β-Adrenergic signaling and thyroid hormones affect HSP72 expression during heat acclimation

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Maloyan, Alina, and Michal Horowitz. β-Adrenergic signaling and thyroid hormones affect HSP72 expression during heat acclimation. J Appl Physiol 93: 107–115, 2002.—Heat acclimation upregulates 72-kDa heat shock protein (HSP72) and predisposes to faster activation of the heat shock response (HSR). This study investigates the role played by β-adrenergic signaling and/or plasma thyroxine level in eliciting these features by using rats undergoing shock response (HSR). This study investigates the role played by β-adrenergic signaling and/or plasma thyroxine level in eliciting these features by using rats undergoing 1) heat acclimation (AC; 34°C, 2 and 30 days); 2) AC with β-adrenergic blockade; 3) AC-maintained euthyroid; 4) hyperthyroid; 5) hyperthyroid; and 6) controls. The hsp72 mRNA (RT-PCR) and HSP72 levels (Western blot) were measured before and after heat stress (2 h, 41°C, rectal temperature monitored). β-Adrenergic blockade during AC abolished HSP72 accumulation, without disrupting HSR. Low thyroxine blunted the HSR at posttranscriptional level, whereas thyroxine administration in hyperthyroid and AC-maintained euthyroid rats arrested heat stress-evoked hsp72 transcription. We conclude that β-adrenergic signaling contributes to the high HSP72 level characterizing the AC state. Thyroxine has two opposing effects: 1) direct repressive on rapid hsp72 transcription after heat stress; and 2) indirect stimulatory via β-adrenergic signaling. Low thyroxine could account for diminished HSP72 synthesis via lower heat production and thermoregulatory set point.

heat shock protein; heat shock response; heat stress; hypothyroid; hyperthyroid; propranolol; β-adrenergic receptors; heat-acclimatory homeostasis

THERE ARE A VARIETY OF PREDISPOSING factors that affect thermal tolerance. Among these, only two adaptations are directly invoked to combat heat stress: 1) the rapid heat shock response (HSR); and 2) heat acclimation (10, 26, 35). Heat acclimation is a long-term developing process leading to an expanded dynamic body temperature regulatory range due to left and right shifts in the temperature thresholds for heat dissipation and thermal injury, respectively (7, 11). In contrast, the HSR is a rapid molecular cytoprotective mechanism and involves the production of heat shock proteins (HSP). Under normothermic conditions, the resting cellular 72-kDa HSP (HSP72) level is low. However, a rise in body temperature increases transcription of the heat shock genes, leading to rapid augmentation of their expression. Their binding to denatured or nascent polypeptides in the cells protects vital structural components and, in turn, physiological functions, from thermal damage. This facilitates survival and recovery after removal of the stressor. Prior induction of the HSP72 by mild stress should, therefore, be protective against subsequent, more severe stress (20).

Recently, our laboratory showed (22) that, in rats, heat acclimation leads to a marked upregulation of the basal level of HSP72, an inducible member of the HSP72 family that is considered the most responsive to heat stress, and to a faster HSR. Genetic manipulations to overexpress this protein enhance thermal tolerance in cell cultures and in several animal species (5, 18, 19, 28). Thus preexisting, large HSP72 reserves allow the organism to deal with abrupt changes in core temperature in a hot environment without the need for de novo synthesis of HSP72 (5, 22, 26). This enhanced cytoprotection may be the underlying mechanism involved in the delayed temperature threshold for thermal injury on heat acclimation, implying that the HSP72 defense pathway plays an integral role in the heat-acclimation repertoire (10, 11). It fits with the finding that a variety of species genetically adapted to high ambient temperatures, including ethnic human populations, are characterized by a constitutively higher level of 70-kDa HSP-like proteins, compared with their related species inhabiting temperate or cold environments (40). This may indicate that heat acclimation recapitulates evolutionary adaptation (10, 26).

The mechanisms leading to enhanced heat-acclimation-induced HSP72-related cytoprotection in mammalian species are intriguing for the following reasons. 1) In homotherms, heat acclimation does not involve a marked elevation in body temperature. 2) No correlation was found between heat strain and rectal temperature (T_r) per se and hsp72 transcription. This may suggest that hsp72 transcription is mobilized via intermediate messenger(s) (22). Moseley (26) hypothesized that the evoked cytokines act as a trigger. This could be applicable to whole body severe hyperthermia, although, so far, it has not been evident on acclimation provoked by moderate heat under sedentary conditions. Considering the initial phase of heat acclimation,
during which body temperature is regulated by increased excitability of the autonomic nervous system (10), likely mediator candidates are the accelerated sympathetic system and the release of catecholamines. Sympathetically induced acceleration of hsp72 transcription, via β-adrenergic receptors, has already been reported in brown adipose tissue after cold stress or cold acclimation and in blood vessels on cold stress and surgical stress, whereas β-adrenergic signaling mediates HSP72 induction after exercise stress (23, 24, 32, 38, 39).

Sustained low-plasma thyroxine, characterizing acclimatory homeostasis, plays a pivotal role in the development of several important acclimatory responses (3, 14), including alterations in the density and affinity of the adrenergic receptors, in turn leading to altered responsiveness to sympathetic signaling (3, 4, 13). The interdependence between sympathetic activity and plasma thyroxine level has been documented, including under conditions of cold acclimation (23, 24). Taken together, we hypothesize an effect of thyroxine on the HSP72 level and HSR occurring on acclimation. The influence of thyroxine on the cytosolic and mitochondrial 70-kDa HSP levels (36), and on the HSR, has been defined in cardiac and skeletal muscles (31), thus supporting our hypothesis.

Given the involvement of 1) β-adrenoreceptor activation through sympathetic stimulation or catecholamine release and 2) thyroxine level in many physiological responses to heat acclimation, we hypothesize that β-adrenergic signaling and/or plasma thyroxine level plays a role in establishing the heat-acclimation-induced HSP72 elevation and the altered HSR. To prove this hypothesis, the hsp72 steady-state transcript level and the subsequently encoded HSP72 were measured in hearts of rats subjected to β-adrenoreceptor blockade or to pharmacological manipulations influencing the level of plasma thyroxine during the acclimation regimen, before and on evocation of the HSR. β-Adrenergic blockade during heat acclimation abolished HSP72 accumulation without disrupting the HSR, whereas sustained low-thyroxine level diminished the magnitude of the HSR at the posttranscriptional level. In contrast, long-term thyroxine administration (to both normothermic and acclimating rats) arrested the heat stress-evoked hsp72 transcription. Cumulatively, thyroxine has two opposing effects on hsp72 transcription: inhibitory and, indirectly, stimulatory via β-adrenergic signaling. Thus the cross talk between adrenergic signaling and low-thyroxine level is influential in upregulation of the basal HSP72 level on heat acclimation.

MATERIALS AND METHODS

Male 3-wk-old Rattus norvegicus (Zabar strain, albino var), initially weighing 80–90 g, fed on Ambar laboratory chow and water ad libitum, were randomly assigned to long (30 days) and short-term (2 days) heat-acclimated (AC) and normothermic groups (Fig. 1). The AC groups were divided into 1) AC rats; 2) AC rats with blockade of β-adrenergic receptors [AC propranolol treated (APROP)]; and 3) AC euthyroid rats [AC thyroxine (ATHY)]. AC and APROP included long-term, fully acclimated rats and those that had undergone short-term heat acclimation (AC and APROP vs. 2d-AC and 2d-PROP, respectively). This allowed us to study both the autonomically mediated short-term and the sustained long-term acclimatory responses (10). ATHY rats underwent only long-term heat acclimation to blunt the sustained, low-thyroxine-mediated responses developing in our acclimation experimental model. The normothermic groups included 1) untreated animals, which served as controls (C); 2) hypothyroid [control 6-n-propyl-2-thiouracil-treated (CPTU)] rats; and 3) hyperthyroid [control thyroxine-administered (CTHY)] rats.

HSR was characterized, in the present investigation, by the magnitude of elevation of steady-state hsp transcript [peak-to-basal ratio (peak/basal)] in response to heat stress and the subsequent encoded protein. Hence, to characterize the effect of β-adrenergic blockade and thyroxine levels on the HSR, all groups were subdivided into groups of rats that received no additional treatment and those that were subjected to heat stress. The levels of the transcripted hsp72 mRNA and the expression of the HSP72 protein were measured in these groups before the heat stress session and post-heat stress, after their subjection to several recovery periods at room temperature, as described below (see Experimental protocols). The heart was chosen as the organ model. It is a major effector of the cardiovascular system, responding to acute, as well as chronic, changes in ambient temperature. A large body of data on gene expression in this organ during heat acclimation, including HSP72 and HSP73 (heat stress control) profiles from previous studies, are already available (22). All experimental protocols were approved by the Ethics Committee for Animal Experimentation of the Hebrew University.

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 (12:12 h) for the required time as stated above. This acclimation model was previously characterized at our laboratory for young and old rats for several thermoregulatory physiological parameters, including “classic criteria” for heat acclimation, such as

![Fig. 1. Protocol bar illustrating the experimental plan. C, controls; 2d-AC, 2-day heat-acclimated rats; AC, long-term (30 days) heat-acclimated rats; 2d-PROP, 2-day heat-acclimated rats administered propranolol; APROP, long-term (30 days) heat-acclimated rats administered propranolol; ATHY, long-term (30 days) heat-acclimated euthyroid rats obtained by administering L-thyroxine in the drinking water; CTHY, hyperthyroid rats, obtained by administering L-thyroxine in the drinking water; CPTU, hypothyroid rats obtained by administering 6-n-propyl-2-thiouracil (PTU) in the drinking water; HS, heat stress; HSR, heat shock response. For further details, see text.](http://jap.physiology.org/)

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growth rate, heart rate, body temperature, and metabolic rate (7, 9, 12, 13, 15, 16, 25). In the present study, successful acclimation was assessed by body weight. AC euthyroid and hyperthyroid rats were obtained by administering 3 ng/ml L-thyroxine (Sigma Chemical) in the drinking water for 1 mo, whereas hypothyroid rats were obtained by administering 0.02% PTU in their drinking water for 1 mo (4). β-Adrenergic blockade during acclimation (APROP rats) was achieved by twice daily administration of propranolol (1 mg/100 g body wt, Sigma Chemical). The efficacy of this treatment was validated previously on heart rate (13). Heat stress was attained by subjecting the rat to 41°C for 2 h. During the heat stress session, Trea was monitored on-line, by using a YL 402 thermistor, inserted 6 cm beyond the anal sphincter and attached to a computerized data-acquisition system (22). On termination of the heat stress, the animals were returned to room temperature for different time intervals as described below.

**Experimental protocols.** All rats were killed by cervical dislocation. For mRNA analysis, the rats were killed before and 3, 10, 20, and 60 min after the given heat stress; to determine HSP72 expression, the animals, except for those treated with propranolol, were killed 1, 4, 24, and 48 h after the given stress (22). The 2d-PROP and APROP rats were killed 1 and 4 h after the heat stress, respectively. The hearts were rapidly excised, mounted on a Langendorff perfusion apparatus, retrogradely perfused (for 2 min) to wash out all remaining blood 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 and 5% CO2 at 37°C (3, 4, 22). The left ventricle was carefully excised, frozen, and stored at −70°C until analysis.

**Semiquantitative detection of mRNA by RT-PCR.** To measure hsp72 mRNA, semiquantitative RT-PCR was performed as previously described (22). Briefly, total RNA was extracted with TRI-Reagent (Molecular Research Center, Cincinnati, OH) from the left ventricle homogenate. Total RNA (10 μg) was reverse transcribed in a 50-μl reaction mixture containing 0.5 μg of oligo(dT)15 as primer, together with 400 units of 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 2-deoxynucleotide 5’-triphosphate, 100 μM of each specific primer, and 1.5 units of Vent polymerase (United States Biochemical). We synthesized DNA oligonucleotide primers for HSP72 selected from the published hsp72 gene nucleotide sequence (21). The sense primer was 5’-GCT-GAC-CAA-GAT-GAA-GGA-GAT-C-3’ (corresponding to sequence 546–567), and the antisense primer was 5’-GAG-TCG-ATC-TCC-AGG-CTG-CC-3’ (corresponding to sequence 1017–1038). Amplification was carried out for 40 cycles with denaturation at 94°C for 30 min, annealing at 64°C for 45 min, and extension at 72°C for 1 min. To ensure equal amounts of initial mRNA, parallel actin amplifications were performed (annealing temperature: 62°C, 35 cycles) (30). The PCR products were resolved on 1.5% agarose gel, stained with ethidium bromide, and visualized under ultraviolet light. The density of the bands was computer analyzed by using Tina software (Raytest, Straubenhardt, Germany).

**Western blot analysis.** The left ventricles were homogenized with 20 mM HEPES (pH 7.5), 1.5 mM MgCl2, 0.2 mM EDTA, 0.1 M NaCl, 5 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, and 1.2 mM Na3VO4. NaCl was added to a final concentration of 0.45 M (43). The homogenate was centrifuged at 12,000 rpm for 30 min at 4°C. The supernatant was mixed with an equal volume of buffer solution, as above, together with 40% (vol/vol) glycerol (43). The protein concentration of the myocardial specimens was quantified by using the Bradford reagent (Bio-Rad Laboratories, Richmond, CA). Total protein (50 μg/lane) was run on 12.5% polyacrylamide gels under denaturing conditions (17). After separation by electrophoresis (50 mA for 2 h), the proteins were transferred onto nitrocellulose (190 mA, 4°C, 1 h). The nitrocellulose membranes were then blocked for 2 h in PBS containing 0.1% dried skimmed milk powder and probed overnight, at 4°C, with monoclonal IgG cross-reactive to HSP72 (Stressgen, Victoria, BC) diluted 1:1000. After repeated washings, the membranes were incubated at room temperature for 1 h with horseradish peroxidase-conjugated rabbit anti-mouse IgG (Jackson) diluted 1:1,000. Specific antibody binding was detected by using enhanced chemiluminescence (Amersham) and visualized by exposing X-ray film to the membrane (for further details, see Refs. 3 and 22). The density of the scanned HSP72 band was calculated with Tina software.

**Calculations and statistics.** The heating rate (°C/min) was calculated from the regression lines fitted to the Trea points, starting from normothermic temperatures until the onset of the hyperthermic plateau. The area below the Trea change (ΔTrea) curves during the entire period of heat exposure was used to calculate heat storage (Σ ΔTrea/min × 0.83 × body wt) and was compatible with the cumulative heat strain (22). All data were normalized to 100 g body wt.

**For statistical analysis, one- and two-way ANOVA were used, with 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. The data are expressed as means ± SE. Values of P < 0.05 were considered statistically significant.

**RESULTS**

**Body weight, body temperature, and heating rate.** Body weight, basal Trea on termination of heat stress, and the actual heat strain of all of the experimental animals are presented in Table 1; Fig. 2 illustrates changes in Trea during the course of the heat stress. As previously published (e.g., Refs. 9, 34), the AC rats grew at a slower rate than the normothermic ones. Neither propranolol nor thyroxine affected growth rate. Thus, AC, APROP, and ATHY rats had similar body weight and were significantly smaller than the normothermic or the 2d-AC groups, except for the CPTU rats, which were markedly smaller. Taken together, all experimental groups were age matched but only partially weight matched. The basal Trea of the AC euthyroid rats (ATHY) was significantly higher than that of the matched C rats. In contrast, the hypothyroid state (CPTU) resulted in a marked drop in Trea. β-Adrenergic blockade during long-term acclimation (APROP group) also resulted in a significantly lower Trea than that of the C rats.

**Exposure of the rats to 41°C delineated differences in the rate of heating and the intensity of heat strain among the various experimental groups.** The rate of heating was highest in the AC rats, whereas heat strain was maximal in the APROP rats. The lowest heat strain was found in the CPTU rats. On subjection to heat stress, elevation of Trea to the hyperthermic
plateau (the $T_{re}$ at which the core temperature is regulated during heat stress) (12) in the latter group was only 1.6°C higher than the preheat stress level. The recorded $T_{re}$ plateau of C, AC, and APROP rats exceeded that of the basal value by −3°C.

Steady-state hsp72 mRNA and HSP72 levels. The steady-state levels of hsp72 mRNA and the encoded protein HSP72 in C and AC rats before and after heat stress at 41°C are presented in Fig. 3. Under resting conditions, hsp72 mRNA in the AC group was almost undetectable, whereas HSP72 was pronouncedly elevated with respect to the C group ($P < 0.005$). After being subjected to heat stress, the magnitude of the hsp72 mRNA elevation in AC rats was greater (10- vs. 2.3-fold), and mRNA and the encoded HSP72 peaked earlier than in the matched C rats (mRNA: 40 vs. >60 min; HSP72: 1 vs. 4 h; Fig. 3).

HSP72 was then maintained at the attained peak level 24 and 48 h after the given heat stress. 2d-AC rats were characterized by a marked upregulation of the basal hsp72 transcript (Fig. 4A), with a gradual decline after termination of the superimposed heat stress (41°C). These data are in agreement with our laboratory's previously published findings (22) and provided us with baseline data for comparison with the hsp72 mRNA and HSP72 levels obtained after pharmaceutical manipulations.

Effects of β-adrenergic-receptor blockade on hsp72 mRNA and HSP72 levels. Blockade of β-adrenergic receptors during the acclimation regimen abolished upregulation of the steady-state hsp72 transcript level characterizing the 2d-AC group (Fig. 4A) and attenuated the post-heat stress elevation (Fig. 4B). On long-term heat acclimation, the hsp72 transcript level of the APIPAP rats was similar to that observed in the 2d-AC group, both under basal conditions and after heat stress (Fig. 4B), and there was no significant elevation in basal HSP72 from that measured for C rats (Fig. 4C vs. Fig. 3). In the APIPAP rats, the evoked HSP72 synthesis 1 and 4 h post-heat stress resembled that observed for the short-term acclimated 2d-PROP rats (peak/basal of 1.4 and 1.3 for APIPAP and 2d-PROP, respectively). It is noteworthy that acute propranolol administration (data not shown) failed to block the HSR.

Effects of plasma thyroxine level on hsp72 mRNA and HSP72 levels. Both hypothyroidism and hyperthyroidism alone (CPTU and CTHY groups, respectively) did not significantly affect the basal HSP72 level characterizing the nonacclimated state. The hsp72 transcript in the CTHY group was barely detectable (not shown). The hyperthyroid state also abolished the induction of HSR, and HSP72 failed to increase significantly above control level until 48 h post-heat stress (Fig. 5). In contrast, in the CPTU group (Fig. 6A), heat stress induced a rapid elevation in hsp72 mRNA (mRNA peak/basal of 1.9). No posttranscriptional changes, however, were observed in this group, and the

### Table 1. Body weight, basal rectal temperature, and the effects of ambient heat stress (at 41°C) on rectal temperature, heating rate, and calculated heat strain in the nonacclimated and heat-acclimated experimental groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>CTHY</th>
<th>CPTU</th>
<th>2d-AC</th>
<th>2d-PROP</th>
<th>AC</th>
<th>ATHY</th>
<th>APROP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight</td>
<td>269.0 ± 10.0</td>
<td>270.2 ± 6.5</td>
<td>269.0 ± 7.0</td>
<td>260.5 ± 7.0</td>
<td>261.2 ± 3.5†</td>
<td>220.0 ± 9.0</td>
<td>230.0 ± 5.3†</td>
<td></td>
</tr>
<tr>
<td>Basal $T_{re}$, °C</td>
<td>37.50 ± 0.29</td>
<td>37.175 ± 0.083</td>
<td>36.12 ± 0.15‡</td>
<td>37.86 ± 0.23</td>
<td>37.11 ± 0.088</td>
<td>37.8 ± 0.10</td>
<td>37.93 ± 0.12¢</td>
<td></td>
</tr>
<tr>
<td>$T_{hyper}$, °C</td>
<td>40.97 ± 0.15</td>
<td>40.47 ± 0.05</td>
<td>37.7 ± 0.093‡</td>
<td>39.87 ± 0.27</td>
<td>40 ± 0.023</td>
<td>41 ± 0.43</td>
<td>40.038 ± 0.065</td>
<td></td>
</tr>
<tr>
<td>Heating rate, °C/min</td>
<td>0.045 ± 0.004</td>
<td>0.027 ± 0.006*</td>
<td>0.0274 ± 0.006*</td>
<td>0.037 ± 0.002</td>
<td>0.0347 ± 0.003</td>
<td>0.074 ± 0.016</td>
<td>0.031 ± 0.0039</td>
<td>0.049 ± 0.0086‡</td>
</tr>
<tr>
<td>Heat strain, cal</td>
<td>237.25 ± 3.92</td>
<td>301 ± 1.1*</td>
<td>73.2 ± 0.7‡</td>
<td>196.6 ± 5.3</td>
<td>317 ± 1.53§</td>
<td>306.5 ± 3.8</td>
<td>307.3 ± 1.16</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE and were derived from representative groups of 6–12 animals for each treatment. Heat strain was calculated for each animal. $T_{re}$, basal rectal temperature before heat stress; $T_{hyper}$, hyperthermic $T_{re}$, on termination of 2 h of heat stress; 2d-AC, 2-day heat-acclimated rats; AC, long-term (30 days) heat-acclimated rats; 2d-PROP, 2-day heat-acclimated rats administered propranolol; APIPAP, long-term (30 days) heat-acclimated rats administered propranolol; ATHY, long-term (30 days) heat-acclimated euthyroid rats obtained by administering l-thyroxine in the drinking water; CTHY, hyperthyroid rats obtained by administering l-thyroxine in the drinking water; CPTU, hypothyroid rats obtained by administering 6-n-propyl-2-thiouracil in the drinking water. *Significant difference from control ($P < 0.005$). †Significant difference from AC group ($P < 0.005$). §Significant difference between 2d-AC and 2d-PROP groups ($P < 0.005$).

Fig. 2. Effect of ambient HS (at 41°C) on rectal temperature change in the course of heating in CTHY or CPTU rats, and 2-day and 30-day heat-acclimated rats at 34°C and 35% relative humidity with no further treatment (C, AC) or treated with propranolol (2d-PROP, APIPAP) or thyroxine (ATHY). Data were derived from representative groups of 5 or 6 animals for each treatment. For statistics, see Table 1.
HSP72 level remained at the basal constitutive level, despite the heat stress episode (Fig. 6B). In the ATHY rats (Fig. 5), similar to the AC rats, HSP72 accumulation, characterizing the acclimated state, took place. Basal HSP72 level at the end of the acclimation regimen in this group was 14.2 ± 0.4 vs. 5.6 ± 0.5 in the nonacclimated (P < 0.001). Exposure of the ATHY rats to heat stress, as in the CTHY rats, did not increase hsp72 transcription or HSP72 production, characteristic of the HSR response.

DISCUSSION

Our laboratory’s previous studies (22) indicated that alterations in HSP72 signaling constitute an integral part of the heat-acclimation repertoire. This is evident from the high levels of HSP72 expression without the need for de novo protein synthesis to confer protection and from the faster response to heat stress. In the present investigation, using a nonspecific β-adrenergic antagonist, we provide causal evidence for the significant influence of β-adrenergic signaling on the buildup of the cellular HSP72 reserves and on the magnitude of the HSR. The sustained low-thyroxine level, occurring on acclimation, diminishes the magnitude of the HSR, possibly via its influence on adrenergic signaling. A novel finding in this study is the interference of a high-thyroid level with the HSR. The mechanism leading to this effect is not clear.

β-Adrenergic signaling and HSP72 responsiveness on acclimation. Acclimation is a biphasic process. The initial phase, 2d-AC, is characterized by several significant alterations in sympathetic activity, β-adrenergic signaling, and catecholamine turnover (10, 11). In the heart, this is also reflected by a marked decrease in the affinity of the adrenergic receptors and by impaired chronotropic and inotropic responses (3). Thus accelerated sympathetic activity compensates for these detriments. HSP72 also shows a biphasic profile of acclimation dynamics, with hsp72 mRNA upregulated and downregulated during short- and long-term heat acclimation, respectively. A nearly reciprocal biphasic profile is exhibited by the protein: slight downregulation during the short-term phase of heat acclimation, with pronounced upregulation characterizing acclimatory homeostasis (22). In the propranolol-administered rats, the biphasic acclimation kinetics were blunted (Fig. 7). Sustained propranolol treatment resulted in stabilized steady-state hsp72 transcription throughout the entire heat-acclimation regimen, with the hsp72 mRNA level being essentially the same as in the preacclimation state. Subsequently, the rise in HSP72 level to that characterizing the long-term heat-acclimation phase was attenuated. Mobilization of the molecular machinery to heighten hsp72 transcription after the superimposed heat stress was also abolished, implying the involvement of adrenergic signaling in both processes. Based on the results obtained for the 2d-PROP group (compared with the 2d-AC group), it is likely that the accelerated sympathetic flow, together with the surge in circulating catecholamines occurring during the short-term acclimation via β-adrenergic signaling, contributes to the elevated hsp72 transcription observed at that acclimatory phase and the subsequently sustained high HSP72.

Long-term heat acclimation is characterized by decreased autonomic excitability and an elevated response-to-signal ratio (10, 11). We assume that, during this acclimatory phase, the persistent responsiveness of the adrenergic signaling to thermal challenges is sufficient to maintain a high-HSP72 level. Heat acclimation is a continuum of many parallel processes. Therefore, although adrenergic signaling per se has a pronounced effect on the induction of HSP72, the cross talk among several signaling pathways evoked on acclimation could provide a more comprehensive explanation for their induction and maintenance at a high-cellular level. For example, a large body of evidence indicates that HSP72 expression is influenced by β-adrenergic-receptor intermediates, including cAMP and cAMP-dependent protein kinase, which accumulate via other signaling pathways as well (1, 33). On acclimation, their accretion, even irrespective of β-adrenergic...
seemingly equivocal, data suggest that the pathways through which catecholamines mediate HSP72 expression in vivo are both stress and tissue specific.

**Thyroxine level and HSP72 responsiveness on acclimation.** In the present investigation, the effects of 1-mo-long thyroxine manipulations are compatible with the time-dependent, thyroxine-induced acclimatory metabolic influences (14). Our data show that, in the thyroxine-administered groups CTHY and ATHY, the HSR was arrested. This finding seemingly disagrees with the results of Pantos et al. (31), showing that hearts from long-term hyperthyroid rats overexpress hsp72 mRNA in response to ischemic stress. However, HSP72 synthesis was evoked only in the presence of cardiac hypertrophy, suggesting that the development of hypertrophy rather than increased thyroid level played a role in the induction of transcribed hsp. Our finding may be compatible with that of Dillmann et al. (2), that 3,5,3′-triiodothyronine administration to hypothyroid rats does not enhance either the level or the translational activity of several mRNA species (including 70- to 75-kDa proteins), despite the general increase in total mRNA. The primary effect of thyroxine is on the transcriptional regulation of target genes, via its binding to nuclear receptors (42). We can thus conclude that, in our experiments, indirect cellular thyroxine mediation of the rapid HSR was not brought into play. In contrast, thyroxine administration did not abolish basal HSP72 upregulation in the course of the acclimation. This may imply more than one pathway for HSP72 induction.

An important long-term effect of the plasma thyroxine level is its influence on the density and affinity of the β-adrenergic receptors (8, 37), a long-term hyperthyroid state leading to increased density of these receptors. In light of the results obtained in this inves-

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Fig. 4. A: basal steady-state hsp72 mRNA content in C, 2d-AC, and 2d-PROP rats. B: semiquantitative RT-PCR analysis of hsp72 mRNA steady-state level in left ventricle of hearts excised from 2d-PROP and APROP rats before and 20, 40, and 60 min after HS at 41°C for 2 h. Top: representative set of bands (10 μl/lane). For comparison, representative set of bands of 2d-AC rats is also presented. Bottom: bar graph showing relative amounts of hsp72 mRNA, normalized to 0.3-actin. Values are means ± SE. ∗ Significant difference from the matched APROP, 0.01 < P < 0.03; § Significantly lower than the matched 2d-AC group, P < 0.009 or P < 0.04. C: HSP72 protein accumulation before and 1–4 h after HS at 41°C for 2 h. Top: representative sets of blots (ECL detection). Bottom: bar graph showing HSP72 protein level, normalized to commercial-positive HSP72 control (Stressgen). Values are means ± SE (n = 5–6).

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Fig. 5. Quantification of HSP72 levels in left ventricle of hearts excised from CTHY and ATHY rats before and 1–48 h after HS at 41°C for 2 h. Top: representative sets of blots (ECL detection). Bottom: graph shows HSP72 protein level, normalized to commercial-positive HSP72 control (Stressgen). There was no expression of hsp72 mRNA in these experimental groups. Values are means ± SE (n = 5–6). § Significant difference from AC group, P < 0.05. ¥ Significant difference from C group, 0.0001 < P < 0.05.
In the APROP groups, we hypothesize that the cumulative long-term acclimatory response in the ATHY rats is due to an equilibrium between the direct (repressive) effect of thyroxine on rapid hsp72 transcription and the slow, indirect -adrenergic activation effect leading to high-basal HSP level, compared with that in the C rats. For the ATHY rats, analogous to the CTHY rats, the euthyroid state is "a hyperthyroid state."

In contrast to the thyroxine-administered groups, CPTU rats showed heat stress-induced hsp72 transcript elevation, even if somewhat attenuated, but without subsequent HSP72 induction, suggesting a mismatch between transcription and posttranscriptional processes. The constitutive HSP72 level after 30 days of treatment was higher than that in the C group but lower than that in the 1-mo-acclimated rats.

Taking into consideration the marked effect that low thyroxine has on adrenergic-mediated physiological functions and our results from the APROP 1-mo-treated rats, we hypothesize that the hypothryoid-HSP72 interaction is mediated via the sustained low-thyroxine attenuation of the -adrenergic pathway. Whereas the long-term cumulative hypothryoid influence did not interfere significantly with the accumulation of large HSP72 reserves, desensitization of -adrenergic signaling may serve as a "negative regulator"
of hsp72 transcription during the HSR, as reflected by the lower peak/basal hsp72 mRNA ratio, compared with that in C rats (1.9 vs. 2.3). The cross talk among thyroxine, adrenergic signaling, and hsp72 transcription, as observed in the present investigation, is illustrated schematically in Fig. 8. Out laboratory previously found (22) that, whereas heat-stress-triggered hsp72 transcription is mediated via activation of thermoreceptors, the subsequent posttranscriptional events are correlated with heat strain. CPTU rats showed a low basal T<sub>re</sub> and pronouncedly low heat strain on subject to heat stress. Hence, in agreement with our previous results, hsp72 transcription in the heat-stressed CPTU rats responds to the elevated ambient temperature. The low heat strain in these rats (Table 1), however, is not sufficient to induce posttranscriptional processes. Thus HSR in the CPTU rats was reflected only by changes in transcription. The limited ability of CPTU rats to raise their T<sub>re</sub> to a higher hyperthermic plateau is in agreement with the finding that hypothyroidism, including PTU-induced hypothyroidism, results in lower T<sub>re</sub>, because of reductions in both metabolic thermogenesis and the thermoregulatory set point (6, 41). According to Osafa et al. (29), the interference with cellular metabolism due to PTU lengthens survival during heat stress significantly compared with both nonacclimated and AC rats (29). The drop in T<sub>re</sub> of the CPTU rats is far below the cumulative heat strain of the rats assigned to each logical manipulations used in this investigation affecting with our previous results, hsp72 transcription during the HSR via β-adrenergic mediation. Enhanced survival of these rats during heat stress is, therefore, not due to an increased threshold for thermal injury but due to decreased metabolic rate.

Rate of heating and HSP72. The various pharmacological manipulations used in this investigation affected resting body temperature, rate of heating, and the cumulative heat strain of the rats assigned to each group. When the experimental manipulation did not blunt hsp72 transcription, it was possible to establish a correlation between the extent of the heating effects and HSP72 synthesis. Our data show a high positive correlation between the rate of heating and HSP72 synthesis but not with heat strain or the hyperthermic plateau temperature.

In summary, in this investigation, we provided clues to the mechanisms underlying enhanced HSP72 reserves in the AC heart. Heat-acclimation-induced HSP72 upregulation has been shown in the brain (10) and recently in rat salivary glands and mouse heart (Robinson S, Marmary I, Brumberg Z, and Horowitz M, unpublished observations). Furthermore, the finding that depletion of catecholamines almost completely abolishes hsp72 mRNA accumulation in neonatal piglet brains after hypoxic stress (27) may imply that, on heat acclimation, the brain can share similar mechanisms to enhance its cytoprotection. Collectively, it leads us to hypothesize that there is a beneficial overall HSP72 acclimatory response, leading, in turn, to delayed thermal injury during heat stress.

REFERENCES


