HOW DO ANIMALS ADAPT TO CHRONIC HEAT?
A PHYSIOLOGICAL GENOMICS PERSPECTIVE

Michal Horowitz, Laboratory of Environmental Physiology and Dept of Physiology, The Hebrew University, Jerusalem, Israel

Introduction

Among the various physical environmental stressors, “temperature is ecologically most important, for it is a factor that is all pervasive, and in most environments, lacks spatial or temporal constancy” (Cossins and Bowler, 1987). Concomitantly, upon transfer from one temperature to another for prolonged periods, most animals can adapt physiologically and biochemically to the new environment. This process is termed thermal acclimatization/ acclimation and, if successful, such adaptation enhances thermal tolerance in terms of the extreme of tolerable temperature and the duration of endurance in that temperature (Horowitz, 1998). Although time is required to develop acclimation, the acclimation effect is long acting. Acclimation is a “within life time mechanism”, reversible, and may involve a genetic basis, although not necessarily. We suggest that such a widespread event, which gives rise to a new phenotype, comprises a reprogramming of gene expression and translational processes. In this presentation, the mechanisms underpinning the phenotypic transition in response to persistent exposure to a hot environment (heat acclimation) and the beneficial outcomes are discussed.

Heat acclimation dynamics-molecular physiological linkage

Classically, heat acclimation is considered an autonomic-controlled array of physiological mechanisms working in concert to enhance heat endurance. The criteria for its expression are reduced metabolic and heart rates, as well as body temperature, low temperature-thresholds for activating heat dissipation effectors, and increased cardiovascular reserves and capacity of the evaporative cooling system (Horowitz, 1998; 2002). To elaborate the concept of heat acclimation, one must consider its kinetics over the time of acclimation. Apparent acclimation is observed already at the short-term heat acclimation (STHA) phase. During STHA, temperature homeostasis is achieved primarily via an augmented excitability of the autonomic nervous system to override desensitization of cell membrane receptors and signaling pathways, leading to impairments in peripheral cellular performance (Fig. 1).

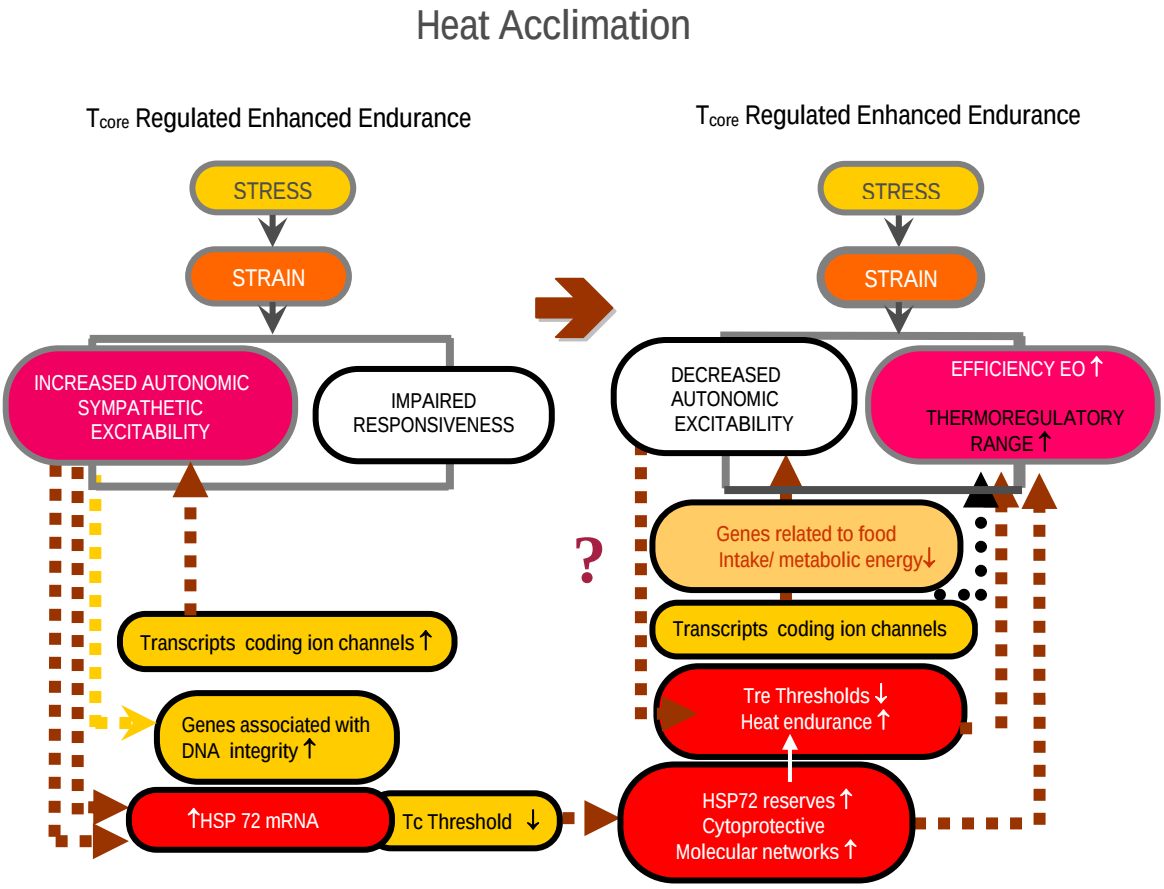


Figure 1. Conceptual model of heat acclimation – molecular physiological linkage.

A broad-scale genomic approach and gene-chip array technology unraveled important molecular events occurring at that acclimation phase. In the hypothalamus, where the thermoregulatory controller is located, a marked transient up-regulation in transcript levels is confined predominantly to genes encoding voltage-gated ion channels, ion pumps, channels, and transporters (Schwimmer et al., 2006). These changes, together with the up-regulation of hormone/neurotransmitter receptors and cellular messengers, collectively point to an enhanced depolarization, leading to the release of neurotransmitters and enhanced neuronal excitability (Fig. 1, left pathway; Fig. 2). This global transcriptional sketch is consistent with the physiological model of the acclimation process demonstrating enhanced excitability to compensate for perturbed cellular performance at STHA. Another important

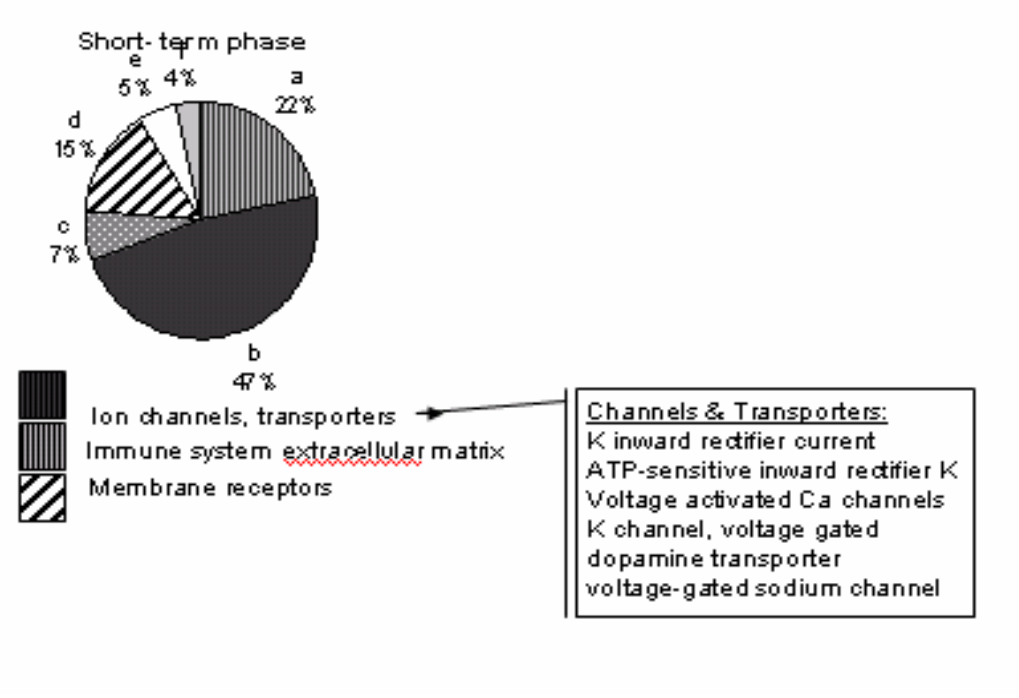


Figure 2. Global genomic response of hypothalamic genes to short-term heat acclimation (STHA). The pie charts demonstrates functional distribution of the up-regulated genes. It is notable that 47% of the up-regulated genes comprise ion channels, transporters and genes linked with transmitter activation. A list of significant up-regulated genes is shown on the right panel-bottom. (Derived from Schwimmer et al., 2006)

outcome of the enhanced excitability is the transcriptional up-regulation of the universal cytoprotective heat shock protein HSP 72 kDa (Fig. 1, right pathway). Note worthily, at the STHA state, enhanced *hsp* transcription is not accompanied by an increased cellular store of the encoded protein (Maloyan et al., 1999). A transient up-regulation of genes linked with maintenance of DNA integrity was also detected at this acclimation phase (Fig. 1; Horowitz et al., 2004).

The long-term heat acclimation (LTHA) process is characterized by a transition from the early transient, inefficient phase – the hallmark of STHA - to highly energy-efficient cellular machinery and integrative processes. In the hypothalamus, a drop in the expression of genes related to various metabolic activities, including those linked with mitochondrial energy metabolism and cellular-maintenance processes, is noteworthy, as is the down-regulation (or resumption to preacclimation level) of transcripts encoding ion channels, ion currents, and transporters, accompanied by a decrease in temperature thresholds for the activation of heat dissipation effectors (Fig. 1, Horowitz, 1998, Schwimmer et al., 2006).

The transition from the STHA phase to acclimation homeostasis unmasks slowly developed, long-lasting molecular cytoprotective signaling networks involving genes encoding proteins that are essential for heat-shock responses (HSR), anti-apoptosis, and antioxidation, collectively leading to enhanced thermal endurance and delayed appearance of thermal injuries (Fig. 1). The induction of a large cytosolic heat shock protein (HSP 72) “reservoir” (Fig. 1) provides sustained cytoprotection without the need for *de novo* protein synthesis (Maloyan et al., 1999). Concomitantly, this constitutive feature is reinforced by a faster HSR (namely faster *hsp* transcription and, in turn, translation of the encoded protein upon superimposition of acute heat stress). The mechanisms leading to these responses are not completely understood. Nevertheless, based on data derived from detailed studies on HSPs, we suggest the following:

1. A basic paradigm of STHA is to up-regulate the transcription of selective cytoprotective genes to increase optimal levels of their encoded proteins.
2. Given the interference of persistent adreno-blockade during the acclimation regimen with this response, the evolvement of the acclimation-protective networks likely involves the sympathetic nervous system (Fig. 1, Maloyan et al., 2000).

Collectively, the global acclimation transcriptional sketch is consistent with the physiological model of the acclimation process — a two tier defense strategy. The first strategy is an immediate transient response, associated with the maintenance of cellular integrity during the strain developed at the onset of acclimation and the second, a sustained response correlated with adaptive, constitutive processes.

Heat acclimation: cross-tolerance and interference

An inseparable outcome of acclimation is that adjusting to one environmental stressor can—in addition to involving primary adaptations—increases the amount of adjustment to additional stressors. Such cross-reinforcement raises the possibility of inducing adaptation to a stressor without prior exposure to that particular stressor. In contrast, certain environmental stressors interfere with and even abolish heat acclimation. Identifying these stressors and understanding the induced mechanisms involved in their interaction with heat acclimation will uncover acclimation “master regulators” and signaling pathways leading to heat acclimation.

Heat acclimation and cross-tolerance

An important beneficial effect of heat acclimation is the development of "cross-tolerance" against oxygen supply/oxygen demand mismatching, hyperoxia and ionized irradiation and its consequences (Eynan et al., 2002; Arieli et al., 2003; Robinson, Marmary, and Horowitz personal communication). Based on our genomic data, we argue that cross-tolerance emerges from an enhanced capacity or responsiveness of molecular signaling shared by adaptation to the primary stress (heat tolerance) and the secondary stress (e.g., oxygen shortage). The number of the alerted shared pathways is small, however, comprising cellular functions associated with the cytoprotective and energy metabolism modules that are enhanced by heat acclimation (Horowitz et al., 2004; Maloyan et al., 2005).

In the heart, significant molecular pathways linked with cross tolerance are the inducible heat shock protein genes, leading to a greater basal HSP 72 cytosolic pool in heat acclimated animals (as mentioned above) and the up-regulation of the hypoxia-inducible transcription factor, HIF 1α, the master regulator of oxygen homeostasis. A greater abundance of the HIF-1α protein and an enhanced transcriptional activation of target genes upon heat stress or ischemia insults characterize this state. The latter includes the up-regulation of *Vascular endothelial growth factor (Vegf*) mRNA, a consensus HIF-1α target, upon acute heat or ischemic stress and basal up-regulation of *Epo* and *EpoR* mRNA *(Erythropoietin and Erythropoietin receptor, respectively)* levels following acclimation, suggesting that the acclimation response consists of chronic and acute adaptive components. In accordance with our concept of heat acclimation-mediated cardioprotection via larger cytoprotective protein reserves, the markedly enhanced levels of EpoR, which mediates the protective function of erythropoietin in this tissue, might be beneficial to the heat-acclimated ischemic heart.

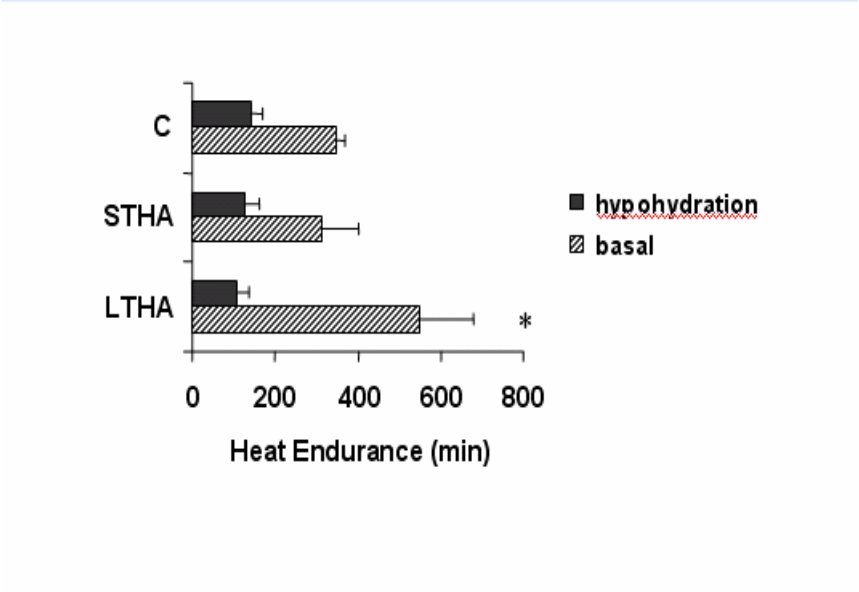
We can conclude that the occurrence of the chronic adaptive component agrees with our concept that greater cellular reserves of key cytoprotective proteins represent an important part of the acclimation cross-tolerance response.

An intriguing issue is the involvement of HIF-1 in heat acclimation. The HIF-1-mediated hypoxic response, which appeared early in metazoan evolution to regulate metabolic responses, is highly conserved (Semenza, 2004; Shams et al., 2004). We hypothesize that HIF-1 could be exploited by a variety of physiological adaptive mechanisms requiring metabolic changes, as in the case of heat acclimation, which shows an enhancement of the metabolic machinery to elevate energy potential upon insults. Our finding that HIF-1 is essential for acclimation of the nematode *C. elegans* (Treinin et al., 2003) confirms that this function developed early in metazoan evolution.

Interference of heat acclimation by hypohydration

Unequivocally, hypohydration overrides heat acclimation and abolishes the beneficial, elongated heat endurance acquired with heat acclimation (Fig. 3). Hence, understanding the signaling pathways that interfere with heat acclimation can provide insight into our understanding of major acclimation signaling.

Figure 3. Hypohydration interferes with heat acclimation.



The phenomenon of hypohydration's interference with heat acclimation at the level of gene expression was recently studied in the hypothalamus of heat acclimating rats. Hypohydration alone results in a significantly larger number of up-regulated transcripts relative to the control euhydrated state, among which a large number of transcripts are linked with ion transports, ion currents, and neurotransmitter activation. This genomic profile predominates when hypohydration is superimposed on LTHA in rats, which might link the impaired heat endurance occurring under these physiological conditions and a specific transcriptome profile**.** Whether this profile is linked with decreased thermal endurance is not yet completely understood. In-vivo studies on the thermoregulatory activity of heat acclimated, hypohydrated rats suggest that during hypohydration, a dichotomy exists between the central thermoregulatory beneficial responses, manifested by an elevated temperature threshold for heat-dissipation mechanisms and a decrease in the metabolic rate and the capacity of the peripheral effectors (Schwimmer et al., 2006). Considering that the evaporating salivary glands of heat-acclimated rats increase their output/stimulus ratio (Horowitz and Meiri, 1985; Kloog et al., 1986) while acclimated, we hypothesize that the disruption of acclimation thermal endurance at the hypohydration state is due to a failure in adjusting secretion at the glandular level rather than to a central failure.

Concluding remarks

To summarize, the heat acclimation profile discussed here points to a two-tier defense strategy. At the molecular level, the immediate transient response is associated with maintaining DNA and cellular integrity during the strain developed at the onset of acclimation, whereas the sustained response correlates with slowly developing, long-lasting cytoprotective signaling networks involving genes encoding proteins that are essential for heat-shock responses, anti-apoptosis, and antioxidation. At the integrative level, the early response is characterized by an immediate transient response, in which rapid transient mechanisms are recruited to alleviate the initial strain and to maintain cellular integrity. When acclimation homeostasis has been achieved, a sustained response correlated with adaptive, constitutive processes is developed. During both phases, cross-talk between molecular and integrative mechanisms has an essential role. Cross-tolerance and interference are inseparable part of the acclimation responses. Understanding these processes unmasks important signaling pathways and thus contributes to our understanding of the heat-adaptive processes. Along this line, an emerging new issue is the awareness that HIF 1α , the master regulator of oxygen homeostasis is an essential component in the pathway to heat acclimation.

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