Editorial Comment

Heat Shock Proteins and the Ischemic Heart
An Endogenous Protective Mechanism

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Protection of the ischemic heart has been the subject of experimental and clinical research for more than a decade. Myocardial infarct size is a function of cell necrosis occurring during ischemia and reperfusion, and numerous investigators have attempted to limit ischemic- and reperfusion-induced injury by pharmacological means. On balance, if the number of articles indicating successful intervention may be considered the arbiter of consensus, then it is unequivocal that ischemic- and reperfusion-induced injury can be attenuated pharmacologically and by induction of a preconditioning ischemic or thermal stress in experimental animal models. However, no drug has yet been approved for use as an "anti-ischemic" agent or as a cardioprotective adjunct to thrombolytic/angioplasty treatment (excepting antithrombotic drugs). The approach taken by Currie and colleagues, as reported in this issue of Circulation (and elsewhere4-7), to limit ischemic heart damage is unique in that protection of the ischemic heart is afforded by an incompletely understood endogenous mechanism activated by whole-body heat shock. Raising the body temperature of anesthetized animals to 42°C for a period of 15 minutes (heat shock) protects the heart against an ischemic insult. Currie and coworkers demonstrate that whole-body heat shock is protective against ischemia/reperfusion injury in vivo, confirming previous studies of cardioprotection against in vitro ischemic injury.4-6 Heat shock–mediated cardioprotection in vivo is transient, and the observed protective effect is no longer present 40 hours after application of the initial heat stress. The hypothesis supported by current4 and previous4,8,9 studies is that myocardial protection is related to the heat shock–mediated increase in cardiac heat shock protein synthesis. The term "heat shock protein" (HSP) refers to a group of proteins of which there are several families, differentiated by molecular weight (i.e., HSP 60, HSP 70, and HSP 90 families). Within families of HSP are subsets; constitutively expressed proteins are referred to as heat shock cognates (HSCs), whereas proteins expressed largely under conditions of stress are referred to as the HSPs.10 Members of this latter group also may be expressed at a lower level under nonstressed conditions. Cells exposed to elevated temperature, ethanol, heavy metals, or other noxious stimuli show increased expression of HSPs. HSCs and constitutively expressed HSPs function under nonstressed conditions in cells to prevent misfolding or aggregation of nascent polypeptides, allowing polypeptides to traverse biomembranes and promoting proper folding and oligomerization of newly synthesized proteins.10 Since protein folding and assembly events in vivo require the participation of accessory components, HSP functioning in this way has been referred to as molecular chaperones. As described in the review by Ang et al,10 subsequent to a heat shock episode (or other insult), HSP expression is increased and HSPs function to protect cellular proteins from denaturation, or if damage has occurred, promote disaggregation and allow refolding back to an active conformation.

Of particular interest from the viewpoint of cardioprotection, HSP mRNA and HSP are increased in the heart after ischemia,11 hypoxia,12 hemodynamic overload,13 myocardial stretch,14 or hyperthermia (heat shock).4 The precise mechanism by which HSPs (in particular, the HSP 70 family) afford cardioprotection in ischemia/reperfusion, if indeed these proteins serve a protective role, remains to be defined. As discussed above, HSPs are involved in maintaining cellular function under normal conditions (nonstressed) as well as under conditions of stress. More recently, it has been demonstrated that products of the 70-kd HSP family function in a variety of immunologic mechanisms.15 The inducible 72-kd HSP is expressed in cells exposed to a variety of stimuli, including cytokines, oxygen free radicals, and inflammatory mediators released from phagocytic cells infiltrating areas of damaged tissue.16 It is unknown whether there are changes in the profile of myocardial HSP expression and function under conditions of chronic disease. It is therefore intriguing to suggest that the brief episodes of ischemia occurring in patients with ischemic heart disease may lead to the induction of HSPs that function to protect the tissue on reperfusion.

The majority of the research demonstrating cardioprotection against ischemia by heat shock has come from the laboratory of Currie and colleagues4-7 and more recently from that of Yellon and coworkers.9 Whole-body heat-stressed hearts show improved post-ischemic left ventricular functional recovery and reduced enzyme loss in response to a subsequent in vitro
ischemic stress. The effect of whole-body heat stress on in vivo myocardial ischemia/reperfusion injury is not as clear or as well defined. Incongruent results using in vivo paradigms have been reported.\textsuperscript{3,6,17} The conclusions of the study of Yellon et al\textsuperscript{17} are at variance with those of Currie et al.\textsuperscript{3} The data were derived using a similar experimental model, with the exception that the ischemic period, in the former, was 45 minutes rather than 30 minutes. Can an increase by 50% in the ischemic period account for this discrepancy? It is possible that a 45-minute ischemic interval resulted in injury beyond which stress proteins could not confer adequate protection. Additional studies using varying periods of regional ischemia (20, 30, 45, and 60 minutes) are needed to address this matter adequately. A second difference between these studies is that Yellon et al\textsuperscript{17} quantitated HSP 72 with a commercially available monoclonal antibody, whereas Currie et al\textsuperscript{3} quantitated HSP 71 using a noncommercial polyclonal antibody. This difference, however, is unlikely to explain the failure of whole-body heat shock to be cardioprotective in the study of Yellon et al.\textsuperscript{17}

The time window after which a period of heat shock confers in vivo cardioprotection against a subsequent ischemic period is now defined, albeit preliminarily.\textsuperscript{3} Previously, whole-body heat shock conferred protection against ischemia in the rat heart in vitro up to 96 hours after the heat shock event.\textsuperscript{7} The time course of heat shock-mediated protection in vivo is considerably less than in vitro since protection was seen at 24 but not 40 hours after heat shock. Others have demonstrated in vivo ischemic protection at 24 hours after heat shock in the rat\textsuperscript{8} but not in the rabbit heart,\textsuperscript{17} as discussed already. Heat shock also failed to protect against 45 minutes of ischemia at 40 hours.\textsuperscript{3} The degree of ischemia against which heat shock is protective is limited. This possibility is supported by in vivo results in rats where heat shock protected against a 35-minute but not 45-minute period of regional ischemia.\textsuperscript{8} It is apparent, therefore, that the cardioprotection conferred by whole-body heat shock in vivo diminishes with time and is dependent on the intensity of the applied ischemic insult. This is not too dissimilar to what has been observed with myocardial preconditioning, in which brief periods of regional ischemia will protect against a subsequent 60-minute ischemic insult but not against a more protracted ischemic insult of 90 minutes.\textsuperscript{18}

The temporal relation between detectable increases in HSP expression and protection from a subsequent ischemic myocardial insult may have important consequences on the final outcome. An unresolved issue is determination of the time at which induced HSP is effective in providing maximal protection. To date, data on cardioprotective effects of whole-body heat shock have been derived primarily from studies in which the isolated heart was subjected to an ischemic insult 24 hours after whole-body hyperthermia. Previous studies have shown a correlation between the expression of HSP and protection against ischemic damage.\textsuperscript{4,5,9} Currie et al\textsuperscript{3} have shown in the current study that increased HSP 71 expression correlates with cardioprotection at 24 hours but not 40 hours after heat shock. Although HSP 71 was increased up to 48 hours after heat shock, no protection was afforded against either a 30- or 45-minute period of ischemia in vivo 40 hours after heat shock.\textsuperscript{3} HSPs unrelated to the HSP 70 family, in particular HSP 71 quantified in the current study, may be involved in the cardioprotection at 24 hours that are not present (or sufficiently present) at 40 hours. The effect of heat shock on the tissue level of proteins not typically referred to as "HSPs" but evidently increased by heat shock (particularly catalase)\textsuperscript{4} also may be important to the observed reduction in infarct size. In this regard, heat shock-mediated cardioprotection against ischemia/reperfusion injury in vitro was dependent on active catalase.\textsuperscript{7} The relation between in vitro and in vivo cardiac effects of whole-body heat shock remains inconclusive.

An issue not addressed by Currie and colleagues is the effect of whole-body heat shock on extracardiac cells and the possible contribution of such effects to cardioprotection. The use of an in vivo ischemia/reperfusion paradigm introduces the possibility that whole-body heat shock affects noncardiac cells, tissue, or organ function that may impinge on cardiac susceptibility to ischemia- and reperfusion-induced damage. Although it is established that whole-body heat shock protects the heart from ischemia-reperfusion damage in vivo (indicative of a direct myocardial effect), the complexity of the in vivo milieu and the pancellular nature of the heat shock response imply that additional mechanisms must be considered. Altered neutrophil function is one possibility. In response to heat, neutrophil HSP expression is increased and neutrophil NADPH oxidase activation is inhibited, indicating that neutrophil superoxide production may be attenuated concomitant with increased HSP expression.\textsuperscript{19} Since neutrophil ablation reduces infarct size,\textsuperscript{20} whole-body heat shock may confer cardioprotection in vivo in part by influencing neutrophil function. However, it is also possible that whole-body heat shock exerts negative effects because the duration of cardioprotection is much less in vivo than in vitro,\textsuperscript{7} and heat shock has failed to protect the heart in vivo.\textsuperscript{17}

Differentiation of the effect of heat shock on cardiac endothelial cells versus cardiac myocytes has not been addressed in studies that have shown cardioprotection by heat shock. The vascular endothelium plays a significant role in cardiac physiology and pathophysiology, and heat shock has been shown to increase HSP expression in vascular endothelial cells.\textsuperscript{21} The relevance, if any, of a putative increase in endothelial HSP expression to cardioprotection is unknown. Furthermore, it remains to be determined if heat shock mediates a change in the expression of cardiac endothelial cell surface adhesion molecules. In view of the role of HSP in the cellular protection and trafficking of proteins\textsuperscript{22} and of the time course of both HSP expression and endothelial adhesion receptors\textsuperscript{23} during reperfusion of the previously ischemic heart, a relation may exist between HSP and cell surface adhesion receptor expression. As alluded to above, such a relation may not be beneficial.

Currie et al\textsuperscript{3} show that whole-body heat shock "affords an ATP-sparing effect" and the preservation of tissue ATP may contribute to enhanced posts ischemic functional recovery. Myocardial ATP content was increased immediately after heat shock and remained increased 48 hours later. The immediate increase in tissue ATP preceded the peak in HSP mRNA and HSP 71 synthesis. Hyperthermic treatment, therefore, has an acute effect on tissue ATP metabolism that is independent of changes
in inducible cardiac HSP 71. If improved postischemic function is related to the ATP-preserving effect of heat shock, then there appears to be a dissociation between this effect and the requirement for increased synthesis of HSP 71. Since tissue ATP content was not determined immediately before or after the ischemic period, it is conjecture that the ATP sparing effect of hyperthermia relates to enhanced postischemic recovery or reduction in infarct size. Previous studies comparing heat-shocked animals with controls showed no change in myocardial tissue ATP content before or after the ischemic insult.6

There is now considerable evidence supporting adenosine as a primary mediator of myocardial ischemic preconditioning.24 whereas HSP generally is not considered in this context. In the rabbit, however, multiple 5-minute periods of ischemia increased the level of HSP mRNA threefold, with significant increases as early as 1 hour after reperfusion.25 Increased HSP 70 protein in the rabbit heart is apparent after 2 hours of reperfusion. The time course of expression of HSP in response to ischemia, therefore, may not be rapid enough to account for the protective effect of ischemic preconditioning. In this regard, cycloheximide and actinomycin D (inhibitors of protein synthesis) did not block ischemic preconditioning in rabbit heart.26 Preliminary results support this conclusion; however, cardiac HSP expression was not specifically quantified in the study by Thornton et al.26 That HSP may be involved in ischemic preconditioning remains possible, however, as a 65-kd protein was induced rapidly after an ischemic preconditioning episode.28 Therefore, as alluded to earlier, HSPs other than HSP 71 may play a role in ischemic preconditioning. The differentiation of the protective role of constitutive versus induced HSP and determination of the intracellular substrates putatively protected by HSPs will provide insight into the mechanism of HSP-associated cardioprotection.

As in previous studies, the current report in Circulation3 confirms that whole-body heat shock confers protection against a subsequent period of myocardial ischemia/reperfusion and extends our understanding of this process by demonstrating that the protective effect in vivo is transient and no longer present 40 hours after heat shock. The approach of conferring cardioprotection against ischemia by heat shock is of particular interest because it speaks to the possibility of a novel mechanism by which the heart may be "preconditioned" to withstand ischemic episodes. It is apparent that cardiac HSP research will continue to define the mechanism by which a sublethal insult confers protection against a subsequent noxious ischemic insult. Issues that remain to be determined include definition of the role and mechanism of HSP in the cardioprotective process and determining whether other proteins (catalase?) are contributory in vivo. To define the role of HSP, future work in this area of research should determine if less noxious stimuli can increase HSP expression and concomitantly protect the heart. Furthermore, experimental protocols must define the end points to be examined since the beneficial effects and duration of protection by HSP induction may differ when functional recovery versus reduction in infarct size are compared. Last, the matter of species differences must be taken into account when comparisons are made regarding the cardioprotective effects of HSP. Ultimately, can a pharmacological intervention be designed (an organic heat shock factor?) to target the responsible genetic element and stimulate discrete expression of the protein or proteins responsible for protection? Clearly, this possibility should stimulate further research in the area of HSP and cardioprotection. Currie and colleagues are to be complimented for stimulating and maintaining interest in this vital area of research.

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