Do Cellular Heat Acclimation Responses Modulate Central Thermoregulatory Activity?

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The classical concept of heat acclimation is of an autonomically controlled array of integrative physiological processes, improving heat tolerance. New evidence suggests that a temporal interplay between opposing autonomic and peripheral cell-originated responses, switched on by heat and autonomic stimulation of the cell membrane, and a marked increase in the stock of the inducible 70-kDa heat shock protein contribute to widening of the thermoregulatory activity span.

Homeotherms are able to maintain their body temperature within a very narrow temperature range. Therefore, when body temperature rises above its normally regulated range, because of either environmental factors or excess metabolic heat production, irreversible thermoregulatory failure may rapidly develop. Operating at a very “high gain,” the thermoregulatory system works in concert with a set of coded behavioral functions to prevent/attenuate this hazardous outcome. A variety of factors affect thermal tolerance. Among these, only two responses are directly provoked to combat the deleterious consequences of heat stress: 1) the rapid heat shock response (HSR) and 2) heat acclimation. HSR is a rapid, short-acting molecular process associated with the synthesis of several families of heat shock proteins (HSPs) of different molecular weights. It is thought to protect cells from noxious stimuli and to accelerate their repair following short, sublethal heat injury. The time course of HSP accumulation and the family to which they belong vary in different cells, but, on average, the HSR in the intact body seems to operate several hours following the stress and retains its activity for a few days. In contrast, heat acclimation is switched on in response to persistent, moderate ambient heat and takes time to develop but is long acting (several weeks). The classical concept of heat acclimation is of an autonomically controlled array of integrative physiological processes working in concert to improve heat dissipation. Cumulative data from recent studies imply, however, that during the process of acclimation thermoregulatory control is modified through interplay among autonomic, cellular, and molecular responses, each of different intensities, time courses, and even direction. Whether HSPs play a role in the latter mechanism is still an open question.

In contrast to poikilotherms, in which acclimation responses are evoked by a wide range of changes in body temperature and therefore can be easily detected individually, homeotherms undergo only very small shifts in their core temperature during acclimatization or acclimation (adaptation, under natural environmental stress vs. under artificial laboratory conditions, respectively). This raises the question, What are the nature and the magnitude of the strain required to elicit acclimation responses? Likewise, the marked acclimation response might result from the additive effects of many small, yet significant, changes at organ and cell levels. Because of the nature of this process, there are conceptual and methodological difficulties that prevent homeotherms from being the “first choice” model for studying cellular or molecular acclimation responses to hot environments. Our knowledge of the role of these processes in acclimating homeotherms is therefore sparse. Some aspects of the cellular heat acclimation responses in mammals, their dynamics during heat acclimation, and their contribution to short- and long-term thermoregulatory adaptive responses provide the central theme for this minireview. In this context, emphasis will be placed on processes associated with autonomic excitability, that is, effector organ feedback relationships. The role of HSP in acquiring thermal tolerance on acclimation will also be discussed.

The integrative acclimation response: a dynamic model

Heat acclimation is achieved through continuous or repeated challenge of the thermoregulatory system by a hot environment, in proximity to...
the upper limit of the thermoneutral zone. It enhances heat tolerance in terms of duration and extremes of toleration to stress and, in turn, delays the development of heat injuries via "widening of the dynamic thermoregulatory range." This is achieved by 1) increasing the capacity of the thermal effectors for heat dissipation, 2) decreasing heat production, and 3) under certain circumstances, expanding the body core temperature safety margins.

The thermoregulatory features of the acclimated status include lowered core temperature, reduced heart rate, elevated cardiovascular reserve, and increased capacity for evaporative cooling. The concept of widened dynamic thermoregulatory range is more readily understood by considering how this is achieved. For example, in the cardiovascular system, augmented thermoregulatory skin blood flow for removal of heat from internal organs is complemented by several coordinated activities, varied in different species. Collectively, these include a decreased vasodilatation temperature threshold leading to an extended body temperature range over which skin blood flow is regulated, increased basal skin vascular tone that allows greater amplitude for peripheral vasodilatation, and augmentation of the share of the cardiac output distributed to the splanchnic organs. Consequently, at the onset of the splanchnic thermoregulatory-vasomotor response, namely, the thermally induced splanchnic vasoconstriction coincidental with skin vasodilation, increased splanchnic vasoconstriction without a reduction of the splanchnic blood flow beyond the preacclimation normothermic level is possible. This coincides with the diversion of a larger circulating volume to the cutaneous vascular beds together with systemic blood pressure adjustment. As a result, in the acclimated state, better adjustment of splanchnic organ temperature partly contributes to the enhanced heat endurance. The adaptation of other thermoregulatory functions follows a similar principle.

To understand the mechanisms involved in heat acclimation, one must consider their kinetics, i.e., their changes over time. In a comprehensive study on humans, Senay et al. (14) provided substantial evidence that acclimation of the different effectors is not simultaneous and that the adaptations of each effector sometimes involve an array of diametrically opposed compensatory processes. Extensive investigations on the dynamics of acclimation in rodent heat-dissipating effectors have revealed that an apparent acclimated state is exhibited soon after short periods of exposure to the new environment. This is brought about by a cascade of transiently recruited mechanisms that alleviate the strain, even in the face of perturbed body homeostasis. Analyses of data obtained from simultaneous studies on intact animals and isolated organs of animals undergoing similar treatments unexpectedly revealed transiently impaired effector responsiveness during the initial phases of heat acclimation. This was compensated by acceler-
ated autonomic activity (Fig. 1). As acclimation progressed, the short-term mechanisms were replaced by efficient long-lasting processes working in tandem to produce the optimal adaptive state. An example of the extent to which enhanced efficiency can be attained (as for the heart) is depicted in Fig. 2.

We can conclude that acclimation is a continuum of processes, varying temporally and differing in efficiency and optimal performance. Ultimate acclimation is a best-choice “compromise.” This concept can be best illustrated by a simplified biphasic model (Fig. 3). In the initial, short-term heat acclimation phase (STHA; 1–5 days), the effector organ output-to-autonomic signal (EO/AS) ratio transiently decreases, i.e., accelerated efferent activity is required to override impaired peripheral responsiveness and to produce adequate effector output. This suggests that, during this period, the autonomic nervous system plays the major role in alleviating the sustained strain. In contrast, after long-term heat acclimation (LTHA; >3 wk), the evoked intrinsic organ adaptations reduce the need for accelerated excitation. This is manifested by increased effector output despite decreased autonomic stimulation (increased EO/AS).

Evidence for alterations in membrane/cellular signal transduction responses on adaptation of thermoregulatory effectors

During STHA, direct stimulation of the isolated submaxillary gland, the evaporative cooling organ of the rat (4), results in depressed water secretion, compared with the preacclimation control level. The results are identical whether the glands were stimulated electrically at a supramaximal rate via the chorda tympani nerve or by pilocarpine, a parasympathomimetic drug (6). This unexpected result drew our attention to the fact that the impaired responsiveness of this important heat dissipation effector implies a postsynaptic event. Cumulative data from studies on both STHA and LTHA rats as well as from a...
variety of heat dissipation pathways support this finding. Bradycardia, an accepted estimate of heat acclimation, is an early event in the evolution of the acclimation response. During STHA, however, higher carbachol (CCh) and noradrenaline (NE) concentrations are required for downregulation and upregulation, respectively, of the atrial beating rates than before acclimation. When the beating rate-transmitter dose relationship is calculated as a percentage of peak response, it emerges that the reduced responsiveness to parasympathomimetic stimulation is due to decreased sensitivity to the transmitters, probably attributable to intrinsic factors within the pacing cells. The drop in the sensitivity to NE is less pronounced, suggesting that, in the NE transduction pathway, postsynaptic events other than decreased sensitivity are responsible for the observed phenomenon (7).

Improved cellular performance also underlies the higher EO/AS observed on LTHA. In the rat evaporative cooling system, the use of $^{86}$Rb, a marker of secretory activity, demonstrated a significant increase in total stimulated glandular output (4). Likewise, in primates and humans, heightened responsiveness to central autonomic stimulation and greater sweating per unit length of the secretory tubule have been documented (13).

The involvement of cellular processes can be demonstrated in vasomotor thermoregulatory reflexes as well. In heat-stressed LTHA rats, the splanchic blood flow, the major circulatory reservoir for the thermoregulatory skin blood flow, remains elevated for a long period despite the induced skin vasodilatation (as opposed to the immediate vasoconstriction required to support skin blood flow before acclimation). When vasoconstriction does occur, it can be more pronounced than before acclimation, due to an enhanced postsynaptic cellular response, allowing for greater vasoconstriction despite the decreased autonomic excitability known to occur following LTHA (15). The improved cellular performance is mirrored by increased isometric vascular contraction, resulting from an augmented responsiveness of $\alpha$-adrenergic receptors and a decrease in number or responsiveness of $\beta$-adrenergic receptors (15). Studies on acclimated cardiac myocytes suggest that, distal to the adrenoceptors, augmented force generation is associated with greater elevation of cytosolic $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) on contraction, owing to changes in sarcoplasmic reticulum properties under these conditions (10). The underlying mechanisms leading to augmented contractility, distal to the receptors in the acclimated blood vessels, have not yet been elucidated.

What then are the target sites of the postsynaptic signal transduction pathways that undergo modulation on acclimation?

**The muscarinic receptors: mobilization of water secretion**

The data at hand pinpoint the cell membrane as the first target of the heat-induced changes. This is indicated by an array of cellular responses as well as by direct changes in the properties of the membrane per se. The predominant avenue for water secretion in the salivary and sweat glands of many mammalian species is the muscarinic receptors, since cholinergic muscarinic receptors are directly coupled to G protein, and is the most extensively studied system. To date, investigations have been carried out largely on the submaxillary gland of the rat (4). Scatchard plots reveal that the specific binding of the muscarinic ligand $^3$H-NMS (nabiline) is compatible with two populations of receptors having similar densities (density of binding sites) but different affinities (dissociation constant) for the ligand. Chronic exposure to heat produces changes in both density and dissociation constants. During STHA, in conjunction with the impaired glandular responsiveness, significant upregulation of the high-affinity receptor population takes place, leading to an increased ratio of high-affinity to low-affinity receptor population density. Concomitantly, a marked decrease is evident in the binding affinity of both the high- and low-affinity receptor populations. This effect is apparent both in direct binding and when muscarinic receptor subtype-selective antagonist competition experiments are used, suggesting a nonspecific, global response. As acclimation progresses, muscarinic receptor density gradually increases and, on LTHA, the binding to the high-affinity site reaches 163% of the preacclimation level. The affinities, however, return to the preacclimation values. Alterations in affinity usually imply receptor or G protein as targets (4). Thus the two transiently altered muscarinic receptor populations may reflect changes of the membrane receptor or a difference in receptor-G protein coupling, possibly of regulatory significance.

Along this line, it has been suggested that occupancy of the active low-affinity binding site is regulated by the high-affinity receptor population. Hence, it is likely that the membrane receptors function as the initial mediators of the altered responsiveness of the water secretory effector organ to the autonomic stimulation observed on heat acclimation.

The study of muscarinic receptors and investigations of other signal transduction pathways...
suggest that steps distal to those involving the cell membrane are subject to acclimation-type changes. Basal \([Ca^{2+}]_{i}\), and CCh-stimulated free cytosolic \(Ca^{2+}\) concentration \(([Ca^{2+}]_{c})\) can be used to assess these effects. A striking phenomenon in our model is the transient decrease, during STHA, of basal \([Ca^{2+}]_{c}\), levels in the secretory cells, accompanied by changes in the evoked \([Ca^{2+}]_{c}\), signal on stimulation (4). This phenomenon does not fully concur with the changes observed at the receptor level. It is possible, however, that the elevated CCh-evoked \([Ca^{2+}]_{c}\), is a cellular compensatory mechanism to produce an adequate secretory \(Ca^{2+}\) response coincidentally with a widened regulatory range. Another complication is the discrepancy between the \([Ca^{2+}]_{c}\), and the reduction in isolated glandular output, indicating that steps distal to \(Ca^{2+}\) mobilization may be affected as well.

The phenomenon of transient receptor upregulation coupled with desensitization induced by short exposure to moderate heat has been observed for both adrenergic and muscarinic receptors by other investigators. In the rat parotid gland, Fujinami et al. (2) attributed desensitization of the adrenergic receptors to receptor-G protein uncoupling, leading to decreased adenosine 3',5'-cyclic monophosphate accumulation. In the muscarinic transduction pathway (3), the same investigators showed that muscarinic receptor upregulation, coinciding with desensitization, is reflected in a reduction in \(Ca^{2+}\) mobilization in response to inositol 1,4,5-trisphosphates (IP3) administration. However, on prolongation of the stress, decreased \(Ca^{2+}\) mobilization is due to diminution of carbachol-stimulated IP3, generation. These data also implicate G protein as a tentative target for heat interference. The use of 5'-guanylyl imidodiphosphate [Gpp(NH)p], a nonhydrolyzable activator of G protein, in our binding experiments provided further support for the role played by G protein as a stress (heat)-strain mediator. By its action, Gpp(NH)p reduces the high-affinity-to-low-affinity ratio of the muscarinic receptors (as measured by \[^{[3H]}\text{NMS}\]). This decrease was much more pronounced on STHA, during which upregulation of the high-affinity muscarinic receptors occurs.

In several confluent rodent fibroblast cell lines studied, noxious heat shock (45°C) has been shown to act via its effect on transmembrane signal transduction associated with IP3 release and a number of \(Ca^{2+}\)-mediated events, providing evidence for \(GTP\)-binding G proteins as mediators in this process (1). Our data imply that prolonged moderate heat also affects the G proteins.

Collectively, the above findings agree with our postulate on the basis of integrative responses, that is, that heat acclimation is a continuum of temporally varying processes, which can be grouped into two phases. Understanding the precise regulatory benefits of each individual step along the signal transduction pathway awaits further investigation. It is likely, however, that the observed changes contribute to the fine tuning of the response or its amplification, thereby widening the thermoregulatory span.

**Are cellular acclimation responses specific?**

The specificity of the acclimation responses observed in the muscarinic transduction pathway was compared with those observed during acute heat stress (4, 8). In nonacclimated submaxillary glands, heat stress induces receptor downregulation. In contrast to acclimation, heat stress leads to an increased high-affinity-to-low-affinity population ratio by reducing the density of the low-affinity receptor population (4). Despite this seemingly opposing effect, when heat stress was superimposed on STHA, it did not abolish the receptor upregulation resulting from the acclimation effect. After LTHA, acute heat stress resulted in the disappearance of the low-affinity muscarinic receptor population without affecting the high-affinity one. Acute heat stress also tends to increase binding affinities during all acclimation periods. These results, coupled with the data on the \(Ca^{2+}\)-evoked signal measured in our system as well as in others [e.g., fibroblasts (1)], suggest that the two heat treatments, acute and chronic, produce mechanistically independent responses.

**Do cellular responses reflect localized or global systemic/neurohumoral events?**

To date, our findings are contradictory. During STHA, receptor upregulation is coincidental with accelerated excitability and desensitization, whereas decreased receptor affinity is concomitant with an augmented \(Ca^{2+}\) signal. These results suggest that, although thermal adaptation in the whole animal is a multisystem integrative response, there is a hierarchy in effector system regulation in which alternating cellular responses may play a local regulatory role. The extent to which effector responses in heat stress are locally mediated is not well documented.

To challenge our hypothesis regarding the existence of a cellular strategy for coping with desensitization, we developed a novel experimental paradigm that precluded the involvement of neurohumoral factors using a cultured salivary acinar cell line (HSY) undergoing simulated acclimating conditions (9). Although cultured cells may respond differently from the whole organism,
some extrapolations to the intact animal can be made. This model's compatibility with receptor responsiveness to STHA has been assessed. Two-day exposure of HSY cells to a heating protocol that simulates changes in body temperature on STHA caused muscarinic receptor upregulation, an alteration of the ratio of high- to low-affinity binding sites, and a nonselective decrease in binding affinity (9). Receptor upregulation was markedly more pronounced than in submaxillary gland preparations. These findings support our hypothesis of a cellular compensatory strategy for coping with heat stress. Preliminary experiments with HSY cells in which protein synthesis was blocked by cycloheximide indicated that a major component of the increased receptor population was newly synthesized. Acid stripping of the liganded receptors suggested that these newly synthesized receptors are localized on the surface of the cell membrane (8).

It can thus be concluded that moderately adverse environmental signals induce membranous strain and an array of cellular compensatory strategies for coping with this membranous strain. In the intact animal, the cell membrane is subjected to both neurohumoral factors and evoked cellular compensatory responses. The observed outcome is the result of these complex dynamics. Our data also suggest that cellular responsiveness plays a role in the temporally varying central-peripheral interactions occurring during heat acclimation, as illustrated in Fig. 4. New experimental paradigms and molecular biology techniques facilitating further study of these events are now being developed.

Do central cellular functions undergo changes?

Compared with the available data on cellular responses of peripheral organs, information on central structures is limited. Although increased central sensitivity to heat stimuli has been documented for heat acclimation, conclusions have been derived primarily from peripheral effector responses to central stimulation. From the arguments presented above, it is now clear that peripheral responses could be misleading. Considering the high plasticity of the temperature characteristic of hypothalamic neurons, one would expect, however, that adaptation to different ambient temperatures might result in some central changes. Two pieces of information are now available. Pierau et al. (12) showed that in warm-adapted rats the number of warm-sensitive neurons is markedly reduced and cold-sensitive neurons disappear. This suggests that the plasticity of the hypothalamic network may enable adjustment to long-term changes in the thermal environment. From another aspect, Patronas et al. (11) found an increased number of angiotensin receptors in the paraventricular nucleus of STHA rats. This finding may indicate that, on acclimation, membranous changes take place in specific central structures.

FIGURE 4. Central-peripheral interactions during heat acclimation. Ambient heat (dark gray arrows) mobilizes the thermoregulatory system (light gray arrows) via its influence on T\textsubscript{core} and the warm receptors. Concomitantly, T\textsubscript{core} has a cumulative direct effect on cell membranes (black arrows). The so-far known targeted sites are membrane receptor density (B\textsubscript{max}) and affinity (dissociation constant, K\textsubscript{d}), cytosolic Ca\textsuperscript{2+} concentration, and agonist-stimulated cytosolic Ca\textsuperscript{2+} concentration. Temporally altered autonomic activity with acclimation stems from thermoregulatory demands (the global thermoregulatory loop) modified by effector organ cell responsiveness. Latter determines effector organ/autonomic signal ratio and, in turn, autonomic excitability required for adequate effector output. POAH, preoptic and anterior hypothalamic regions.
Heat shock response

HSPs are thought to play a central role in cellular heat protection mechanisms. It is therefore tempting to hypothesize that this pathway has a dual role in the acclimation process. Although very early during acclimation this pathway confers rapid thermotolerance, once acclimation has been achieved, altered thresholds for the mobilization of the HSR or protein accumulation may dampen the intensity of other cellular responses. Surprisingly, a recent study conducted in our laboratory on heat-acclimating rats (5) showed that, in the initial phase of heat acclimation, a noticeable improvement in heat tolerance coincided with complete desensitization of the HSR. The 70-kDa HSP (HSP70) family is thought to be the most responsive to heat stress. Nevertheless, on STHA, the basal level of the inducible HSP70 (iHSP70), as measured in the heart, was lower than that of control animals and remained almost unchanged at that lowered level for at least 48 h following exposure to heat stress. These results do not support that this HSP plays an active role in enhancing heat tolerance during this phase of heat acclimation. However, careful follow-up of HSP levels with the progression of heat acclimation showed that, after the initial decline, iHSP70 upregulation occurred. After LTHA, rat heart and brain displayed ~240% more iHSP70 than occurred in nonacclimated rats. Concomitantly, the rate of increase following heat stress was significantly faster, with HSP reaching a peak level 1 h after stress termination. In contrast, nonacclimated rats acutely exposed to heat attained a maximal HSP level only 4 h after heat stress but were able to produce almost threefold more HSP than their basal level. HSP70 are markers of injury. Concomitantly, their presence is protective. Hence, these data suggest that, in parallel with the development of other protective mechanisms, chronic exposure to moderate heat increases the stock of HSP. In cultured cells, HSPs render cells less temperature sensitive and elevate the degree of the lethal temperature. Hence, their accumulation on LTHA could also contribute to the elevation of the upper temperature safety margin and, thus, further widen the thermoregulatory range, as already discussed at the beginning of this article.

The extent of this beneficial effect is still not clear, since our knowledge of the level of hyperthermia at which heat stroke develops in the acclimated body is still rather controversial and varies under different experimental conditions. There is evidence, however, that the threshold for thermal injury can be elevated.

The finding that chronic, moderate heat induces HSP accumulation (5) suggests that this protein-dependent pathway, in addition to its role in the rapid HSR, follows a long-term adaptive process. The nature and the role of this process are not yet understood. It is noteworthy that comparisons of levels of HSP70 between invertebrates, poikilothermic vertebrates, and humans inhabiting either normothermic or hot environments showed that the hot environment species have higher HSP levels compared with species of the same genus inhabiting normothermic environments. This may suggest that the production of a larger stock of HSP on acclimation recapitulates the evolutionary adaptation.

Summary and conclusions

In this brief review, we provide evidence indicating that changes take place in cell membranes when they are subjected to STHA. These changes seem to switch on cellular responses: initially, a cascade of transient acclimation compensatory responses and, later, long-acting mechanisms. These alterations, which are heat acclimation specific, modify autonomic excitation according to a biphasic pattern. The HSR seems to develop independently, conjointly with other avenues of heat acclimation. Changes in the efficiency of this response stem from a marked increase in the stock of HSP. The short-term acclimation phase appears to be the most conspicuous. This is likely to be the “time window” during which a variety of long-acting processes are switched on. Indeed, recent novel findings from our laboratory provide evidence of a marked elevation in the expression of several gene products, including the mRNA for HSP, and changes in the lipid composition of the cell membrane during this phase.

I regret that, due to editorial constraints, investigators who have made important contributions to the field of heat acclimation could not be cited.

The constructive comments of Drs Y. Oron, R. Arieli, and E. Shohami and of my students while writing this review are highly appreciated.

Most of my studies, which provided the basis for this article, were supported over the years by the Israel Science Foundation, founded by the Israel Academy of Sciences and Humanities.

References


