, 42). NA, not applicable; VO2max, maximum rate of O2 consumption.
**Table 2. Summary of 6-wk exercise training protocol**

<table>
<thead>
<tr>
<th>Week</th>
<th>Final Duration, min</th>
<th>Speed</th>
<th>Estimated Training Intensity, %V̇O₂max</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15</td>
<td>Warmup: 2 min @ 15 m/min</td>
<td>61</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>5 min @ 24 m/min alternated with 5 min of sprints:</td>
<td>77</td>
</tr>
<tr>
<td></td>
<td></td>
<td>a) 3 min @ 33 m/min</td>
<td>&gt;100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b) 1 min @ 39 m/min</td>
<td>&gt;100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>c) 1 min @ 45 m/min</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cooldown: 2 min @ 15 m/min</td>
<td>61</td>
</tr>
<tr>
<td>4</td>
<td>40</td>
<td>Warmup: 2 min @ 15 m/min</td>
<td>94</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
<td>5 min @ 33 m/min alternated with 5 min of sprints:</td>
<td>&gt;100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>a) 1 min @ 39 m/min</td>
<td>&gt;100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b) 1 min @ 45 m/min</td>
<td>&gt;100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>c) 1 min @ 53 m/min</td>
<td>&gt;100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>d) 1 min @ 59 m/min</td>
<td>&gt;100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>e) 1 min @ 66 m/min</td>
<td>&gt;100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cooldown: 2 min @ 15 m/min</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>

Estimates of the workloads at each stage in the protocol were made by creating regression equations based on previously published measurements of oxygen uptake at various workloads in trained and untrained rats (22, 33, 34, 42).

**Immunoprecipitation.** To obtain enriched precipitates of JNK from heart homogenates, 200 µg of protein were incubated with 5 µl of the stock primary antibody directed at p46 and p54 JNK in RIPA buffer (10 mM Tris, pH 7.20, 150 mM NaCl, 1% deoxycholic acid, 1% Triton X-100, and 0.1% SDS) in a shaker/rotator at 4°C for 2 h. Protein A agarose (25 µl) was added, and shaking was continued for another 30 min. After a 5-min centrifugation at 1,000 g and 4°C, the supernatant was discarded. The pellet was washed three times with the buffer used for homogenization, three times with LiCl wash buffer (0.5 M LiCl, 100 mM Tris·HCl, pH 7.6, 0.1% Triton X-100, 1 mM DTT), and three times with Final Wash Buffer (20 mM Tris, pH 7.2, 2 mM EGTA, 10 mM MgCl₂, 1 mM DTT, 0.1% Triton X-100).

**Western blotting.** Immunoblotting was carried out as described previously with minor modifications (4). Heart samples were homogenized in buffer (62.5 mM Tris, 10 µg/ml Aprotinin, 10 µg/ml leupeptin, 1 mM PMSF, 1 mM orthovanadate, and 10% Triton X-100) for ~45 s with a polytron. Samples were then subjected to centrifugation for 5 min at 5,000 g. The supernatant was used for subsequent studies after the protein concentration was determined using the bichinchoninic acid reagent with BSA as a standard.

To determine levels of JNK protein, 10 µg of LV homogenate were resolved in 10%, 0.75 mm SDS-polyacrylamide gels and transferred to nitrocellulose membranes, and blocked with phosphate-buffered saline containing 1% BSA and 0.1% Tween 20 for 1 h at room temperature (ImunoBlok). The membranes were washed three times for 5 min each in Western buffer (50 mM NaCl, 10 mM Tris, pH 7.0, 1 mM EDTA, 0.1% Tween 20) and then incubated for 1 h in a 1:10,000 dilution of primary anti-JNK antibody at room temperature. After being washed, membranes were incubated in a 1:10,000 dilution of anti-rabbit secondary antibody conjugated with horseradish peroxidase for 1 h at room temperature. Detection was carried out with the use of enhanced chemiluminescence. The membranes were subsequently stained with Coomassie blue to ensure equivalent loading of protein.

For the phosphospecific antibody experiments, 12% SDS-polyacrylamide gels were used. Membranes were incubated in ImmunoBlok for 1 h. Membranes were washed three times for 5 min with 1× Western buffer. Respective membranes were incubated for 1 h in a 1:1,000 dilution of phosho-SAPK/JNK or a 1:2,000 dilution of phosho-SEK1/MKK4 primary antibodies. After incubation, membranes were washed three times for 5 min with 1× Western buffer and incubated in the anti-rabbit secondary antibody at a 1:20,000 dilution. The membranes were then washed three times for 5 min with 1× Western buffer, dried on plastic wrap, and stained with Fluoroblot Substrate Solution according to the manufacturer’s procedure. Detection was carried out using the enhanced chemiluminescence mode in a Fluor-S multimager (Bio-Rad) and quantified with Quantity One software (Bio-Rad).

**RNA blotting.** RNA blotting was performed as described previously with modifications (3). RNA was isolated from left and right ventricles by the method of Chomczynski and Sacchi (8). Ten micrograms of total RNA were size fractionated by electrophoresis through 1% agarose gels, transferred to nitrocellulose membranes, and hybridized with [3P]-radiolabeled probes overnight at 68°C for cDNA probes and 42°C for oligonucleotide probes using PerfectHyb Plus (Sigma). Hybridization intensity was quantified with a Personal Phosphoimager FX (Bio-Rad). Signals visualized on computer screen were identified by position relative to 18S and 28S rRNA migration, delineated by rectangles, and quantified after background subtraction. Each blot was subsequently stripped and reprobed. The signal from each sample was normalized to the signal obtained with an oligonucleotide specific for the 3’ untranslated region of GAPDH and an oligonucleotide probe specific for the 18S rRNA. Complementary DNA probes were synthesized from a template by the random primed method (Promega). The template for the c-Jun probe was a full-length cDNA obtained from American Type Culture Collections (28).

**Statistics.** Values are expressed as means ± SE. Comparisons between various running intensity protocols were made with one-way or two-factor ANOVA and least-significant difference post hoc comparisons (Systat). Comparisons of sedentary and trained groups were made with an independent samples two-tailed *t*-test.

**RESULTS**

**JNK is activated in rat heart by treadmill exercise.** To determine whether treadmill running would activate JNK in the heart, untrained rats were first familiarized with the treadmill and then subjected to a single bout of exercise and euthanized at the end of the bout. JNK activity was initially assessed using a phos-
phosphorylated with a phospecifíc antibody directed at the 46 and 54 kDa isoforms of JNK. Immediately after the 10-min bout of exercise, phosphorylation of the p46 and p54 isoforms of JNK were significantly elevated (Fig. 1). The detection of phospho-JNK suggests that it is activated in the rat heart by treadmill exercise.

To extend this finding and to examine the effect of exercise intensity on the activation of JNK, an in vitro activity assay was employed. Serum-activated 3T3 cells (21) were used as a positive control (Fig. 2A). The running intensities used are summarized in Table 1. JNK was activated in the rat heart by treadmill running at moderate (MI) and high intensity (HI), but not at low intensity (LI) for 10-min or 30-min long duration (LD) (Fig. 2, B and C). The data provide compelling support for the conclusion that JNK is activated in the rat heart by treadmill exercise. Moreover, the data indicate that the response of JNK activation to treadmill running is intensity dependent.

To determine whether the treadmill running protocols of different intensity elicited different patterns in the time course of JNK activation, rats were euthanized at various times after a single bout of treadmill exercise. For each of the 10-min exercise bouts (MI or HI), JNK activation was most pronounced immediately after exercise and declined thereafter (Fig. 3). For the LI 30-min bout of exercise (LD), a modest activation of JNK was observed with a delayed time course. These differences in activation patterns suggest that a latent effect with a potentially different mechanism may be active in the longer duration exercise bout.

**Effect of treadmill exercise on phosphorylation of SEK1.** SEK1 is the kinase immediately upstream of JNK that phosphorylates and activates JNK. To determine whether it was activated, a phospecific antibody directed at SEK1 was employed. Although a pattern emerged that suggested an intensity-dependent activation of SEK1 by treadmill running, none of the changes were significant (Fig. 4). This may reflect the
downstream amplification that often occurs in signaling pathways.

Exercise-induced activation of JNK is followed by increased mRNA levels of c-Jun. The immediate early gene c-Jun is both a downstream gene target of JNK and the substrate for phosphorylation by JNK. To determine whether a single bout of exercise capable of activating JNK would result in upregulation of c-Jun mRNA, rats were subjected to a single bout of exercise and euthanized at various times after the exercise bout. Northern blot analysis revealed a twofold increase in the abundance of c-Jun mRNA that peaked at 30-min postexercise (Fig. 5). Thus the postexercise activation of JNK and the increase in c-Jun mRNA appear to be temporally related.

JNK is not activated by treadmill exercise in hearts of trained rats. To determine whether the cardiac activation of JNK in response to an acute bout of exercise would be augmented or attenuated by exercise training, rats were trained by running (Table 2). Six weeks of treadmill running resulted in significant increases in the dry mass of both heart ventricles (Table 3). Trained rats were then subjected to a single 10-min bout of treadmill running at 0% grade that consisted of 5 min at a speed of 33 m/min and 5 min of progressively faster sprints, culminating in a final minute at a speed of 66 m/min. When hearts were harvested immediately after the exercise bout, JNK activity was not different from baseline JNK activity levels in untrained, cagesedentary rats (Fig. 6). To determine whether the
trained hearts exhibited activation of JNK at a later time point or after a longer bout of exercise, rats were euthanized at various times after a 1-h bout of high-intensity treadmill exercise (Fig. 7). No evidence of exercise-induced JNK activation was observed. Collectively, these data strongly suggest that chronic treadmill exercise training desensitizes the JNK response to treadmill running in rats.

Exercise training does not alter relative abundance of JNK protein in rat heart. Because there is a basal level of JNK activity in nonexercised rats, the abundance of JNK protein is an important factor that can influence the net activity of JNK per gram of tissue. To determine whether training altered the levels of JNK proteins in the heart, immunoblot experiments were conducted. The abundance of both p46 and p54 JNK was similar in hearts of sedentary and trained rats (Fig. 8). This is consistent with the general understanding that signaling kinases are regulated primarily as “on/off switches” and not by fluctuations in their abundance.

Thus the exercise-trained heart would not be expected to have an increased capacity for JNK activation compared with its sedentary counterpart.

DISCUSSION

This study demonstrated that a single 10-min bout of treadmill exercise was sufficient to activate JNK in the left ventricle of the rat heart. The activation of JNK in the rat heart caused by running was directly related to the intensity of the exercise as dictated by speed and grade of the treadmill. The activation of JNK was temporally related to an increase in c-Jun mRNA, a likely target of JNK signaling, and an immediate-early gene thought to alter expression of genes that appear later in the growth response, such as atrial natriuretic factor. This is the first demonstration that physical activity-induced changes in hemodynamic load can activate the mechano- and stress-transducing JNK signaling pathway in the rat heart. The activation of JNK by acute exercise was not observed in trained rats, even though the trained rats were subjected to a bout of running at an intensity that exceeded their maximal aerobic capacity. The data suggest that JNK signaling after an acute bout of exercise may contribute to the acute cardioprotective effects of exercise as well as the adaptive response of the heart to chronic exercise.

Mechanism of activation of JNK in heart by exercise

The molecular mechanisms by which the complex stimulus of whole body exercise activates JNK in the heart are not known but are likely to involve many of the

Table 3. Heart weight and body weight of sedentary and treadmill-trained female Wistar rats

<table>
<thead>
<tr>
<th></th>
<th>Sedentary</th>
<th>Trained</th>
<th>Change</th>
<th>P Value (t-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final BW, g</td>
<td>291 ± 10</td>
<td>290 ± 3</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>LVW, mg</td>
<td>559 ± 24</td>
<td>623 ± 13</td>
<td>+12</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>RVW, mg</td>
<td>161 ± 8</td>
<td>177 ± 6</td>
<td>+10</td>
<td>0.0547</td>
</tr>
<tr>
<td>LVW/BW, mg/g</td>
<td>1.92 ± 0.64</td>
<td>2.15 ± 0.04</td>
<td>+12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RVW/BW, mg/kg</td>
<td>554 ± 19</td>
<td>610 ± 19</td>
<td>+10</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>LVDW/BW, mg/kg</td>
<td>437 ± 9</td>
<td>482 ± 12</td>
<td>+10</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Values are means ± SE. BW, body weight; LVW, left ventricular weight; RVW, right ventricular weight; LVDW, LV dry weight; NS, not significant.
stimuli associated with exercise that have been studied in isolation, such as agonist binding to α- and β-adrenergic receptors and other G protein-coupled receptors, generation of reactive oxygen species, and mechanical stretch (13, 27, 37). The precise contribution of each of these likely mechanisms will require cardiac-specific loss-of-function studies in intact transgenic animals targeted at upstream candidate JNK pathway members. Much additional work will be required to determine the intermediate steps that relate tension and other mechanical, hormonal, and possibly immune signals to the activation of JNK in the heart and to define the set of downstream genes influenced by JNK in the context of the intermittent stress imposed by exercise. It will also be important to determine whether JNK activation is required for any of the long-term adaptations that occur in the exercise-trained heart.

**Downstream effects of JNK.** Although the downstream effects of JNK phosphorylation are not completely understood, a growing body of knowledge indicates that JNK is mechanistically involved in such diverse processes as embryonic morphogenesis, cell proliferation, apoptosis, hypertrophic growth, and in normal functioning of differentiated cells (9, 35, 40). Many of these effects have been attributed to the action of JNK to activate transcription factors that then bind to the DNA of target genes (9, 37). JNK phosphorylates several transcription factors, including c-Jun, JunB, JunD, ATF2, and Elk1 (37), and is a key regulator of the AP-1 transcription complex. JNK is also required for caspase-9 activation in the mitochondrial apoptosis cascade (9). How JNK manages to integrate multiple signals, initiate both pro- and antisurvival responses, and differentiate a physiological stimulus from a pathological stimulus is the intermittent nature of exercise versus the chronic nature of disease. The transient activation of JNK by a daily bout of exercise may alter the balance between signaling pathways and feedback loops to yield the net positive effects evoked by regular exercise training. Delineating these processes will require detailed knowledge of the signaling entities that interact with the JNK pathway and molecular gain- and loss-of-function approaches that dissect each aspect of JNK signaling in an intact animal model.

**JNK as “dose-dependent” mechanotransducer.** Several lines of evidence suggest that JNK is tightly coupled to mechanical perturbation of the cell. Stretching of cardiac myocytes in culture activates JNK at a level proportional to the stretch. Interestingly, the activation of JNK by stretch in cardiac myocytes does not seem to be dependent on secreted angiotensin II (13). Martineau and Gardiner (15, 16) have used a skeletal muscle system to demonstrate elegantly and compellingly that JNK activation is most closely related to peak force generation and the tension time integral. Of note is the observation that eccentric (or lengthening) contractions elicited the greatest force and evoked the greatest levels of JNK activation. The present findings are consistent with the hypothesis that force generation is a key factor regulating JNK, in particular because exercise increases the venous return to the heart, invoking an augmented passive stretch on the heart in diastole and eliciting greater force in systole via the Frank-Starling mechanism. Confirming the causal relationship suggested by these findings, however, will require a creative approach that is capable of isolating force generation from other variables.

**Potential role of JNK activation in exercise-induced cardioprotection.** An acute bout of exercise induces cardioprotective effects against ischemia-reperfusion injury that are mediated in part by activation of heat shock proteins (HSPs) by heat shock factor-1, a transcription factor that binds to the heat shock elements in the promoters of heat shock genes (36). The cardio-

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**Fig. 8.** Abundance of p46 and p54 JNK proteins in hearts of trained and untrained rats. A: levels of JNK proteins were measured by immunoblotting with an antibody that detected p46 and p54 JNK proteins regardless of phosphorylation state. B and C: graphs of mean data for p46 and p54 JNK, respectively. Values are means ± SE for hearts of 13 sedentary (SED) and 12 TRN rats per group. No significant differences were detected between the groups (independent samples two-tailed t-test).
protective effects conferred by heat shock proteins are intensity dependent, i.e., a greater degree of cardioprotection is conferred by a more intense exercise stimulus (18). Activation of JNK may play a role in the heat shock-mediated cardioprotective response by virtue of its phosphorylation of heat shock factor-1 (25). Indeed, the cardioprotective effects of prior heat shock in mouse hearts appear to require activation of JNK (39). In mice subjected to pressure overload, JNK activation appears to be required to protect against apoptosis, inflammation, and for a robust cytokine response (29).

In an in vivo rat model of ischemia-reperfusion, the activation of cytosolic JNK correlated strongly with cardioprotection (11). The observation that cardiac JNK activation by treadmill running exhibits an intensity-dependent response in the heart to acute exercise correlates with the intensity-dependent nature of cardioprotection. It will be important to determine whether JNK activation is required for the cardioprotective response to an acute bout of exercise.

Effects of training on response of JNK to single bout of exercise. The loss of JNK activation by an acute bout of exercise in the trained state might be termed “desensitization” of the JNK pathway to exercise. This is not surprising in light of the fact that the exercise-trained heart is less responsive to a number of stimuli, including the chronotropic action of adrenergic agonists, but the mechanism on JNK abatement is not readily apparent. One potential explanation for the loss of JNK responsiveness may relate to the cardioprotective heat shock response that is invoked by acute exercise. Exercise training increases the accumulation of HSP-70 in the heart (11). JNK may be suppressed by training-induced increases in levels of HSP-70, because the heat shock response is reported to suppress JNK (14). Similarly, other feedback loops may also exist that act to suppress the activation of JNK. Alternatively, there may be an exercise-induced attrition of upstream stimuli that activate JNK, such as desensitization of a G protein-coupled receptor.

Limitations of study. To address concerns that the activation of JNK was primarily a response to stress associated with encountering the shock grid and other aspects of treadmill running rather than exercise per se, three steps were taken. First, the rats were familiarized with the treadmill for 1 wk before the experimental exercise bout. Second, nonrun control rats were placed on the nonmoving treadmill for 10 min before harvest. Third, different intensities of exercise were used that were arguably more different with respect to exercise intensity than with respect to stress. It should be noted, however, that none of these steps completely eliminates the possibility that treadmill-associated stresses independent of the exercise-associated changes in hemodynamics played a role in the activation of JNK. This must be considered a limitation of the study. Another limitation of the study is that JNK was studied only in left ventricular homogenates. The activation pattern and the downstream consequences of JNK stimulation are likely to be quite different in the various cell types that are present in ventricular tissue. Future studies could employ histological and immunocytochemical methods to add this dimension to our knowledge about the effects of exercise on JNK activation in the heart.

In summary, the data presented herein are the first to show that treadmill exercise is sufficient to activate the JNK signaling pathway in the rat heart. Moreover, the magnitude of the activation of JNK in the heart is dependent on the intensity of the exercise bout. The response of cardiac JNK to an acute bout of exercise is abolished in highly trained rats. Together, these data suggest that the JNK pathway may be an important point of regulation in the acute cardioprotective cascade stimulated by acute exercise, as well as in the adaptive response of the heart to regular exercise.

The authors are grateful to Antony F. Scalia, Georgina Cirrincione, Sarah Kostrzeba, and Paul Kolar for help with technical aspects of the project, and to Mary Capriotti for help in preparing the manuscript.

DISCLOSURES

This study was supported by the American Heart Association (Midwest Affiliate).

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