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Isoproterenol potentiates exercise–induction of Hsp70 in cardiac and skeletal muscle

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Abstract The response to exercise stress is characterized by an increase in circulating catecholamines and rapid synthesis of the inducible member of the 70 kDa family of heat shock proteins (Hsp70). Cell culture studies indicate that Hsp70 expression is influenced by β-adrenergic receptor intermediates including cyclic AMP (cAMP) and cAMP dependent protein kinase (PKA). Thus, in the present investigation, the effect of a β-adrenergic agonist, isoproterenol (ISO; 10 mg/kg) and a β-adrenergic antagonist, nadolol (NAD; 25 mg/kg), on the in vivo expression of Hsp70 in rodent cardiac and skeletal muscle following moderate (MOD; 17 m/min) and exhaustive (EXH; 30 m/min) exercise was examined. While ISO alone did not induce Hsp70 synthesis, ISO treatment potentiated Hsp70 expression following MOD in the white vastus and heart (395 ± 29 and 483 ± 29% greater than control respectively, $P < 0.05$). Furthermore, this effect was reversed with combined β-adrenergic agonist and antagonist treatment (ISO+NAD) indicating that the isoproterenol induced increase in post-exercise Hsp70 expression was mediated via β-adrenergic receptor activity. However, there were no differences in Hsp70 levels among treatment groups following EXH. The failure of NAD to attenuate Hsp70 accumulation following EXH suggests that β-adrenergic receptor activity is not the main signal in the induction of Hsp70 following exercise. Hsp70 induction was dependent on exercise intensity and ISO administration prior to MOD resulted in Hsp70 levels similar to those observed following EXH. The results from the present investigation indicate that β-adrenergic receptor stimulation does not induce Hsp70 synthesis per se, but may be one factor involved in the complex regulation of the stress response to exercise in vivo.

INTRODUCTION

The increase in circulating catecholamines in response to stress results in stimulation of β-adrenergic receptor activity and consequently, increased intracellular concentrations of cyclic AMP (cAMP; Hoffman and Lefkowitz 1990). Certain forms of stress have been shown to produce rapid synthesis of the highly conserved, inducible member of the 70 kDa family of heat shock proteins (Hsp70; Udelsman et al. 1993, Locke et al. 1995). Since heat treatment (Kiang et al. 1991) and exercise (Goldfarb et al. 1986; Goldfarb et al. 1989) result in increased intracellular cAMP concentrations and since both perturbations have also been shown to induce Hsp70 synthesis (Flanagan et al. 1995; Kelly et al. 1996; Locke et al. 1990), cAMP may be involved in mediating Hsp70 induction.

Direct evidence for the involvement of cAMP on the expression of Hsp70 comes from in vitro studies. Choi et al. (1991), stimulated *hsp70* promoter-linked reporter gene activity through cAMP-elevating agents. The investigators (Choi et al. 1991) proposed that the ATF consensus sequence, (–37)GTGACGA(–31), within the promoter of the heat shock transcriptional unit, conferred this responsiveness to cAMP. However, evidence for the involvement of β-adrenergic receptor activity on the induction of Hsp70 in vivo remains equivocal (Udelsman et al. 1994b; Inaguma et al. 1995; Murphy et al. 1996; Hastie et al. 1997).

We have previously demonstrated Hsp synthesis following a single bout of treadmill running (Locke et al. 1990). Given the involvement of β-adrenergic receptor activity in the physiological response to exercise and given the evidence for β-adrenergic receptor mediation of Hsp70 synthesis in vitro we examined the effects of the β-adrenergic

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agonist isoproterenol (ISO), and the β-adrenergic antagonist nadolol (NAD) on cardiac and skeletal muscle Hsp70 expression in vivo following an acute bout of exercise.

MATERIALS AND METHODS

Animals and exercise

The study was approved by the University of Western Ontario Committee on Animal Care and was performed in accordance with the guiding principles of the Canadian Council on Animal Care. Male Sprague-Dawley rats (Charles River, Quebec; 250–300 g), were housed 3–4 per cage in an environmentally controlled room with a 12:12-h dark–light cycle, and fed and watered *ad libitum*.

Animals were randomly assigned to either a control or to one of two exercise groups. The moderate exercise (MOD) group performed treadmill running at 17 m/min on 0% grade for 60 min and the exhaustive exercise (EXH) group ran at 30 m/min on 0% grade until exhaustion. These two exercise protocols were employed in order to minimally and maximally activate the stress response to exercise (Skidmore et al. 1995). Animals in each of the experimental groups were further subdivided into sham, isoproterenol (ISO) and nadolol (NAD) treatment groups. A combined treatment group (ISO+NAD) was subjected to MOD in an attempt to suppress the enhanced expression of Hsp70 observed in isoproterenol treated rodents following MOD. The non-specific β-adrenergic agents ISO and NAD were chosen in order to manipulate β_1 and β_2 adrenergic receptor activity in cardiac and skeletal muscle respectively (Elfellah and Reid 1987; Hoffman and Lefkowitz 1990).

Animals in the EXH groups were familiarized to a motorized treadmill with two, 10 min sessions of walking and moderate running conducted a minimum of 3 days prior to the experimental run. We have determined that this familiarization protocol does not result in increased Hsp70 expression (unpublished observations). Exhaustion was defined as an inability to continue running despite mild electrical stimulation. All rats were run between 08:00 and 11:00.

Injections

Animals were administered either 10 mg/kg body weight isoproterenol hydrochloride crystalline (ISO; Sigma Chemical; I 6504), 25 mg/kg body weight nadolol (NAD; Sigma Chemical; N 1892), 10 mg/kg body weight isoproterenol and 25 mg/kg body weight nadolol (ISO+NAD) in 155 mM NaCl and 2.5 mM ascorbate, or sham treatment of 155 mM NaCl and 2.5 mM ascorbate alone (Sham). Injections were made subcutaneously, above the right hindlimb, 30 min prior to the acute exercise bout.

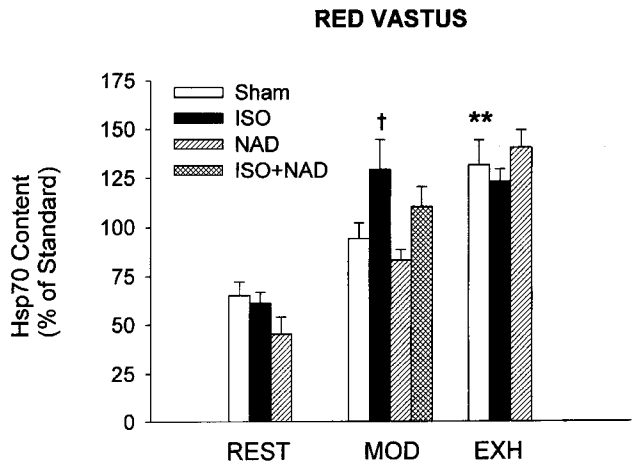

Fig. 1 Hsp70 content of red vastus as determined by immunoblotting with anti-Hsp70. Data are percentages of standard (40 μg male Sprague-Dawley soleus; means ± SE). Gels were loaded with 30 μg of red vastus homogenates. Empty bars are sham treated animals (sham), solid bars are isoproterenol treated (ISO), striped bars are nadolol treated (NAD) and cross-hatched bars are combined isoproterenol and nadolol treated. Bars are grouped according to rest, MOD and EXH as indicated. †ISO treated rodents demonstrate significantly greater Hsp70 than NAD treated animals. **Sham-EXH animals had significantly greater Hsp70 levels compared with Sham-MOD at $P<0.05$ ($n = 6–9$ per group).

Sacrifice and tissue analysis

Twenty-four hours following completion of the experimental sessions, animals were anaesthetized via intraperitoneal injection of Somnotol (60 mg/kg body weight). The red portion of the vastus lateralis (red vastus) composed of primarily fast-twitch oxidative glycolytic fibers, white portion of the vastus lateralis (white vastus) composed of primarily fast-twitch glycolytic fibers (Armstrong and Phelps 1984), and heart were quickly removed, frozen in liquid nitrogen and stored at −80°C. Tissue samples were homogenized in 20 vol of 600 mM NaCl and 15 mM Tris(hydroxymethyl)aminomethane, pH 7.5. Protein concentrations were determined using the technique described by Lowry et al. (1951), with bovine serum albumin as a standard. Immunoblotting and protein quantitation were performed as described by Locke et al. (1991), using a commercially purified anti-heat shock 72-kDa polyclonal antibody (SPA-812; Stress Gen).

Statistical analysis

Hsp70 content was reported as percent of standard (40 μg male Sprague-Dawley soleus; means ± SE) and compared within a given tissue by one-way analysis of variance (ANOVA) among exercise and drug treatment groups

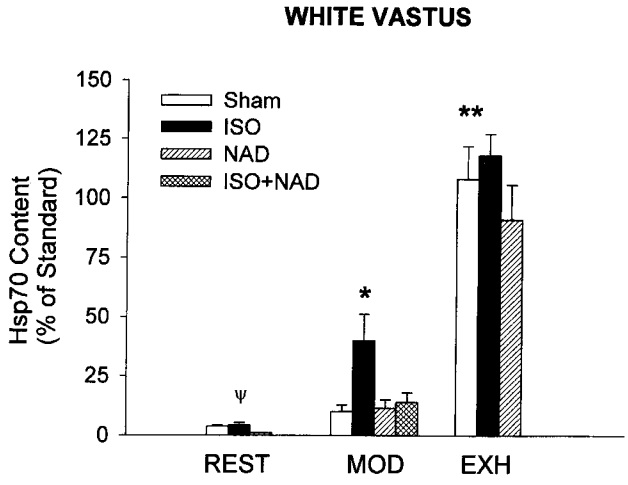

Fig. 2 Hsp70 content of white vastus as determined by immunoblotting with anti-Hsp70. Data are percentages of standard (40 μg male Sprague-Dawley soleus; means ± SE). Gels were loaded with 40 μg of white vastus homogenates. Empty bars are sham treated animals (sham), solid bars are isoproterenol treated (ISO), striped bars are nadolol treated (NAD) and cross-hatched bars are combined isoproterenol and nadolol treated. Bars are grouped according to rest, MOD and EXH as indicated. *ISO treated rodents demonstrate significantly greater Hsp70 than sham, NAD and combined ISO+NAD treated animals. **Sham-EXH had significantly greater Hsp70 levels compared with Sham-MOD. ψISO treated control animals had significantly greater Hsp70 content than NAD treated animals at $P<0.05$ ($n = 6–9$ per group).

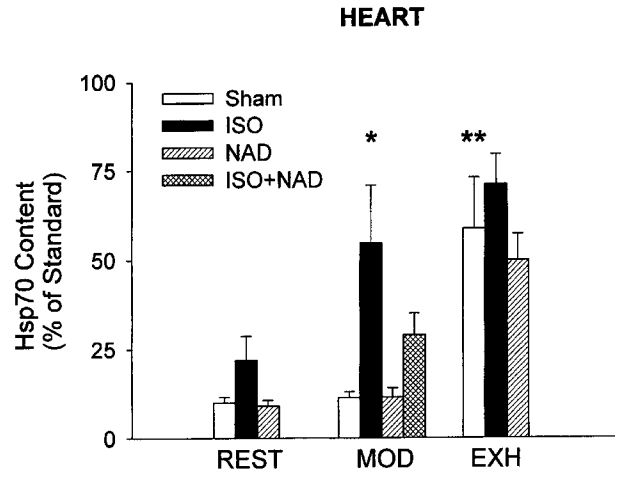

Fig. 3 Hsp70 content of heart as determined by immunoblotting with anti-Hsp70. Data are percentages of standard (40 μg male Sprague-Dawley soleus; means ± SE). Gels were loaded with 50 μg of heart homogenates. Empty bars are sham treated animals (sham), solid bars are isoproterenol treated (ISO), striped bars are nadolol treated (NAD) and cross-hatched bars are combined isoproterenol and nadolol treated. Bars are grouped according to rest, MOD and EXH as indicated. *ISO treated rodents demonstrate significantly greater Hsp70 than Sham and NAD treated animals. **Sham-EXH had significantly greater Hsp70 levels compared with Sham-MOD at $P<0.05$ ($n = 6–9$ per group).

individually. Post-exercise colonic temperatures and run times to exhaustion were also compared by one-way ANOVA among experimental groups. Pairwize comparisons were conducted using a Tukey post-hoc test. Significance was defined as $P<0.05$.

RESULTS

Effect of β-adrenergic manipulation on Hsp70 expression

Pharmacological treatment alone had no affect on Hsp70 levels in cardiac or skeletal muscle in non-exercised animals, although NAD treatment resulted in statistically lower Hsp70 expression compared with ISO treatment in the white vastus. Following MOD, ISO resulted in greater Hsp70 levels than NAD in the red vastus (Fig. 1), but the difference between ISO-MOD and Sham-MOD was not statistically significant. However, ISO administration prior to MOD resulted in significantly greater exercise-induced Hsp70 synthesis in the white vastus and heart compared with sham treatment (395 ± 29 and 483 ± 29% respectively; Figures 2 & 3). NAD treatment alone had no effect on post-exercise Hsp70 content in any of the examined tissues. However, the enhanced Hsp70 response to MOD with ISO treatment was reversed by combined administration of β-adrenergic agonist and antagonist in the white vastus (Fig. 2; ISO+NAD). Although not statistically significant, combined treatment also tended to suppress the isoproterenol induced potentiation of post-exercise Hsp70 expression in the heart (Fig. 3).

Despite the longer run time to exhaustion for the Sham-EXH group compared with ISO-EXH and NAD-EXH groups (Table 1), high intensity exercise resulted in similar Hsp70 levels among treatment groups. Animals subjected to EXH also exhibited similar post-exercise temperatures among treatment groups (Table 2). In contrast, ISO-MOD rodents demonstrated statistically higher colonic temperatures following exercise compared with Sham-MOD animals (40.14 ± 0.16 versus 39.28 ± 0.20°C respectively). ISO and combined treated rodents (ISO+NAD) exhibited similar colonic temperatures following MOD despite demonstrating significantly different post-exercise expression of Hsp70 in the white vastus.

Effect of exercise intensity on Hsp70 content

Rodents running at 30 m/min had significantly higher post-exercise Hsp70 expression than those running at 17 m/min in the red vastus, white vastus and heart (140 ± 10, 1,072 ± 13 and 519 ± 24% respectively; Sham-EXH versus Sham-MOD). Interestingly, ISO treatment prior to MOD resulted in red vastus and cardiac Hsp70 levels similar to those observed in rodents which ran to exhaustion at the

higher exercise intensity (ISO-MOD and Sham-EXH; Figures 1 and 3).

DISCUSSION

The major finding of the present investigation was that the β-adrenergic agonist, isoproterenol, enhanced Hsp70 expression in vivo in cardiac and skeletal muscle following MOD exercise. Moreover, combined β-adrenergic agonist and antagonist treatment suppressed this effect in the white vastus, indicating that the ISO-induced potentiation of post-exercise Hsp70 was mediated through β-adrenergic receptor activity. Other in vivo work has also demonstrated involvement of catecholamines in the induction of Hsp70 in response to other forms of stress. Depletion of catecholamines by AMT administration nearly completely abolished *Hsp70* mRNA accumulation in neonatal piglet brain following hypoxia (Murphy et al. 1996). Inhalation of the β-adrenergic agonist, albuterol, prior to bronchoscopy resulted in increased Hsp70 levels in human bronchial epithelial cells (Hastie et al. 1997). Thus, these observations suggest a role for the cAMP responsive element on the promoter region of the *Hsp70* transcriptional unit, as determined by Choi et al. (1991), in mediating the stress response to exercise, hypoxic and pharmacological perturbations in vivo.

In contrast, Udelsman et al (1994b), suggested that aortic Hsp70 synthesis following surgical stress was α_1-not β-adrenergic dependent and Inaguma et al. (1995), demonstrated that induction of Hsp70 in heat stressed rodents was not inhibited by β-adrenergic blockade. As epinephrine and norepinephrine stimulate both α- and β-adrenergic receptor activity (Hoffman and Lefkowitz 1990), these equivocal findings suggest that the pathways through which catecholamines mediate Hsp expression in vivo are stress and tissue specific.

While evidence for direct cAMP mediation of *Hsp70* promoter activity has been established (Choi et al. 1991; Pizurki and Polla 1994), an alternative pathway for adrenergic mediation of the stress response has been proposed. A number of laboratories have reported involvement of second messenger related protein kinase activity in the phosphorylation of heat shock transcription factor (HSF 1), a requisite step for the transcription of Hsp genes (Cotto et al. 1996). Using expression vectors for cAMP dependent protein kinase (PKA) inhibitors, Choi et al. (1991), demonstrated a significant reduction of heat-induced promoter activity of the *Hsp70* gene. In contrast, Lee et al. (1994), reported reduced *Hsp70* mRNA accumulation following inhibition of calcium/phospholipid dependent protein kinase (PKC) while PKA inhibition had no effect. More recently, Ohnishi et al. (1998), suggested that heat-induced HSF 1 activation and Hsp70 accumulation are predominantly dependent on PKC and to a lesser extent on PKA, and that the discrepancy between the findings of Choi et al. (1991), and those of Lee et al. (1994), may have been due to differences in cell lines used (Ohnishi et al. 1998).

Table 1 Run times to exhaustion at 30 m/min

Treatment	Run Time (min.)
Sham-EXH	82.38 ± 10.30*
ISO-EXH	43.38 ± 3.18
NAD-EXH	58.25 ± 3.65

Values are means ± SE; n = 8 per group. Sham-EXH, sham injected; ISO-EXH, isoproterenol injected; NAD-EXH, nadolol injected. *Sham-EXH had significantly longer run times to exhaustion than ISO-EXH and NAD-EXH animals.

Pizurki and Polla (1994), demonstrated that cAMP production did not increase Hsp synthesis by itself, but enhanced Hsp70 accumulation following heat shock in human monocytes. While unable to determine any direct effect of cAMP on the promoter of *Hsp70* and/or the phosphorylation of HSF 1 by PKA, the results from the present investigation indicate a similar involvement of β-adrenergic receptor activity on in vivo Hsp70 expression in cardiac and skeletal muscle following exercise. While ISO alone did not induce Hsp70 synthesis per se, the β-adrenergic agonist potentiated the stress response to MOD. Thus, although β-adrenergic receptor stimulation enhanced post-exercise Hsp70 expression, the failure of NAD to attenuate Hsp70 accumulation following EXH suggests that factors other than β-adrenergic receptor activity play a greater role in the stress response to exercise.

This conclusion is supported by the differential Hsp response of the red and white vastus following MOD and EXH in the present study. The red vastus, being a fast-twitch oxidative muscle is recruited almost maximally during MOD exercise (Terjung 1976). Thus, further increasing intracellular second messenger activity with ISO had little effect on the stress response to MOD in this tissue, as ISO and Sham Hsp70 levels in the red vastus were not statistically different. Factors associated with exercise other than β-adrenergic receptor activity such as muscle damage, altered calcium and metabolic related activity, regional hypoxia and/or reductions in intracellular ATP concentration, may also be involved in the induction of Hsp70 following exercise (Locke and Noble 1995). The white vastus, however, being a fast-twitch glycolytic muscle recruited to a lesser extent during MOD exercise (Terjung 1976), was responsive to β-adrenergic manipulation with respect to post-exercise Hsp70 accumulation. At higher intensity exercise wherein the white vastus is recruited to a greater extent, these other factors maximally activated the stress response, and thus, any effect of increased β-adrenergic receptor activity on Hsp synthesis could not be determined.

Table 2 Post-exercise colonic temperatures

Treatment	Colonic Temperature (°C)
Sham-MOD	39.28 ± 0.20
ISO-MOD	40.14 ± 0.16*
NAD-MOD	39.31 ± 0.28
ISO+NAD	39.70 ± 0.17
Sham-EXH	41.52 ± 0.11
ISO-EXH	41.73 ± 0.20
NAD-EXH	41.58 ± 0.08

Values are means ± SE; *n* = 8–9 per group. Sham-MOD, sham injected and exercised at 17 m/min; ISO-MOD, isoproterenol injected and exercised at 17 m/min; NAD-MOD, nadolol injected and exercised at 17 m/min; ISO+NAD, isoproterenol and nadolol injected and exercised at 17 m/min. Sham-EXH, control injected and exercised at 30 m/min; ISO-EXH, isoproterenol injected and exercised at 30 m/min; NAD-EXH, nadolol injected and exercised at 30 m/min. Colonic temperatures taken immediately post-exercise. *ISO-MOD temperature greater than Sham-MOD and NAD-MOD.

The most studied stressor on Hsp synthesis has been temperature. With EXH there were no differences in post-exercise colonic temperatures (Table 2) or Hsp70 levels despite differences in run time to exhaustion among treatment groups (Table 1). Thus, either elevated temperature or other factors associated with EXH were more important in regulating Hsp expression than adrenergic activity. Following MOD, rodents receiving ISO treatment had higher post-exercise colonic temperatures than sham treated rodents (Table 2). Thus, it is possible that ISO influenced Hsp70 expression indirectly by further elevation of body temperature during exercise. While the present study cannot address this issue, no animal in the ISO-MOD group approached the 41.5°C heat shock threshold established in passively heated rodents (Locke et al. 1995, Locke and Tanguay 1996). Moreover, post-exercise colonic temperatures between ISO and combined (ISO+NAD) treated rodents were not different despite differential Hsp70 expression following exercise. The above observations and the blocking of the ISO-induced potentiation of the Hsp response to MOD with combined treatment in the white vastus suggest that the potentiation of exercise-induced Hsp70 expression with ISO in the present investigation occurred via increased β-adrenergic receptor activity.

Previous reports have indicated that ISO treatment alone results in increased Hsp70 synthesis in the heart, possibly due to ISO-induced myocardial hypoxia and increased cardiac work (White and White 1986). Although there was a tendency for ISO treated control rodents to exhibit elevated myocardial Hsp70 levels in the present investigation, myocardial Hsp70 levels between ISO and Sham treated animals were not statistically different (Fig. 3; REST group). The differential effects of ISO on myocardial Hsp70 levels between the present study and that conducted by White and White (1986) is likely due to the much greater dosage employed in the latter investigation (10 versus 500 mg/kg body weight).

The results from the present examination of exercise-induced Hsp70 synthesis indicate that exercise intensity is an important factor in the degree to which Hsp70 is expressed (Figures, 1–3; Sham-EXH versus Sham-MOD). We have recently reported that rodents which ran a given total distance at a high rate, exhibited marked elevations in myocardial Hsp70 levels relative to those which ran similar distances at a slower rate (Noble et al. 1999). An underlying mechanism for this intensity-dependent expression of Hsp70 may involve exercise-induced cAMP levels, as intracellular cAMP increases have been shown to be exercise intensity dependent (Goldfarb et al. 1986; Goldfarb et al. 1989).

Figure 4 illustrates the pathways through which the exercise and pharmacological perturbations employed in the present investigation may have affected post-exercise Hsp70 expression. Cell culture studies have demonstrated a direct relationship between the β-adrenergic receptor intermediates cAMP and PKA and the transcription of the *Hsp70* gene (Choi et al. 1991; Ohnishi et al. 1998). ISO affects physiological function in a manner similar to exercise, by increasing body temperature, cardiac output and glycogen utilization (Hoffman and Lefkowitz 1990). Furthermore, these effects have been associated with induction of Hsp70 (Locke and Noble 1995). Since ISO increases β-adrenergic receptor activity and stimulates physiological processes associated with the stress response to exercise, the pharmacological manipulations used in the present study likely influenced post-exercise Hsp70 content through a complex interaction of intracellular and physiological events.

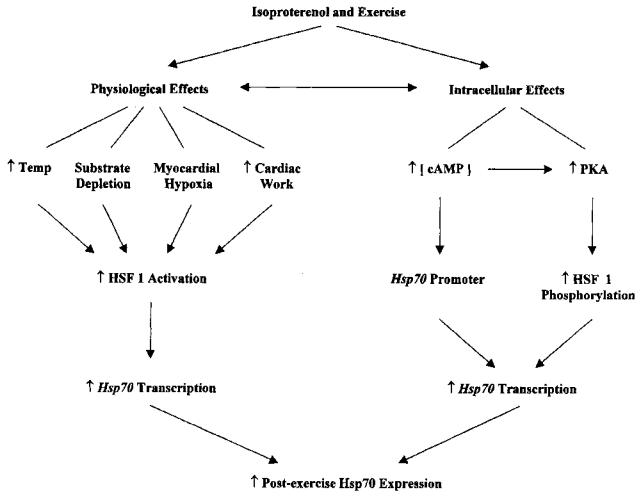

Fig. 4 Diagrammatic representation of the common pathways through which isoproterenol and exercise may influence post-exercise Hsp70 expression in vivo.

In summary, the results from the present investigation suggest that β-adrenergic activity may mediate Hsp70 expression in vivo in cardiac and skeletal muscle following moderate intensity exercise. These results are in line with work done in culture, indicating that β-adrenergic receptor activity does not stimulate Hsp synthesis per se and is not the main signal for the induction of Hsp70. However, β-adrenergic receptor activity may be one factor involved in the complex regulation of the stress response to exercise in vivo.

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