Heat acclimation increases the basal HSP72 level and alters its production dynamics during heat stress

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Maloyan, Alina, Aaron Palmon, and Michal Horowitz. Heat acclimation increases the basal HSP72 level and alters its production dynamics during heat stress. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R1506–R1515, 1999.—It has been previously shown that heat acclimation leads to an elevated basal level of 72-kDa heat shock protein (HSP72). Augmented expression of HSP72 is considered as a cytoprotective response. This led us to hypothesize that alterations in the heat shock protein (HSP) defense pathway are an integral part of the heat acclimation repertoire. To investigate this, we studied the temporal profile of basal HSP expression upon acclimation and the dynamics of their accumulation subsequent to acute heat stress (HS). In parallel, HSP72 mRNA level before and after HS was measured. For comparison, HSC mRNA (the constitutive member of 70-kDa HSP (HSP70) family) was measured in similar conditions. Heat acclimation was attained by continuous exposure of rats to 34°C for 0, 1, 2, and 30 days. HS was attained by exposure to 41 or 43°C for 2 h. Thermal regulatory capacity of the rats was defined by rectal temperature, heating rate, and the cumulative heat strain invoked during HS. HSP72 and HSP70 gene transcripts were measured in the left ventricle of the heart by means of Western immunoblotting and semiquantitative RT-PCR, respectively. The resultant acclimatory change comprised a higher resting level of the encoded 72-kDa protein (∆175%, P < 0.0001). After HS, peak HSP72 mRNA level was attained, 40 and 20 min post-HS at 41 and 43°C, respectively, vs. 60 and 40 min in the nonacclimated group. The subsequent HSP synthesis, however, was dependent on the severity of the cumulative heat strain. At the initial phase of heat acclimation, augmented HSP72 transcription unaccompanied by HSP synthesis was observed. It is concluded that upon heat acclimation, the HSP defense pathway is predisposed to a faster response. At the initial phases of heat acclimation, inability to elevate the HSP cytosolic level rules out their direct cytoprotective role.

72-kilodalton heat shock protein; 72-kilodalton heat shock protein messenger ribonucleic acid; 73-kilodalton constitutive heat shock protein messenger ribonucleic acid; heat stress; heart; rats

There are a variety of factors that can affect thermal tolerance. Among these, however, only two classes of adaptations are directly evoked to combat heat stress (HS): 1) the heat shock response (HSR), and 2) heat acclimation. The HSR (4, 30, 32) is compatible with the development of rapid transient thermotolerance subsequent to acute HS. It is due, at least in part, to heat shock proteins (HSP) binding to denatured or nascent polypeptides in the different compartments of the cell. While the resting cellular HSP level is rather low, a rise in body temperature increases the transcription of the heat shock genes, leading ultimately to augmentation of their level in the cell. Among the HSP, the inducible isoforms of the 70-kDa family are considered as the most responsive to HS (as well as to ischemia) and a variety of pharmacological stresses (e.g., Ref. 22). In contrast, the constitutive HSP, the 73-kDa form (HSC73), is expressed constitutively in all cells, and in most species increased only slightly subsequent to HS. Recently, HSP such as 23 and 104 kDa have attracted interest in conferring thermotolerance (18, 25). The protective HSR cascade starts within minutes (16), although its temporal value in the whole, intact body, as well as in cells in culture, is not immediate. In cells, thermotolerance is best observed 12–24 h (16) after HS, whereas in intact animals, it is fully expressed ~24 h after the given stress. It is then manifested by a significant increase in the ability to withstand HS (+60% in rats and mice; Horowitz, unpublished observations; and Ref. 38). The share of HSP in this thermal preconditioning-induced response is the “cellular attempt” to protect vital components from thermal damage in a way that facilitates survival and subsequent recovery after the stress is removed.

In contrast to the HSR, heat acclimation (6–8, 10) is a slowly developing response, evoked by chronic exposure to moderate heat. Our extensive studies on the acclimating rat suggest that acclimation is a biphasic process (8). A similar pattern is inferred for humans (e.g., Ref. 39). Collectively, heat acclimation comprises an initial transient phase characterized by accelerated autonomic activity to rapidly control heat dissipation effector(s). This enhanced activity is reduced when developing biochemical processes improve effector organ efficiency. This is when acclimation has been achieved. At that phase, enhanced heat tolerance is conferred primarily by the improved integrative physiological activity of the heat dissipation effectors. In the cardiovascular system of the rat, for example, this is manifested by elevated splanchnic blood flow to allow better regulation of deep core temperature (34), increased stroke volume, and decreased heart rate (33). Concomitantly, intrinsic cellular physiological and biochemical modalities of adaptations lead to increased cardiac pressure development and increased arterial force generation in the face of lowered oxygen consumption. Collectively, these suggest increased cardiovascular efficiency (8).

Evidence is available that heat-tolerant species, invertebrates as well as a variety of vertebrates including...
ethnic human populations genetically adapted to high ambient temperatures, are characterized by a higher content of 70-kDa HSP (HSP70)-like proteins compared with their related species inhabiting moderate or cold environments (20, 37). Heat acclimatization also induces HSP elevation. In the goby fish, for example, this was shown for 90-kDa HSP together with an altered temperature threshold for their synthesis compared with that of the same species inhabiting normothermic environments (1–3). Likewise, we showed a marked elevated resting level of 72-kDa HSP (HSP72) in hearts (11) and brains (31) of heat-acclimated rats, suggesting that heat acclimation, similar to evolutionary adaptation to a hot environment, produces changes in the HSP system. This may imply that the HSP defense pathway is an integral part of the heat acclimation repertoire.

With the consideration of the biphasic nature of heat acclimation (7, 10), it is tempting to hypothesize that at the very early period of acclimation, HSP confer rapid thermotolerance. When acclimation has been achieved, an altered threshold for HSP production, or their elevated basal level, may buffer the intensity of other cellular responses.

The aims of this investigation were as follows: 1) to elucidate whether members of the HSP70 family play a role in the initial, stressful phases of the acclimation process and 2) to determine whether the acclimation process alters basal HSP expression and the dynamics of their accumulation subsequent to acute HS. The rat heart was chosen as a model. The rationale for selection of this organ was twofold: 1) during the process of heat acclimation, the heart (as well as the entire cardiovascular system) is volume overloaded and, hence, may reflect the body response to heat strain (12, 39); and 2) heat acclimation was shown to confer ischemic tolerance (17). We therefore wanted to examine whether accumulating HSP are part of the cross-tolerance repertoire.

The results obtained show that heat acclimation increases the stock of the inducible HSP72 in the cells and alters both rate and magnitude of HSP gene transcription upon HS. In contrast, the steady-state level and dynamics upon stress of the mRNA encoding for the constitutive HSC73 showed no changes.

**MATERIALS AND METHODS**

Male 3-wk-old Rattus norvegicus (Zabar strain, albino var) initially weighing 80–90 g, fed on Ambar laboratory food and water ad libitum, were used. The animals were randomly divided into two groups: 1) control-normothermic (C) and 2) heat acclimated (AC). The latter group included fully acclimated rats (long-term heat acclimated; LTHA) and those that underwent only the initial phases of heat acclimation (short-term heat acclimation; STHA). Each rat group was subdivided into those rats that received no additional treatment and those which were subjected to HS. The levels of the transcribed HSP72 and HSP73 mRNA and the expression of HSP72 protein were measured before and subsequent to several heating protocols as described below.

Experimental conditions. The C group was held at an ambient temperature of 24 ± 1°C; heat acclimation was attained by continuous exposure to 34 ± 1°C and 30–40% relative humidity in a light-cycled room (12-h:12-h light-dark cycle) for 1, 2 (STHA), and 30 days (LTHA) as previously described (6). To characterize differences between the controls and the acclimating rats, rats were studied before and after exposure to heat stress at 41°C for 2 h. To further characterize the C and the fully AC rats, additional rats, of these groups only, were exposed to HS at 43°C for 2 h. Exposure to these two temperatures produced different rates of heating and heat strain, depending on the physiological thermal capacity of the acclimated vs. the nonacclimated animals. Therefore, within- and between-group comparisons were made. During the heat stress, rectal temperature (Tre) was monitored on-line using a YL402 thermistor, inserted 6 cm deep beyond the anal sphincter, and attached to a computerized data acquisition system.

Experimental protocol. All rats were killed by cervical dislocation. For mRNA analyses, the heat-stressed rats were killed 20, 40, and 60 min after the given stress, whereas to determine HSP expression, the animals were killed 1, 4, 24, 48, 72, and 96 h after the given stress. Hearts were rapidly excised and placed in ice-cold (4°C) physiological saline. The hearts were then mounted on a Langendorff perfusion apparatus and retrogradely perfused to wash all remaining blood out with Krebs-Henseleit buffer containing (in mM) 120 NaCl, 4.7 KCl, 1.2 MgSO4, 1.2 KH2PO4, 1.25 CaCl2, 25 NaHCO3, and 11 glucose, at pH 7.4, and aerated with a mixture of 95% O2–5% CO2 (13). The perfusate was kept at 37°C by a circulating water bath. After 2-min perfusion, the hearts were removed from the apparatus, and the left ventricle was carefully excised, frozen, and stored at −70°C until analysis.

Semi-quantitative detection of mRNA by RT-PCR. To measure the transcribed mRNA for HSP70 (constitutive and inducible), semi-quantitative RT-PCR was used. L. ventricular tissue from five hearts of each group was homogenized with a polytron (Kinematika, Lucerne, Switzerland). Total RNA was extracted with TRI-REAGENT (Molecular Research Center). A quantity of 10 µg of total RNA was reverse transcribed in a 50 µl reaction mixture containing 0.5 µg of oligo(dT)15 primer, together with 400 U Moloney murine leukemia virus reverse transcriptase, according to the manufacturer’s instructions (United States Biochemical, Cleveland, OH). For the PCR, 5 µl of the cDNA mixture were added to 50 µl of a master mix containing 200 µM of each dNTP, 100 pm of each specific primer, as well as 1 mM MgCl2 and 1.5 U Vent polymerase (United States Biochemical). We synthesized DNA oligonucleotide primers for HSP72 selected from the published HSP72 gene nucleotide sequence (19). The sense primer was based on the sequence number 546–567 5′-GCT-GAC-CAA-GAT-GAA-GGA-GAT-C-3′ and the antisense number 1017–1038 5′-GAG-TCG-ATC-TCC-AGG-CTG-3′. The DNA oligonucleotide primers for HSC70 (HSP73) were selected from the published sequence of the HSC70 gene (36). The sense primer was based on the sequence number 1023–1080 5′-CAA-TCA-GAT-GAT-GAT-GAT-GAT-C-3′ and the antisense number 1201–1256 5′-AAA-AAT-GTC-GTT-GGG-GTT-CA-3′. The primers were designed to amplify a product that crosses introns to avoid confusion between the mRNA transcript and genomic DNA. The PCR reaction conditions were optimized for each set of primers. To check the sensitivity and linearity of the amplification, PCR was performed in a range of different numbers of cycles, input RNA, cDNA, annealing temperatures, and concentration of Mg2+. Calibration curves are presented in Fig. 1. The optimal reaction conditions chosen were an annealing temperature of 64°C, Mg2+ concentration of 1 mM, and cycle number 40 and 28 for the 72- and 73-kDa mRNA, respectively. Samples were amplified in an automated thermal cycler (Perkin Elmer Cetus, Emeryville, CA). To ensure different amounts of initial...
mRNA, parallel actin amplification was performed (annealing temperature, 62°C; 35 cycles) with the following oligonucleotides: 5'-GAG-ACC-TTC-AAC-ACC-CCA-GCC-3' (sense) and 5'-GGC-CAT-CTC-TTG-CTC-GAA-GTC-3' (antisense) (31). The PCR products were separated on 1.5% agarose gel and stained with ethidium bromide. The stained gels were photographed under ultraviolet illumination using Polaroid 667 film. The prints were scanned by a VISTA 8S scanner (Umax), and the optical density of the bands was computer analyzed by NIH 1.6 Image Software (NIH). The relative intensity of bands for the relevant mRNA was correlated by the relative intensity of the internal control, actin. A number of studies (e.g., Ref. 24) have already proven that hyperthermia does not affect the steady-state level of the mRNA of this housekeeping gene. We (M. Eynan, A. Palmon, and M. Horowitz, unpublished data) could not show any apparent change in β-actin mRNA level (see also Fig. 4) with heat acclimation.

Western immunoblotting. The left ventricles were homogenized with SDS sample buffer (20% glycerol and 6% SDS in 0.12 M Tris at pH 6.8), centrifuged at 12,000 rpm for 20 min at 4°C, and boiled for 10 min. The protein concentration of the myocardial specimens was quantified by the Bradford method (Bio-Rad Laboratories, Richmond, CA). Prepared samples were further diluted in sample buffer to allow loading of 50 µg of total protein per gel track. Protein was separated on 12.5% polyacrylamide gel under denaturing conditions according to the method of Laemmli (15). The samples were diluted in dissociation buffer (10% SDS, 200 mM EDTA, 10% glycerol, 5% 2-mercaptoethanol, and 0.001% bromphenol blue), vortexed, and heated at 95°C for 2–3 min. Electrophoresis was conducted at 50 mA for 2 h. After separation by electrophoresis, proteins were transferred onto nitrocellulose (190 mA, 4°C, 1 h) by Western blotting. The nitrocellulose sheets were then washed for 2 h in PBS containing 0.1% dried skimmed milk powder (Marvel) to block nonspecific binding sites. After washing, membranes were incubated at 4°C overnight with monoclonal IgG cross-reactive to the inducible HSP72 anti-body (Stressgen, Sidney, Canada) at 1:1,000 dilution. After repeated washing in PBS with 0.2% Tween 20, the membranes were incubated with horseradish peroxidase-conjugated rabbit anti-mouse IgG (Sigma) at 1:10,000 dilution at room temperature for 1 h. The membranes were then washed and developed to enhance chemiluminescence (Amersham, Bucks, UK) detection and exposed to X-ray film (Kodak). The HSP72 level was measured by scanning the immunoblots with laser densitometry. Band density was calculated by integrating the area (in pixels) and normalized to the level of human recombinant HSP72 loaded in the same gel each time. Each band density was measured five separate times and averaged.

Calculations and statistics. Heating rate (°C/min) was calculated from the regression lines fitted to T re points, starting from normothermic temperatures until the onset of the hyperthermic plateau. The area below the ΔT re curves during the entire heat exposure yielded heat storage (°C/min × 0.83 × body wt) normalized for 100 g body wt and was compatible with the cumulative heat strain (5).

For statistical analysis, two-way ANOVA was employed using commercially available computer software. Treatments were taken as the fixed effects, and the individual hearts were assumed to be random samples from the animal heart population. Student's unpaired t-test was used for individual matched-group comparisons. Data are expressed as means ± SE. Values of P < 0.05 were considered to be statistically significant.
RESULTS

Body temperatures and heating rates. Basal rectal temperatures, rectal temperature upon termination of the HS, and the actual heat strain of all experimental animals are presented in Table 1, and Fig. 2 illustrates Tre change over time with the course of the HS. Normothermic Tre did not differ significantly between any of the experimental groups except for day 1 of the acclimation. This fits with the temporal Tre profile with acclimation: initial overshoot, followed by stabilization at a Tre that is slightly elevated compared with the preacclimation level (6). Exposure of the rats to 41°C delineated differences in the Tre of the hyperthermic plateau (the Tre at which core temperature is regulated during HS; Ref. 9), the rate of heating, and the amount of heat strain between the various acclimating groups. At a similar ambient HS (41°C), 1-day AC (AC1) rats attained a hyperthermic plateau Tre that was only 0.6°C higher than the pre-HS level, whereas 30-day AC (AC30) rats showed the highest elevation from basal to plateau Tre, 3.2°C. Rate of heating and heat strain in these groups matched these changes. Further exposure of the C and AC30 rats to HS at 43°C resulted in a significant change in their rate of heating compared with that attained upon exposure at 41°C. In the C rats at 43°C (C-43), rate of heating slightly increased. In contrast, rate of heating of the AC30–43 showed a profound decrease. Under these physiological conditions, the calculated heat strain in the C rats increased by almost 50%, whereas that of the AC rats decreased by 20% compared with the developed heat strain in the matched 41°C exposed groups. The latter phenomenon was observed in several previous investigations in our laboratory (Ref. 27, and T. Moses, Y. Shapiro, D. Moran, and M. Horowitz, unpublished data).

Steady-state HS provoked mRNA changes with acclimation. The steady-state HSP72 mRNA profile in hearts of the nonstressed rats with heat acclimation showed temporal variations as depicted in Fig. 3. On day 1 of the acclimation, HSP72 mRNA slightly decreased. Marked upregulation was observed on day 2, whereas on day 30 of the acclimation, the mRNA was almost

Table 1. Effects of ambient heat stress (at 41 or 43°C) on rectal temperature, heating rate, and calculated cumulative heat strain in nonacclimated and heart-acclimated rats

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>1 Day (41°C)</th>
<th>2 Days (41°C)</th>
<th>30 Days (41°C)</th>
<th>Control</th>
<th>1 Day (43°C)</th>
<th>2 Days (43°C)</th>
<th>30 Days (43°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal Tre, °C</td>
<td>37.50 ± 0.46</td>
<td>38.04 ± 0.17*</td>
<td>37.86 ± 0.23</td>
<td>37.80 ± 0.10</td>
<td>40.17 ± 0.12</td>
<td>42.04 ± 0.16*</td>
<td>39.18 ± 0.27</td>
<td>40.86 ± 0.42§</td>
</tr>
<tr>
<td>T_hyper, °C</td>
<td>0.045 ± 0.004</td>
<td>0.052 ± 0.01</td>
<td>0.019 ± 0.002*</td>
<td>0.037 ± 0.002</td>
<td>237.25</td>
<td>344.8</td>
<td>70</td>
<td>196.6</td>
</tr>
<tr>
<td>T Heat strain, cal</td>
<td>41.00 ± 0.43t</td>
<td>40.86 ± 0.42§</td>
<td>40.37 ± 0.007†</td>
<td>306.5</td>
<td>250.4</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE and were derived from representative groups of 6–12 animals in each treatment. Heat strain was calculated from mean values. Nonacclimated rats were maintained at 24 ± 1°C. Heat-acclimated rats were maintained at 34°C and 35% relative humidity. Basal rectal temperature (Tre), Tre before subjecting to heat stress (HS); T_hyper, hyperthermic Tre upon termination of 2-h HS. * Significant difference from control (at HS of 41°C). † Significant difference from matched group at 41°C (*P < 0.001, †P < 0.025, ‡P < 0.05, §P < 0.1).
undetectable. HS increased HSP72 mRNA concentration. The magnitude of the mRNA concentration, however, varied for the various acclimating groups (Fig. 4). After HS at 41°C (Fig. 4, right), C hearts showed a continuous increase in HSP72 mRNA level during the first hour after HS. In the LTHA group (AC30), peak HSP72 was observed 40 min after HS. The peak reached by the mRNA, however, was similar to that of the C rats. Percent mRNA rise at its peak in the AC30 hearts compared with the nonstressed steady-state level was markedly greater than in the C group (Table 2). To further pinpoint the differences between C and AC30 rats, HSP72 transcription was measured after HS at 43°C. Under this condition, peak HSP72 transcript, in both C and AC30 hearts, was observed 20 min after HS (Table 2 and Fig. 5). The relative peak value (peak-to-resting ratio) in C rats was markedly higher than that measured for the 41°C

Table 2. Peak/basal HSP72 mRNA and HSP72 ratio in control and heat-acclimated rats after heat stress at 41 or 43°C

<table>
<thead>
<tr>
<th></th>
<th>HSP72 mRNA</th>
<th>HSP72</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Peak/basal</td>
<td>Time to peak, min</td>
<td>Min</td>
</tr>
<tr>
<td>C</td>
<td>41°C</td>
<td>2.34 ± 0.36</td>
<td>&gt;60</td>
</tr>
<tr>
<td></td>
<td>43°C</td>
<td>3.47 ± 0.12</td>
<td>20–40</td>
</tr>
<tr>
<td></td>
<td>41°C</td>
<td>10.57 ± 1.67*</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>43°C</td>
<td>10.04 ± 1.62*</td>
<td>20–40</td>
</tr>
</tbody>
</table>

Values are means ± SE. Heat shock protein (HSP) mRNA density (in pixels) is relative to β-actin density of same heart sample. HSP72, 72-kDa heat shock protein. Statistical analysis for differences in peak-to-resting ratio between C to AC30: *P < 0.0005, †P < 0.005. For statistical difference between peak and basal values, see Figs. 4 (mRNA) and 7 (HSP72).
HS-exposed C rats. Thus the augmented ambient heat load affected both rate and magnitude of increase in mRNA concentration.

In the STHA groups (Fig. 4 and Table 3), during HS, steady-state HSP72 mRNA levels varied from day to day. AC1 group showed 50% mRNA elevation, peaking 20 min after HS. On day 2 of the acclimation (AC2), HSP72 mRNA gradually declined after the first sampling time (0 min).

Comparable measurements of HSC transcript showed no significant change, either on heat acclimation or after HS, in all the experimental groups (Fig. 6). This may suggest that in this model, this member of the HSP70 family does not confer thermotolerance.

Basal and HS-provoked HSP72 cytosolic levels. Subsequent to our finding that heat acclimation induces alterations in the inducible HSP72 mRNA, the level of the HSP72 gene product was measured (Fig. 7). The steady-state level in AC30 hearts was almost twofold higher than before heat acclimation. Likewise, after HS at 41°C, peak HSP level was detected 1 h after the stress, compared with 4 h in the C hearts (Fig. 8). Hence, the faster increase of translation of HSP in the AC hearts after the HS corresponded to the accelerated increase in mRNA in this group. The relative increase with time after HS, however, was greater in the nonacclimated groups (1.77 vs. 3.95 in AC30 and C hearts, respectively; P < 0.005, Table 2). The elevated protein level after a single HS lasted for at least 48 h. After 96 h, no HS effect was observed in either group. Increasing the HS temperature from 41 to 43°C produced unexpected results. C rats produced about five-fold increase in their HSP during the first hour after HS, whereas the LTHA rats reached their peak HSP level only 4 h later. Peak HSP levels in both groups, however, were approximately the same (Fig. 9 and Table 2). Interestingly, in AC rats, the different HSP dynamics (compared with that observed after the HS at 41°C, Fig. 7) did not correlate with the earlier attainment of peak mRNA in this group (Fig. 9).

The STHA rats differed from the C and the LTHA rats in respect to both basal HSP level and the dynamics of their appearance and disappearance. On day 1, the HSP level was lower than that in the C group before and after the HS (Figs. 7 and 8), although the relative increase after HS was similar to that of the controls (with peak after 4 h). After 2 days of heat acclimation, accumulation of HSP70 was higher than in control hearts, but it was still significantly lower than in AC30 rats. The rate of increase after HS was similar, however, to that of the AC30 rats (peak after 1 h). In contrast to C and LTHA rats, HS-induced HSP72 of the

| Table 3. HSP72 mRNA and HSP72 dynamics with heat acclimation at 34°C and 35% humidity for 1, 2, and 30 days: a summary of the major changes observed |
|---------------------------------|------------|------------|-------------|
| Basal HSP72 transcript | Control | 1 Day | 2 Days | 30 Days |
| Basal HSP72 | | | | |
| Time to peak (post HS at 41°C) | 60 min (S) | 20 min (NS) | 40 min (S) |
| 72-kDa mRNA | 4 h (×3.3) (S) | 4 h (2.06) (S) | 1 h (×1.34) (NS) | 1 h (×1.85) (S) |
| HSP72 | | | | |
| Maintained at elevated level | 48 h | 24 h | 24 h | 48 h |

Arrows denote changes compared with control group. S, significant difference from nonacclimated; NS, nonsignificant difference from nonacclimated.
two STHA rats reverted to its initial level 48 h after HS.
A summary of the major changes observed in the STHA
compared with the control and the LTHA rats is
presented in Table 3.
To rule out a possible acute effect of the acclimation
temperature, all groups were exposed to 34°C for 2 h.
There were no changes in HSP level after this exposure
in all groups (data not shown).

DISCUSSION
The concept underlined in this investigation is that
HSP are an integral pathway in the evolution of heat
acclimation. The striking findings are that heat acclima-
tion leads 1) to a larger steady-state stock of the
inducible HSP72 (Figs. 6 and 7) and
2) to an acceler-
ated transcription of the HSP72 gene (Fig. 4). This
makes the acclimated HSP/HSP system better able to
respond to acute HS. The arguments supporting this
issue are discussed below.
Acclimatory features of the HSP70 family. Character-
ization of the HSP70 family and the HSR under the
acclimated state was approached by studying the mRNA
encoding HSP70 and the HSP70 gene product profile
before and after rats were subjected to two levels of
environmental HS. Under the stressful conditions,
invocation of the HSR was due to the cumulative heat
strain induced by the rate of heating and the hyperther-
mic level over time of the animals, depending on the
capacity of the physiological mechanisms for heat dissi-
pation. Thus the examination of the HSR in the context
of the global thermoregulatory response allows analy-
ysis of the differences in HSP/HSP stress responsiveness
between the acclimated and nonacclimated groups.
These differences might allow pinpointing of the ther-
mal physiological parameters involved in the invoca-
tion of the HSR. Likewise, the correlation between the
thermoregulatory span, namely, the length of the pe-
riod during which body temperature is regulated and
the injury temperature threshold of the AC30 and C
rats, and the HSP level/production rate, may provide
some cues for evaluation of their survival values in the
acclimated state.
The resultant change after heat acclimation com-
prises a higher resting level of the encoded 72-kDa
protein and earlier appearance of peak HS-induced
HSP72 transcription, compared with the precedi-
level, without a significant change in the constitu-
This is demonstrated by a shorter time to peak mRNA level compared with that of the C rats, suggesting increased transcription rate in the acclimated state. In the 41°C heat-stressed AC30 rats, transcription was accompanied by faster elevation of the encoded protein. This was not the case for the 43°C heat-stressed acclimated rats, which showed attenuated rise in protein. Hearts of nonacclimated rats, in contrast, demonstrated an increase of both transcription and translation with elevation of environmental stress to 43°C.

The two heating protocols used in this investigation affected thermoregulation of the AC30 and the C rats differently. HS at 41°C accelerated the heating rate of the acclimated rats; HS at 43°C, in contrast, attenuated heating in this group most profoundly, without a change in the hyperthermic plateau temperature, thus leading to decreased heat strain. In the C group, elevation of the ambient temperature resulted in a rise in the plateau temperature and heat strain (Table 1). These differences among the groups are likely to be the manifestation of the thermoregulatory capacity of the acclimated and nonacclimated animals. With these responses taken into consideration, some general conclusions as to the activation of the HSR (for both C and AC rats) can be drawn. 1) Time to peak HSP72 transcript level is faster with the elevation of environmental stress. 2) HSP72 accumulation is faster with the augmentation of the heat strain. This is illustrated in the trend lines in Fig. 10. Thus the mismatching between the temporal profiles of transcription and translation in the 43°C heat-stressed AC30 rats stems from their enhanced physiological capacity to attenuate body temperature rise and, in turn, heat strain (26), via the improved activity of heat dissipation effectors (9), and possibly depressed heat production. In a different heating protocol, Flanagan et al. (4) showed, in nonacclimated rats, a positive correlation between heating rate and HSP accumulation. Their data, however, do not contradict the present findings, since all comparisons were made within one physiologically homogeneous group, and the heating protocol was markedly more aggressive.

The absence of correlation between transcription and heat strain or $T_{re}$ per se is hard to reconcile. This may...
suggest that HSP72 transcription is mobilized via intermediate messenger(s). A likely candidate is the accelerated sympathetic activity to the heart, occurring with $T_{re}$ rise (12). Sympathetically induced accelerated HSP72 transcription has been reported in brown adipose tissue and blood vessels upon cold stress (21–23) as well as in response to application of other stressors (38).

The role of activated $\alpha$-adrenoreceptors in this pathway was assessed (23). Tissue selectivity in the HS induction of HSP (4) and their occurrence in organs exhibiting accelerated sympathetic activity at the onset of HS further support a role played by the sympathetic branch of the autonomic nervous system in provocation of HSP transcription.

Another emerging issue in this investigation was that upon HS, the magnitude of HSP72 mRNA and HSP elevations varied between the acclimated and nonacclimated groups. While a 10-fold HSP72 mRNA rise in AC hearts was accompanied by a 1.77-fold rise in the protein level, C hearts showed two- to threefold and four- to fivefold rises in the HSP72 mRNA and HSP, respectively. HSP dynamics upon chronic thermal tolerance in mammalian tissues has only been studied in heat-tolerant cell lines. Similar to AC rats, these cells contain high resting levels of HSP72. Applying heat shock to these cells (A 431) attenuates further production of the proteins, apparently via attenuation of heat shock factor translocation from the cytosol to the nucleus, thus blunting mRNA and, in turn, protein production (14). This is apparently not exactly the case in AC rats in which transcription is sensitized, while translation is desensitized, compared with the nonacclimated state. Moseley et al. (30) provided data that in addition to HS effects on transcription, there is a heat-induced stressor mechanism of posttranscriptional control of HSP70 synthesis, utilizing the untranslated region (3'-UTR) of the HSP72 mRNA. This lends support to differential transcriptional and posttranscriptional acclimatory responses. This issue, however, is beyond the scope of this investigation.

Thus far, our data suggest that the acclimated HSP/HSP system is predisposed to rapid activation, thus providing a cellular “protective infrastructure,” both as a protein stabilizer (35) and as an activator of other cytoprotective processes (28). We suggest that a larger HSP stock in the AC30 rats may contribute to delayed thermal injury upon heat stress. We cannot assess, however, the share of the induced HSR vs. the integrative acclimatory physiological response to enhanced heat endurance. The observations that after very severe HS, AC30 rats can attenuate heat strain and in turn delay HS synthesis, lead us to hypothesize that subsequent to a rapid autonomically controlled response, the HSR provides a second window of protection.

Dynamics of acclimation of the HSP70 family. Upon STHA, the HSP level did not show marked change, either before or after acute HS. Although the first acclimating day was characterized by a low steadystate level of both mRNA and HSP (72 kDa), on day 2 the HSP72 transcript was markedly enhanced, with only a minor increase in protein expression. These modulations can be explained by initial desensitization of both transcription and translation, followed by nonsynchronized recovery of the transcription and then translation processes. Lowered HSP level on day 1 of the acclimation triggers marked transcription, unaccompanied, on the second acclimation day, by intense HSP production. This interference with the translation step fits with the results of other investigations (e.g., Ref. 16), demonstrating that heat shock primarily halts translation. The interfering effect of the initial acclimation strain is also exhibited upon HS when HSP rise is insignificant. Conversely, however, on day 2 of the acclimation, HSP peaked, similarly to the AC30 rats, 1 h after HS, compared with 4 h in C and AC1 rats. Hence, a clue to the HSP72 expression of acclimatory response is already manifested on the second acclimation day.

Both thermoregulation and HSR of the AC1 rats is puzzling. Currently, we cannot speculate whether the minute HSR stems from the attenuated rise in $T_{re}$, or both the attenuated $T_{re}$ rise and HSR are additional to the already known features characterizing the nonstable STHA phase (10). At that initial acclimatory phase, many processes are impaired (8, 12, 10). The attenuated $T_{re}$ rise and the lowered hyperthermic plateau observed seem to be part of this general biphasic pattern. For the rat, which relies on a hyperthermic plateau for thermoregulation, an attenuated rise in $T_{re}$ fits with this notion.

Considering the biphasic nature of heat acclimation dynamics (7, 10), we were tempted to hypothesize that at the very early period of acclimation, HSP confer rapid thermotolerance. When acclimation has been achieved, an altered threshold for the mobilization of HSP, or their accumulation, may contribute to delayed thermal injury.

The data presented in this investigation confirm enhanced responsiveness of the HSP system on long-term acclimation. A protective effect of HSP72 upon STHA, however, is not feasible, since during that period, HSP production is markedly attenuated. During that acclimation phase, increased heat endurance is achieved by enhanced activation of the autonomically controlled heat dissipation effectors. The similarities between evolutionary-adapted and heat-acclimated species suggest that LTHA is a rapid recapitulation of the evolutionary process.

Perspectives

Except for few studies in intact animals, suggesting that HSP72 can be used as markers for the severity of heat injury, there is no whole animal study providing a mechanistic explanation of their cytoprotective role. In the past, the lack of specific pharmacological HSP inhibitors made this prospective very difficult. The recent development of antisense oligodeoxynucleotides and their delivery technologies may now push forward studies along this line. An additional interesting aspect in the study of HSR in mammals is the interrelationships between members of the different HSP classes in cytoprotection. In yeast, hierarchical cytoprotective
defense strategies by the HSP70 and 104-kDa HSP classes have been assessed. This has not been substantiated for mammals, although high-molecular-mass HSP in mammals have been observed. Heat acclimation, which predisposes rapid HSP expression, may provide a useful experimental model for addressing these questions.

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