Exercise, Estrogen, and Ischemic Cardioprotection by Heat Shock Protein 70

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Acute ischemic injury and myocardial infarction arising from coronary artery disease, despite significant strides in medical and surgical management, remain a major public problem. Sex disparity has long been recognized for coronary ischemic events: premenopausal women are at reduced risk for atherosclerotic disease than men of similar age, an advantage that vanishes after menopause. The beneficial effects of estrogen are known to modify vasomotor tone, vascular integrity, blood pressure, lipid profiles, and cholesterol metabolism. However, far less is known about the sex-related effects estrogen has on stress-activated pathways such as the chaperone defense mechanism, which plays fundamental roles in reducing oxidative damage ensuing from myocardial ischemia and reperfusion injury.

In this issue of Circulation Research, Paroo and coworkers have addressed the hypothesis that sexual dimorphism in cardiac heat shock protein 70 (Hsp70) expression induced by exercise is modulated by estrogen and that such effects confer sex-specific protection against ischemic injury. These investigators have shown that a single exercise regimen, 30 m/min for 60 minutes, leads to the induction of Hsp70 expression by 2-fold (24 hours) in male but not in female rats. Whereas exercise upregulates Hsp70 expression in ovariectomized female rats, estrogen replacement abrogates similar effects, suggesting that estrogen plays a key role to repress exercise-induced stress response (in a sex-specific manner). Furthermore, a direct causal mechanism by Hsp70 in mediating the recovery of postischemic cardiac function was convincingly shown using antisense oligonucleotides, an approach that had previously been reported to abrogate Hsp70-dependent protection against simulated ischemia in adult cardiac myocytes but not yet in the intact postischemic heart (the present study by Paroo et al).

What are the mechanisms by which sex-linked Hsp70 expression mediate ischemic protection? Paroo et al have focused their study on Hsp70, which belongs to the multigene family of heat shock genes (hsp), encoding intracellular proteins triggered by heat shock and various other stressful conditions. Molecular chaperones such as HSP might be cytoprotective agents, which effectively retard protein conformational abnormalities implicated, in part, in the pathogenesis of age-related oxidative damage, atherosclerosis, and myocardial ischemia and reperfusion injury. The findings reported here are consistent with previous studies that have reported forced overexpression of Hsp70 affords effective protection against myocardial injury, reduces postischemic ventricular dysfunction, and improves ATP synthesis (see reviews). At the molecular level, antiapoptotic effects of Hsp70 might mediate cardioprotection by directly counteracting the functions of proapoptotic Apaf1, caspase recruitment domain (CARD), and apoptosome formation.

Several additional aspects of this study deserve comment. First, it is surprising to some that the exercise regimen does not stimulate Hsp25/7 expression, which others have reported in different postexercise skeletal muscles. As shown for several HSPs, a high constitutive level of Hsp25/7 expression correlates inversely with its stress-inducible upregulation and synthesis (see data in Xiao et al). Another notable limitation is the assumption that cardiac myocytes per se are the principal targets of protection against ischemia/reperfusion injury. Last, in addition to Hsp70, the salutary effects exerted by exercise-induced cross-tolerance after 24 hours might also include adaptive changes driven by nitric oxide.

The physiological roles of stress “heat shock” proteins are inextricably linked to their transcriptional regulation, which is mediated by a family of transcriptional regulators, collectively known as heat shock transcription factors (HSFs). Various stressful conditions that promote protein instability and denaturation release HSF1 from interacting partners, enabling it to oligomerize as transcriptionally competent homotrimers. Activated HSF1 exhibits increased phosphorylation and a high-affinity binding to heat shock elements arranged as arrays of inverted pentanucleotide repeats (nGAAAn) in the promoters of target hsp genes. Indeed, increased oxidative stress can play an important role, as reactive oxygen species (ROS)-mediated HSF1 activation has been reported during upregulation of heat shock proteins by myocardial ischemia/reperfusion. In addition to pathological stimuli, physiological conditions such as exercise can also stimulate Hsp70 expression in the myocardium, skeletal muscle, even in human leukocytes, probably through HSF1 activity.

How might estrogen per se attenuate Hsp70 expression induced by exercise? The present study does not provide direct answers, but the investigators have commendably addressed a few important possibilities. The effects of estrogen could be mediated by its receptors, estrogen receptor α and estrogen receptor β, homologous members of the superfamily of steroid hormone receptors, which are expressed in...
Hypothetical model for sex-specific regulation of Hsp70 by HSF1-dependent transcription. Under physiological conditions, HSF1 transcriptional activity is controlled by intramolecular interactions involving disulfide bond between cysteine residues, which mediates its redox-sensitive regulation. Intermolecular interaction between HSF1 and a multichaperone complex including HSP90 provides another mechanism for repression of HSF1 activity. HSP90, in turn, has multifunctional roles in protein folding and stabilization of many signal transduction pathways involving steroid hormone receptors. Thus, ligand-dependent interactions have been proposed to modify the equilibrium between HSP90 and its molecular partners, HSF1 and steroid receptors. Stressful conditions that increase oxidative stress (ROS) and denatured proteins shift the equilibrium for HSF1 release, facilitating its activation by homotrimerization, translocation, and high-affinity DNA-binding activity at target hsp genes such as hsp70. Sex-specific differences in redox state, HSP90 content, and constitutive expression of HSP are likely pertinent mediators in determining the regulation of Hsp70 expression by physiological and pathological stimuli.

all major cell types such as vascular endothelial, smooth muscle, and cardiac myocytes. Studies using tamoxifen, an estrogen receptor inhibitor, and other maneuvers in ovariec-
tomized female rats were designed to assess the potential role of estrogen receptors. Their results seem to exclude a significant role for ligand-dependent requirements in the sex-specific expression of Hsp70. However, some caution is warranted because the independent effects of either estrogen or progesterone have been reported to induce Hsp70 expression.

Paroo et al have suggested that the lipophilic properties of estrogen mediate cardioprotection by a mechanism that stabilizes the cellular membrane. In female rats, this stabilizing effect is suggested to protect membrane-bound proteins against denaturation and, therefore, counteracts a major signal for HSP induction. Although the hypothesis remains intriguing, direct evidence for such estrogen-dependent effects is presently unavailable.

That Hsp70 expression is attenuated by estrogen suggests an alternative hypothesis based on emerging lines of evidence for multiple layers of regulation of chaperone defense mechanisms by the transcription factor HSF1 (see Figure). One possibility is that the sex differences in the levels of oxidative stress, a key stress signal for HSF1 activity, can exert direct regulatory effects on transcriptional activation of hsp70 gene expression. A complementary mechanism is based on inter-
molecular interactions between HSF1 and a multichaperone complex including chaperone HSP90. Therefore, it is conceivable that, in female rats, a higher HSP90 content sequesters HSF1 during nonstressful conditions but that lower HSP90 in male rats facilitates the kinetics of derepression such that HSF1-dependent upregulation of Hsp70 is robust in response to exercise-induced oxidative stress. Thus, sex-specific upregulation of Hsp70 could be due to sex differences either in the threshold for HSF1-dependent gene expression or in the intracellular milieu (eg, redox state), or both.

Notwithstanding, a critical finding observed by Paroo et al, but not sufficiently emphasized in their study, is that the protection against ischemic injury observed after exercise in male rats is actually similar to that which is observed in naive (or exercised) female rats. In other words, exercise is required for the male rats to reach the same level of endogenous protection as observed in female rats. Consistent with our hypothesis mentioned above, a lower level of oxidative stress might also contribute to this endogenous protection in female rats. This hypothesis would not preclude several other effects exerted by estrogen. Deleterious cardiac-specific effects by overexpression of β2-adrenergic receptor, which causes increased contractility and ischemic vulnerability, were mitigated in female but not male mice through an endothelial nitric oxide synthase (eNOS)-dependent mechanism. In addition, increased parasympathetic activity may account for reduced heart rate and mean arterial pressure and account for decreased vulnerability to myocardial injury in females compared with males after ischemia/reperfusion. Finally both observational and clinical studies have fueled the current impetus for hormonal replacement therapy (HRT), an intervention that reduces cardiovascular events by 30% to 50% but at the same time has raised many unresolved issues and introduced new controversies, which are beyond the scope of this commentary.

In summary, the foregoing discussion provides new evidence implicating sex-specific differences in experimental models and regulation of chaperone defense yet leaves unresolved questions about the fundamental mechanisms accounting for sexual dimorphism in ischemic vulnerability. Hormonal actions take center stage in different fields ranging from evolutionary physiology, dealing with dynamics in gene-environment interactions in the wild, to cardiovascular physiology. A better understanding for the actions of estrogen on relevant stress response pathways should come from the systematic analysis of sex differences in transcriptome and proteome in response to physiological (exercise) and pathological (ischemia) conditions.

Meanwhile, the potent effects on cardioprotection by sex-related Hsp70 cannot be ignored, summoning the importance to trade the couch for the treadmill, gender differences aside.

References


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