Heat shock proteins and heat adaptation of the whole organism

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Moseley, Pope L. Heat shock proteins and heat adaptation of the whole organism. *J. Appl. Physiol.* 83(5): 1413–1417, 1997.—Adaptation to heat may occur through acclimatization or thermotolerance; however, the linkage of these phenomena is poorly understood. The importance of heat shock proteins (HSPs) in thermotolerance and differences in their accumulation in organisms adapted to the heat suggest a role for HSPs in acclimatization as well. The role of HSPs in heat adaptation of the whole organism and the interrelationships among heat adaptation, endotoxin tolerance, and cytokine resistance through HSPs are reviewed.

acclimatization; thermotolerance

THE INTEGRATION OF CELL BIOLOGY with whole organism physiology has allowed investigators to pursue fundamental questions regarding the impact of cellular changes on the organism's adaptive capabilities. One such question is the impact of the cellular stress response on the whole organism's adaptation to high environmental temperatures and on the capacity of the organism to perform work in the heat. Heat adaptation is divided into thermotolerance and acclimatization. Thermotolerance is a cellular adaptation caused by a single, severe but nonlethal heat exposure that allows the organism to survive a subsequent and otherwise lethal heat stress. In contrast, acclimatization is an organism's ability to perform increased work in the heat because of improvements in heat dissipation brought on by repeated mild elevations in core temperature (Table 1). This review examines the differences between thermotolerance and heat acclimatization, possible links between these forms of thermal adaptation, and potential mechanisms to explain these links. Central to understanding thermotolerance and perhaps to the cellular role in acclimatization are the heat shock or stress proteins (HSPs).

THE HSPs

The HSPs have been studied extensively, especially regarding their regulation, localization, and function in the cell (34, 49). HSPs range in size from 27 to 110 kDa and can be divided into five groups based on both size and function (Table 2). Initially, stress-induced HSP accumulation was associated with thermotolerance, the ability to survive otherwise lethal heat stress, and later with tolerance to a variety of stresses, including ischemia (31), ultraviolet irradiation (2), and cytokines such as tumor necrosis factor-α (TNF-α) (22). The fact that overexpression of various HSPs confers tolerance in the absence of conditioning stress and that inhibition of HSP accumulation through blocking antibodies impairs stress tolerance strongly support the hypothesis that HSPs themselves confer the stress tolerance. The mechanism by which the HSPs confer stress tolerance is not completely understood but may relate to the important role of HSPs in the processing of stress-denatured proteins (33). Microinjection of denatured albumin into *Xenopus* oocytes is sufficient to induce the HSP70 promoter (1). HSPs are also thought to manage the protein fragments occurring as the result of stress-induced translational arrest (11, 37). The maintenance of structural proteins may also be a key to HSP-associated stress tolerance. In this regard, HSP27, a protein homologous with α-crystalline, prevents actin microfilament disruption under stress conditions (27). This effect on the cytoskeleton may be important not only in individual cell tolerance to stress through cytoskeletal stabilization but may also be integral to the protection of the whole organism through the maintenance of endothelial and epithelial barrier functions.

A third mechanism of cellular protein management is through the chaperone function across cell membranes. HSP70 and HSP60 perform a unique relay in the movement of cellular proteins through the mitochondrial membrane, with HSP70 transporting the protein to the outer mitochondrial membrane and participating in the protein's unfolding and insertion into the membrane. HSP60 accepts the protein and participates in the refolding of the protein within the mitochondria (13). Similarly, HSP70, as well as other chaperone proteins, participates in the movement of proteins across the endoplasmic reticulum (11). HSP90 has been

Table 1. *Thermotolerance vs. acclimatization*

	Thermotolerance	Acclimatization
Time to induction	Hours	5–7 days
Duration of adaptation	2–7 days	Indefinite, provided individual continues to have periodic mild temperature elevations
Physiological adaptations		↑ Sweat rate ↓ Heart rate and core temperature
Measurement of adaptation	Survival of an otherwise lethal heat stress	Ability to maintain thermal equilibrium at a given work rate
HSP induction	Yes	?

HSP, heat stress protein.

well described as the chaperone for steroid hormone receptors, serving not only as a chaperone but as an important regulatory protein for the receptor (5).

Given the important role of HSPs in the cell's tolerance of an otherwise lethal heat stress through mechanisms including denatured protein management, cytoskeletal stabilization, protein translocation across membranes, and receptor regulation, one can envision a role for the cellular stress response in the intact organism's adaptation to less intense but, nonetheless, significant thermal challenges that threaten numerous protein-requiring pathways. Furthermore, the demonstration of HSP70 accumulation in muscle tissue of humans after eccentric muscle exercise (39) as well as in humans (40) and animals (12, 15) exercising in the heat supports a role for HSPs in the adaptation to temperatures encountered under normal physiological conditions.

THERMOTOLERANCE VS. ACCLIMATIZATION

The ability of the HSPs to confer thermotolerance in both cultured cells and in animals is well documented (28, 50). Thermotolerance refers to an organism's ability to survive an otherwise lethal heat stress from a prior heat exposure sufficient to cause the cellular accumulation of HSPs. Regardless of stimuli, the hallmarks of thermotolerance are *1*) survival of the cell or organism exposed to an otherwise lethal heat stress; *2*) synthesis of HSPs; and *3*) a relatively short duration of the thermotolerant state (hours to days) that correlates

Table 2. *Heat shock proteins by size, function, and cellular localization*

Size, kDa	Major Function	Cellular Localization
27–28	Stabilization of microfilaments Cytokine signal transduction	Cytosol and nucleus
60	Protein assembly	Mitochondria
70–73	Protein folding and translocation	Cytosol, nucleus, endoplasmic reticulum, mitochondria
90	Protein translocation Receptor regulation	Cytosol, nucleus, endoplasmic reticulum
100–104	Protein folding	Cytosol

with the presence of elevated cellular HSPs and declines with the decrease in HSPs. The requirement of HSPs for thermotolerance and the role of HSPs in protein folding, assembly, and transport support the hypothesis that the thermotolerant state is dependent on one or all of these HSP-related functions, especially through the management of both denatured proteins and of partially synthesized protein fragments.

In marked contrast to thermotolerance, heat acclimatization refers to the organism's ability to perform work in elevated environmental temperatures as well as to continue work under elevated but nonlethal core temperatures (Table 1). Unlike thermotolerance, where cell or organism survival is the measured end point, acclimatization is determined through a work heat-tolerance test demonstrating the organism's ability to achieve and maintain thermal equilibrium at a given work rate in the heat. In addition, heat acclimatization results from a series of elevations in core temperature, generated by performing work in the heat (3, 17). Passive hyperthermia is normally associated with only partial acclimatization. Unlike thermotolerance, which undergoes a rapid decay correlating with a decline in HSPs, heat acclimatization can be maintained for prolonged periods as long as the organism continues to undergo periodic elevations in core temperature. Finally, there is no cellular model of heat acclimatization.

Heat acclimatization not only reduces resting core temperature and provides for greater heat transfer to the skin or heat-dissipating capacity but also allows the organism to tolerate a higher core temperature. Increased heat dissipation occurs through systemic alterations including a decrease in sweating threshold, an increased sweating output at a given core temperature, a reduced threshold for cutaneous vasodilation, and greater skin blood flow at a given core temperature (3, 17, 36). The ability to work at higher core temperatures seen in both rats (17) and humans (32, 38), however, mirrors the thermotolerant state and suggests that cellular mechanisms of adaptation such as those related to HSPs may be at work.

There is a growing body of literature supporting the role for HSPs in the whole organism's adaptation to heat other than through thermotolerance. These inferences are based on interspecies differences in patterns of HSP accumulation and the association of these differences with habitation in a hot climate. A survey of lizard species inhabiting a variety of environments, including highlands, forests, and deserts, demonstrated a remarkable diversity of constitutive HSP70 levels that were correlated with the lizard's environmental temperatures (44). That is, the higher the temperature of the environmental niche, the greater the amount of constitutive HSP70 family found during nonstress conditions. In contrast, the temperature needed to induce an HSP response required for thermotolerance was 2–3°C higher in these same lizards. These data are consistent with a model of heat adaptation of the whole organism through HSPs, whereby the increased levels of constitutive HSP70 allow the poikilothermic organism to deal with abrupt changes in core temperature

associated with escape or hunting in a hot environment. Such core temperature changes would presumably be too rapid to allow the animal to benefit from the de novo induction of the heat shock response, whereas a preexisting elevation in constitutive HSP70 might allow the animal to continue to perform work under acute temperature elevations.

A link between HSPs and heat adaptation through a mechanism other than thermotolerance is also found in the Saharan ant *Cataglyphis bombycina*, one of the most heat-resistant animals known (20). *C. bombycina* is able to forage during the Saharan midday, tolerating body temperatures of 53–55°C, while all other desert ants cease activity when surface temperatures exceed 45°C. Unlike thermophilic bacteria, whose stable protein systems continue to function at elevated temperatures in the absence of an HSP response, *Cataglyphis* shows elevated levels of HSP70 at basal temperatures of 25°C. This elevation of HSP70 at lower temperatures, as described in lizard species inhabiting hot climates, does not reflect an acute response to cellular injury or protein denaturation but appears to be an adaptive response allowing the organism to perform work at elevated temperatures during temperature changes too abrupt to give the animal an opportunity to benefit from de novo HSP synthesis. Finally, a provocative study in humans (30) suggests an ethnic difference in HSP70 accumulation that is also based on environment. Skin fibroblasts obtained from Turkmen living in the Central Asian desert and from European Russians living in temperate climates reveal differences in HSP70 after a 42.5° heat shock, with greater increases in inducible HSP70 in Turkmen fibroblasts after heat shock (30). This increase in inducible HSP70 is associated with the preservation of normal protein synthesis even at these elevated temperatures. The impact of these differences on heat acclimation is unknown. Furthermore, the fact that the cells were studied after 10–15 passages suggests that differences in HSP70 accumulation in these populations reflect genetic differences. However, these studies, taken together, suggest that there are species and intraspecies differences in HSPs, which form part of the adaptive response allowing the organism to live and perform work in high-temperature environments.

MECHANISMS OF HEAT ADAPTATION

If HSP accumulation is integral to the acclimatization of the whole organism to heat exposure by mechanisms other than thermotolerance, how might this adaptation occur? Again, the integration of molecular biology, cell physiology, and studies in the intact organism have allowed us to examine a number of possible mechanisms by which cellular HSP accumulation could result in the whole organism's ability to tolerate or achieve a higher core temperature before exhaustion through mechanisms other than thermotolerance. Central to this question is the mechanism by which heat exhaustion and heat injury occur. One mechanism may be related to the association among heat stress, the subsequent release of endotoxins from the gastrointes-

tinal tract, and cytokine production. A number of studies have demonstrated the presence of systemic endotoxemia (6, 7) and elevations in circulating cytokines (7) with heat exhaustion and heat stroke. Strenuous exercise has also been shown to elicit a cytokine response (8, 46). This elevation in circulating cytokines could be the direct result of the hyperthermia itself due to increases in heat-associated cytokine export from the cell or through increases in cytokine transcription. Alternatively, the increases in cytokines could occur in response to circulating endotoxins translocated across the gut barrier due to heat-induced alterations in gut permeability. Prophylactic gut sterilization (19) or the administration of anti-endotoxin antibodies (18) allows animals to tolerate higher core temperatures. Thus it is intriguing to speculate that heat acclimatization is the result of the organism's ability to dissipate heat more effectively as well as the organism's ability to either block or tolerate gut-associated endotoxin translocation, downregulate cytokine production, or develop an increased tolerance to cytokine exposure. It is in this endotoxin and cytokine response that HSPs may play an important role. Elevations in cellular HSP70 are associated with an attenuation in heat-induced permeability of an epithelial monolayer (35). Unlike thermotolerance, which is measured by cell survival of an otherwise lethal heat stress, this heat-induced epithelial permeability is a reversible phenomenon that occurs at temperatures that are not lethal to the individual cells (35). Thus the association of HSP70 accumulation with the maintenance of epithelial barrier integrity suggests a means to confer heat tolerance in a multicellular system that is associated with HSPs and distinct from thermotolerance. The preservation of the epithelial barrier through an HSP-associated mechanism, possibly through stabilization of the cytoskeleton or through the preservation of important cell-to-cell contacts, may be an important factor in preventing heat-associated endotoxin translocation across the gut. Because HSP27 also functions in the signal transduction of a number of cytokines, including interleukin-1 (IL-1) and TNF-α (42), heat-induced cytokine upregulation in the whole organism activates HSP27, which, in turn, stabilizes cellular actin microfilaments (27). Thus, through direct heat effects or through cytokine upregulation, conditioning stresses resulting in cellular HSP accumulation may attenuate the loss of epithelial barrier integrity during subsequent heat challenges, allowing the organism to adapt to elevated temperatures.

In addition to HSP-mediated barrier integrity, HSP-associated heat adaptation may also involve endotoxin tolerance. Conditioning stresses that result in HSP accumulation or the overexpression of the HSP70 gene in cells confer tolerance to endotoxins in animals (21, 41, 46) and cells (9). This endotoxin resistance may reflect a tolerance to the direct effect of endotoxin or may reflect HSP-associated changes to cytokine production and resistance. In this regard, macrophages stimulated to accumulate HSPs show both transcriptional inhibition and decreased secretion of the inflammatory cytokines TNF-α and IL-1 (14, 43). Similarly, animals

that have undergone a conditioning heat stress sufficient to cause HSP70 accumulation show a decrease in circulating TNF-α after endotoxin exposure (25). In addition to decreased cytokine production by inflammatory cells and resultant decreases in circulating TNF-α, the cellular accumulation of HSP renders cells resistant to the cytotoxic effects of TNF-α (23, 26). Finally, TNF-α and IL-1 upregulate the HSPs (16, 42).

It remains to be seen whether HSPs are directly responsible for these cellular mechanisms of adaptation or are, instead, important markers of the effects. Other lipopolysaccharide-inducible genes such as secretory leukocyte protease inhibitor also inhibit TNF-α secretion by macrophages (24). Similarly, studies of TNF-α resistance have shown that blockade of nuclear factor-κB (NF-κB) signaling in cells results in increased sensitivity to TNF-α (4, 45), whereas TNF-α-induced apoptosis is suppressed through prior NF-κB activation by a conditioning stress such as exposure to radiation, daunorubicin, or IL-1 (48). Such data support the concept that the HSP response is linked to cellular pathways controlling endotoxin tolerance, cytokine production, and cytokine sensitivity.

Viewed as a whole, this literature demonstrates the following: *1*) reversible heat-induced changes in epithelial permeability are attenuated by HSP accumulation; *2*) cells and animals become endotoxin tolerant after HSP70 accumulation; and *3*) this endotoxin tolerance may be related to tolerance to the direct effects of endotoxin, tolerance to cytokine exposure, or inhibition of cytokine production by inflammatory cells. Whereas these studies used a single, relatively intense heat exposure to generate an HSP response, the cellular alterations that were associated with the HSP response were distinct from thermotolerance in that they did not result in the organism's survival of an otherwise lethal heat exposure but rather demonstrated alterations in cell physiology.

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