Changes in cardiac mechanics with heat acclimation: adrenergic signaling and SR-Ca regulatory proteins

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ACCLIMATION TO AN ENVIRONMENTAL stressor is a dynamic process encompassing a continuum of events to enable better coping with the hostile environment. Along with this concept, a large body of evidence on heat acclimation suggests that the process can be delineated as biphasic, comprising short (STHA)- and long-term heat-acclimation (LTHA) phases. During STHA, a multitude of transient, sometimes-opposing mechanisms are recruited to alleviate the initial strain. In this phase (2–5 days of heat exposure), the intact animals show a clear acclimated physiological state, although impairments in cellular signal transduction processes are evident. Hence, to gain adequate thermoregulatory effector output for strain alleviation, compensatory accelerated autonomic excitability is evident (12). On LTHA, long-standing adaptations are generated (7–9, 11, 12). Enhanced biochemical efficiency in a multitude of processes underlies this stable adaptive state (10, 14).

The effects of LTHA on the heart have been extensively investigated in the rat model. On acclimation, left ventricular compliance and systolic pressure are increased while oxygen consumption is lowered. Concurrently, however, the LTHA heart shows lowered velocity of systolic contraction and delayed diastolic relaxation. Thus LTHA improves efficiency of cardiac mechanics, even though this is at the expense of contractile velocity.

A sustained low level of plasma thyroid hormones, induced by heat acclimation (3, 14), plays a major role in the emergence of these important cardiac adrenergic responses, apparently via the influence of thyroid hormone levels on the transcription of critical genes involved in cardiomyocyte contraction and relaxation (e.g., see Refs. 1, 19, and 21). These include 1) redistribution of cardiac myosin isoforms, manifested by a transition from the predominantly fast myosin isoform (V1) in the control (C) hearts to the predominance of the slow myosin isoform (V3) in the LTHA hearts (in turn, the contractile velocity of the heat-acclimated heart decreases (14, 23)), and 2) alterations in transcript of mRNAs coding for the expression of two sarco-
plasmic reticulum (SR)-Ca\(^{2+}\) regulatory proteins, sarco-(endo)plasmic reticulum Ca\(^{2+}\)-ATPase (SERCA) and phospholamban (PLB). On heat acclimation, SR-Ca\(^{2+}\)-ATPase mRNA and the encoded protein are downregulated, whereas transcription and translation of PLB, which in its nonphosphorylated form inhibits SERCA, are upregulated, collectively leading to an elevated PLB/SERCA ratio in the LTHA compared with the C hearts. This elevated ratio fits with the attenuated velocity of relaxation (3). Subjection to heat acclimation, during the maintenance of a euthyroid state, blunts the emergence of the abovementioned molecular and mechanical acclimatory responses (3, 14).

Gross (4) showed that STHA hearts, similar to LTHA hearts, develop higher systolic pressure compared with that of the C rats’ hearts. Two to five days of STHA are insufficient to induce changes in plasma thyroxin level. It is thus unlikely that changes in cardiac myosin isoforms or in Ca\(^{2+}\) regulatory proteins will take place via thyroxin hormone mediation. Observations on cardiomyocytes (O. Cohen, U. Meiri, and M. Horowitz, unpublished data, and Ref. 13), however, showed that greater pressure generation on STHA, similar to the situation in LTHA, involves evocation of greater Ca\(^{2+}\) transients. This may infer that short-term mechanisms other than thyroxin-mediated changes occur in the levels of Ca\(^{2+}\) regulatory proteins. Altered adrenergic signaling, which affects PLB phosphorylation, calcium influx, and, in turn, Ca\(^{2+}\) handling in the STHA myocytes, may be considered.

The aim of the present study was twofold: 1) to question whether changes in the mechanical performance of the rat heart, accruing during STHA, involve molecular changes in the expression of Ca\(^{2+}\) regulatory proteins and in adrenergic signaling and 2) to evaluate the impact of Ca\(^{2+}\) regulatory proteins versus adrenergic signaling on cardiac mechanics during the STHA and LTHA. Our data show that both β-adrenergic signaling and transcription of mRNAs coding for calcium regulatory proteins and their expression are altered. The relative importance of each adaptation varies in the course of the acclimation process.

MATERIALS AND METHODS

Animals. Male Rattus norvegicus (Zabar strain, albino variation) weighing 250 g on termination of heat-acclimation procedure and fed on Ambar laboratory chow and water ad libitum were used. The animals were divided into four groups comprising C, two STHA groups (heat acclimation for 2 days (AC\(_2\)) and for 5 days (AC\(_5\)), and LTHA (heat acclimated for 30 days (AC\(_{30}\)). The C group was maintained at an ambient temperature of 24 ± 1°C; the AC\(_2\), AC\(_5\), and AC\(_{30}\) groups were kept in a climatic chamber at 34 ± 1°C for 2, 5, and 30 days, respectively. Rectal temperatures of the LTHA group were 0.3–0.5°C higher than those of the normothermic group (37.8 ± 0.2°C and 37.5 ± 0.2°C, respectively), whereas the STHA rats temperature was 37.9 ± 0.2°C.

To follow cardiac mechanics with acclimation and to gain insight into adrenergic responsiveness and/or the involvement of cytosolic calcium regulatory mechanisms in the acclimatory responses observed, the mechanical performance of the hearts of each experimental group was studied under the challenge of noradrenaline before and after β-adrenergic blockade. Likewise, steady-state levels of SR-Ca\(^{2+}\)-ATPase and phospholamban mRNAs and the expression of the associated proteins were measured in both the STHA and the LTHA hearts. The level of plasma thyroid hormones was monitored throughout the acclimation process to reconfirm or refute their possible role in the induction of cardiac acclimatory responses. The listed specific aims were applied to all experimental groups. However, to avoid misinterpretation due to work on damaged ventricular tissue or deteriorating experimental preparations, each experimental animal underwent one experimental protocol only. Separate experimental series were assigned to the hormonal analysis as well.

Left ventricular mechanics. The animals were killed by cervical dislocation. Hearts were rapidly exiripitated and placed in a physiological solution at 4°C. The hearts were then mounted on a Langendorff perfusion apparatus and retrogradely perfused through the aorta at a perfusion pressure of 100 cmH\(_2\)O with Krebs-Henseleit bicarbonate buffer containing (in mM): 118 NaCl, 24 NaHCO\(_3\), 1.2 MgCl\(_2\), 2.5 CaCl\(_2\), 4.2 KCl, and 5.5 glucose at pH 7.4, maintained at 37°C and bubbled with 95% O\(_2\)-5% CO\(_2\) (23, 24). Heart temperature was continuously monitored using a thermocouple (Omega Digicator).

Once perfusion was started, an atrioventricular block was induced by electrical coagulation of the membranous interventricular septum with a fine-tipped soldering iron. A decompressed latex balloon (Hugo Sacks Electro-nics no. 4) attached to a Statham P23db pressure transducer (with PE-190 tubing) was inserted into the left ventricle by a left atrial incision. The balloon was inflated with saline until the diastolic pressure reached 0 mmH\(_g\) and then until the systolic pressure was maximal at that diastolic pressure. The inflation volume was thus determined by both left ventricular chamber size and compliance. Although the inflation volume was not the same in all hearts, the left ventricular preload (that at the point of maximal systolic pressure at 0 diastolic pressure) was. This technique allowed, therefore, similar preload among hearts of varying size and compliance. Hearts were paced at 200, 300, and 400 beats/min via stainless steel electrodes with the aid of a Grass S-88 stimulator. Left ventricular developed pressure was recorded using the CODAS data-acquisition system (DATAQ) on an IBM/PC computer. All hearts were perfused under these conditions until a steady state was reached, usually 10–15 min before the experiment was begun.

Adrenergic induced inotropic response. Left ventricular mechanics were measured as described. Two experimental series were conducted. In the first experimental series, cardiac performance was measured initially during perfusion with Krebs-Ringer bicarbonate (KRB) containing 10\(^{-7}\) norepinephrine. After return to basal level following KRB wash, the β-adrenoceptors (AR) were blocked by 5-min perfusion with KRB containing 10\(^{-6}\) propranolol. The perfusate was then replaced by a cocktail of norepinephrine and propranolol as before for α-adrenergic stimulation.

Adrenergic receptors binding. Measurements were performed on left ventricle homogenates. Briefly, each ventricle was cut and minced in an ice-cold Tris (pH 7.7) solution by Polytron homogenizer and centrifuged for 10 min at 1,000 g at 4°C. A membrane pellet was obtained after centrifugation at a speed of 49,000 g at 4°C for 10 min (Beckman L5-50 B ultracentrifuge). The pellet was then suspended in Tris-HCL 1 mM buffer (pH 7.4) and kept frozen (−70°C) until measure-
ments began. In the binding assay, the β-adrenergic antagonist 3H-CGP 12177 was used. For nonspecific binding, l-alpranolol (10⁻⁴ M) was employed. After incubation (30 min, 25°C), the reaction was terminated by the addition of cold buffer and filtration (with GF/C microfiber filters, Whatman) to separate bound from free ligand. Radioactivity was then determined with a liquid scintillation counter (Beckman Analytic). Protein was assayed according to Bradford method (Bio-Rad Laboratories, Richmond, CA). Receptor densities and dissociation constants for the ligands were calculated from Scatchard plots.

Semi-quantitative determination of Ca²⁺-ATPase and PLB mRNA by RT-PCR. To measure transcription of Ca²⁺-ATPase and PLB, semi-quantitative RT-PCR was performed as previously described (3). Briefly, the left ventricle tissue from the hearts of each experimental group was carefully excised, dissected, and homogenized with a polytron (Kinematica, 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(dT15) as primer, together with 400 U of MMLV reverse transcriptase, according to the manufacturer’s instructions [United States Biochemical (USB), Cleveland, OH]. For the PCR, 0.3–1 µl of the cDNA mixture was added to 50 µl of a master mix containing 200 µM of each dNTP, 100 pM of each specific primer, and 1.5 mM MgCl₂ for Ca²⁺-ATPase and 1 mM MgCl₂ for PLB, and 1.5 U of Vent polymerase (USB). We synthesized DNA oligonucleotide primers selected from the published sequence of the Ca²⁺-ATPase gene (27). The sense primer was based on the sequence no. 2079–2104: 5'-ATG-AGA-TCA-CAG-CTA-TGA-CTG-GT3' and the antisense no. 2707–2732: 5'-GCA-TTG-CAC-ATC-TCT-ATG-GTG-AGT-3' and the antisense no. 318–339: 5'-CAG-AAG-CAT-CAC-AAT-GAT-GCA-G-3'. The primers were designed to cross introns to avoid confusion between mRNA transcript and genomic DNA. The optimal conditions for each set of primers were for PLB: annealing temperature 62°C, 20 cycles and 1 µl cDNA, and for Ca²⁺-ATPase, annealing temperature 60°C, 22 cycles, and 0.3 µl cDNA. MgCl₂ concentration was 1.5 and 1.0 mM for PLB and SR-Ca²⁺-ATPase, respectively. Negative and positive controls were included in every run. Each sample was amplified three times in an automated thermal cycler (Mastercycler 5330, Eppendorf, Hamburg, Germany). To ensure a fixed amount of initial mRNA, parallel β-actin amplification was performed (annealing temperature 62°C, 35 cycles) using the following oligonucleotides: 5'-GAG-ACC-TTC-ACC-ACC-CAC-GGC-3' (sense) and 5'-GTC-ATC-TTC-ACC-ACC-CAC-GGC-3' (antisense) (32). PCR products (10 µl each) were separated on 1.5% agarose ethidium-bromide gel, visualized under ultraviolet light, and photographed on high-speed film (Polaroid 667). The prints were scanned by a VISTA 8S scanner (Umax), and the density of the bands was computer analyzed by National Institutes of Health Image software. The relative intensity of bands for each mRNA was divided by the intensity of the band for the control, β-actin.

Western immunoblot. The levels of SERCA and PLB proteins in left ventricular tissue from the hearts of rats in each experimental group were obtained by quantitative immunoblotting (3). Anti-PLB and -SERCA monoclonal antibodies were purchased from Affinity Bioreagents.

A quantity of 50 µg cardiac homogenate proteins from each heart, separated by 12.5% SDS-polyacrylamide gel electrophoresis, was transferred to nitrocellulose membranes and reacted with anti-SERCA or anti-PLB monoclonal antibodies at 1:1,000 dilution. After repeated washings, the membranes were incubated at room temperature for 1 h with horseradish peroxidase-conjugated rabbit anti-mouse IgG (Sigma) diluted 1:1,000. The membranes were then washed and developed to enhance chemiluminescence (Amersham, Bucks, UK), and X-ray film was exposed to the membranes. The SERCA and PLB levels were estimated by laser densitometry of the immunoblots. A pooled cardiac homogenate from the control animals was used as a reference. The protein concentration of the myocardial specimens was quantified according to the Bradford method.

3,5,3'-Triiodothyronine and l-thyroxine measurements. During the acclimation period, plasma levels of 3,5,3'-triiodothyronine (T₃) and l-thyroxine (T₄) were measured at 1-wk intervals. For measurement of T₃ and T₄ levels, 0.5-ml blood samples were withdrawn by cardiac puncture under short ether anesthesia. Samples were then centrifuged, and the plasma was kept at −70°C until analysis. Analysis of all samples was performed at the same time. Total plasma T₃ (ng/dl) and T₄ (µg/dl) levels were measured by radiimmunoassay (Coat-A-Count, Diagnostic Products). The sensitivity of the determinations was 7 ng/dl for T₃ and 0.25 µg/dl for T₄.

Statistics. One- and two-way ANOVA were 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. For individual matched-group comparisons, Student's t-test with Bonferroni correction or Dunnett's test was used. Values of P < 0.05 were considered to be statistically significant. Data are expressed as means ± SE.

RESULTS

Myocardial performance. The mean left ventricular pressure (LVP) developed by heat-acclimated 2-, 5-, and 30-day-old (AC₂, AC₅, and AC₃₀, respectively) rat hearts was significantly greater than that of the C hearts at all stimulation frequencies. For example, at 300 beats/min (Fig. 1), the pressure generated by the AC₂ and AC₅ hearts was significantly greater than that of control hearts, respectively (C: 59.62 ± 11.38, AC₂: 78.98 ± 5.8, and AC₅: 77.15 ± 6.2 mmHg). P < 0.01 and 0.05, respectively. The AC2 (P < 0.01) and AC5 (P < 0.05) hearts were significantly greater than that of control hearts, respectively (C: 59.62 ± 11.38, AC₂: 78.98 ± 5.8, and AC₅: 77.15 ± 6.2 mmHg, P < 0.01 and 0.05, respectively).
Table 1. The rate of pressure generation and relaxation control and heat-acclimated groups at 200, 300, and 400 beats/min

<table>
<thead>
<tr>
<th>Rate (beats/min)</th>
<th>C</th>
<th>AC2</th>
<th>AC5</th>
<th>AC30</th>
</tr>
</thead>
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<tr>
<td>200</td>
<td>+dP/dt/P</td>
<td>62.3 ± 4.3</td>
<td>69.7 ± 1.9</td>
<td>67.8 ± 2.4</td>
</tr>
<tr>
<td></td>
<td>−dP/dt/P</td>
<td>−42.2 ± 3.3</td>
<td>−46.3 ± 1.5</td>
<td>−44.5 ± 1.7</td>
</tr>
<tr>
<td>300</td>
<td>+dP/dt/P</td>
<td>72.2 ± 5.4</td>
<td>76.8 ± 3.2</td>
<td>69.2 ± 3.5</td>
</tr>
<tr>
<td></td>
<td>−dP/dt/P</td>
<td>−47.1 ± 3.9</td>
<td>−51.3 ± 1.3</td>
<td>−49.7 ± 2.1</td>
</tr>
<tr>
<td>400</td>
<td>+dP/dt/P</td>
<td>78.1 ± 8.2</td>
<td>79.6 ± 7.7</td>
<td>72.2 ± 2.9</td>
</tr>
<tr>
<td></td>
<td>−dP/dt/P</td>
<td>−48.2 ± 3.4</td>
<td>−49.1 ± 3.8</td>
<td>−48.3 ± 1.8</td>
</tr>
</tbody>
</table>

Values are means ± SE for n, no. of rats (control (C; n = 14) acclimated for 2 (AC2; n = 8), 5 (AC5; n = 8), and 30 (AC30; n = 14) days. The rates of pressure generation measured in +dP/dt/P (mmHg·s⁻¹·mmHg⁻¹) and relaxation measured in −dP/dt/P (mmHg·s⁻¹·mmHg⁻¹). Statistical comparisons: *P < 0.05.

respectively). These values were not significantly lower than the LVP generated by the AC30 (90.42 ± 20.4 mmHg) hearts. The rates of pressure generation and relaxation (+dP/dt-P and −dP/dt-P) were similar for C, AC2, and AC5, but slower in the AC30 hearts (∙P < 0.05, Table 1). Representative records of individual hearts ascribed to each experimental group are demonstrated in Fig. 2.

Adrenergic inotropic response. There were temporal variations in cardiac adrenergic responsiveness to noradrenaline stimulation. Marked initial decreased responsiveness of the cardiac positive inotropic response was observed on day 2 of the acclimation (28% vs. 54% in C hearts, P < 0.05), although baseline pressure on that day was significantly higher than that of the control group. An upward shift in cardiac responsiveness to the drug was observed from day 5 of the acclimation and onward, achieving a peak on day 5 of the acclimation, achieving a peak on day 5 of the acclimation, but remained greater than in the controls on LTHA. Measurements of the negative inotropic response to propranolol administration yielded similar results (not shown).

Adrenergic receptor binding. The β-adrenergic receptor density is presented in Fig. 4. There was no significant change in the β-AR receptor density during either STHA or LTHA. In contrast, receptor affinity, as inferred from the marked drop in 1/Kd (by 2.4-fold, P < 0.05) decreased.

SR-Ca²⁺-ATPase and PLB mRNA transcription and SERCA and PLB protein expression. To determine whether changes in the mechanical properties of the STHA hearts were associated with altered expression of SERCA and PLB, as was previously found for the LTHA hearts (3), the mRNAs coding for these proteins and SERCA and PLB expression were measured. Repre-
sentative results of the steady-state mRNA levels obtained for Ca\(^{2+}\)-ATPase and PLB and the averaged density of the Ca\(^{2+}\)-ATPase and PLB mRNA bands relative to the density of the housekeeping gene β-actin are shown in Fig. 5. STHA resulted in a pronounced increase in the Ca\(^{2+}\)-ATPase mRNA level, up to 120% (AC2, \(P < 0.05\); AC5, \(P < 0.01\)). Concomitantly, the PLB mRNA levels in the STHA hearts increased threefold compared with C hearts. On LTHA, the expression of the Ca\(^{2+}\)-ATPase steady-state mRNA level decreased to 65% of the control group level (\(P < 0.01\)). The PLB steady-state mRNA level was lower compared with that of the STHA hearts (\(P < 0.01\)), however, it maintained a significantly higher level (Δ84%) than that of the controls.

The level of SERCA and PLB proteins was measured by quantitative immunoblotting (Fig. 6). In the STHA, AC\(_2\) hearts, unlike their steady-state Ca\(^{2+}\)-ATPase transcript levels, maintained SERCA protein at a level similar to that of the C hearts. In contrast, AC\(_5\) hearts displayed a significant decrease in SERCA expression, down to 65% of that of the C group (\(P < 0.01\)). During that phase, PLB levels did not differ significantly from the preacclimation level. On LTHA, similar to previous observations, the SERCA level was significantly lower than the SERCA control levels, whereas PLB showed significant upregulation. It is thus evident that the ratio of PLB to SERCA (Fig. 7) increased in the AC\(_5\) and AC\(_{30}\) but remained unchanged in the AC\(_2\) hearts compared with that in the C group.

Plasma thyroid hormones. Table 2 presents thyroid hormone levels in nonacclimated and heat-acclimating rats. The plasma \(T_4\) level in the C rats (ranging between 4.1 and 3.9 ± 0.25 µg/dl) concurred with the results of previous studies on plasma \(T_4\) concentration in euthyroid rats (5). In the AC group, \(T_4\) levels decreased gradually and were significantly lower than those in the C group after 2 wk of heat exposure. The plasma \(T_3\) levels displayed a similar picture.

Fig. 4. β-Adrenergic receptor density (B) and affinity (A) in C and heat-acclimated rats. Each bar represents the mean ± SE; \(n = 4\) or 5 for each group. *Significant difference from the controls (\(P < 0.05\)).

Fig. 5. Semiquantitative RT-PCR analysis of Ca\(^{2+}\)-ATPase (A) and phospholamban (B) mRNAs in C and heat-acclimated rats. The bar graphs show the relative amounts of Ca\(^{2+}\)-ATPase (A) and phospholamban (B) mRNAs normalized to β-actin in the different experimental groups. The mRNA of each individual animal was measured independently 3 times. Each bar represents the mean ± SE; \(n = 5\) for each group. Symbols denote significant difference from the controls: *\(P < 0.05\), **\(P < 0.01\), ***\(P < 0.03\), ****\(P < 0.005\). In A and B, representative sets of bands (10 µl/lane) for each transcript are presented.
DISCUSSION

This investigation provides solid evidence for temporal variations in cardiac mechanical performance in the course of heat acclimation. In the rat heart model, both STHA and LTHA lead to enhanced LVP. However, the share of each mechanism contributing to the contractile performance, as manifested by the velocity of contraction and relaxation, differs in short- and long-term acclimation. This investigation shows that during STHA, the velocity of contraction and relaxation is similar to that of the control hearts, whereas on LTHA, both these parameters are markedly lowered. These differences stem from different expression of the sarcoplasmic Ca²⁺ regulatory proteins and from altered adrenergic responsiveness of the myocardium as well as from redistribution of myosin isoforms, as previously shown (13). A qualitative comparison of these contractility-associated features between STHA and LTHA is presented in Table 3. Whereas velocity of contraction depends on the distribution of cardiac myosin isoforms (2, 6), velocity of relaxation is associated with the rate of decline of cytosolic calcium during each beat (22). This is largely dependent on the control of the SR-calcium pump (SERCA) by PLB (22, 35). Hence, many investigators have accepted the PLB/SERCA ratio as a

Table 2. T₄ and T₃ levels in the course of heat acclimation

<table>
<thead>
<tr>
<th>Weeks</th>
<th>T₄, µg/dl</th>
<th>T₃, ng/dl</th>
</tr>
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<tbody>
<tr>
<td>C</td>
<td>AC</td>
<td>C</td>
</tr>
<tr>
<td>1</td>
<td>4.16±0.13</td>
<td>4.20±0.15</td>
</tr>
<tr>
<td>2</td>
<td>4.30±0.14</td>
<td>3.87±0.47</td>
</tr>
<tr>
<td>3</td>
<td>3.86±0.17</td>
<td>3.56±0.31</td>
</tr>
<tr>
<td>3</td>
<td>3.90±0.27</td>
<td>3.03±0.25*</td>
</tr>
<tr>
<td>4</td>
<td>3.97±0.25</td>
<td>2.62±0.29*</td>
</tr>
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</table>

Values are means ± SE. *Significant difference from the matched control (P < 0.05). T₃, 3,5,3'-triiodothyronine; T₄, L-thyroxine.

STHA and LTHA lead to enhanced LVP. However, the share of each mechanism contributing to the contractile performance, as manifested by the velocity of contraction and relaxation, differs in short- and long-term acclimation. This investigation shows that during STHA, the velocity of contraction and relaxation is similar to that of the control hearts, whereas on LTHA, both these parameters are markedly lowered. These differences stem from different expression of the sarcoplasmic Ca²⁺ regulatory proteins and from altered adrenergic responsiveness of the myocardium as well as from redistribution of myosin isoforms, as previously shown (13). A qualitative comparison of these contractility-associated features between STHA and LTHA is presented in Table 3. Whereas velocity of contraction depends on the distribution of cardiac myosin isoforms (2, 6), velocity of relaxation is associated with the rate of decline of cytosolic calcium during each beat (22). This is largely dependent on the control of the SR-calcium pump (SERCA) by PLB (22, 35). Hence, many investigators have accepted the PLB/SERCA ratio as a

Table 3. Cardiac performance dynamics in the course of heat acclimation; a qualitative comparison of cardiac mechanics, adrenergic signaling, and calcium regulation with respect to preacclimation

<table>
<thead>
<tr>
<th>STHA</th>
<th>AC₂</th>
<th>AC₅</th>
<th>LTHA</th>
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<tbody>
<tr>
<td>LVP</td>
<td>↑</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>+dP/dt/P</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>-dP/dt/P</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Ca inotropic response</td>
<td>↑</td>
<td>↑</td>
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<td>Ca transients</td>
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<td>Na inotropic response</td>
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<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>β-Adrenergic contribution</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>SR-Ca²⁺-ATPase mRNA</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>SERCA</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>PLB mRNA</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>PLB</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>PLB/SERCA</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>V₃ myosin</td>
<td>↑</td>
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</table>

STHA, short-term heat acclimation; LTHA, long-term heat acclimation; LVP, left ventricular pressure; ↑, greater than C; ↓, lower than C; =, equal to C; SERCA, sarcoplasmic reticulum Ca²⁺ ATPase; PLB, phospholamban; V₃, slow myosin isoform. All data are from this investigation except Ca inotropic response (M. Eynan, C. Gross, and M. Horowitz, unpublished data), Ca transients (O. Cohen, U. Meiri, and M. Horowitz, unpublished data and Ref. 13), and V₃ myosin (13). Arrows grade the magnitude of change.

Fig. 6. Quantification of sarcoplasmic reticulum Ca²⁺-ATPase (SERCA; A) and phospholamban (PLB; B) levels in hearts isolated from C and heat-acclimated rats. The bar graphs show the SERCA and PLB protein levels in the different groups of rats normalized to the C sample. Each heart homogenate was tested 3 times in separate runs. The data are expressed as means ± SE; n = 5 for each group. Symbols denote significant differences from the matched controls: *P < 0.05, **P < 0.005. In A, representative sets of blots of the various groups (enhanced chemiluminescence detection) are presented.

Fig. 7. Percent change in PLB/SERCA ratio in hearts of heat-acclimated rat hearts compared with C hearts (baseline = 100%). The data (derived from values presented in Fig. 6) are expressed as means ± SE. For n, see Fig. 6. *Significant difference from the matched control (P < 0.03).
major determinant of cardiac lusitropy (velocity of relaxation) (19, 21), irrespective of the changes leading to the establishment of this ratio (26). The two studied STHA groups, AC₂ and AC₅, similar to the controls, showed fast myosin (V₁ isoform) predominance (13). This is compatible with the preacclimation velocity of contraction measured in these groups. In contrast, the maintenance of the preacclimation velocity of relaxation in the STHA groups cannot be simply attributed to the PLB/SERCA ratio calculated. This ratio is similar to that of the controls in the AC₂ group only. In the AC₅ group, the PLB/SERCA ratio is markedly elevated and somewhat exceeds that of the LTHA hearts, in which slow velocity of relaxation is documented. This mismatching may suggest that the PLB/SERCA ratio does not always fit with the state of lusitropy. This view is supported by our previous observation in hypothyroid rats, in which a euthyroid PLB/SERCA ratio accompanies a marked negative lusitropic effect (3).

PLB is a prominent mediator of the transduction of cardiac β-adrenergic signaling via its phosphorylation by β-adrenergic stimulation. Phosphorylation of PLB relieves SERCA inhibition by PLB, enhances calcium uptake to the SR pool, and, in turn, establishes an adequate SR-Ca²⁺ pool for the expression of the β-adrenergic inotropic response (20). Accordingly, upregulation of PLB subjects the SR to greater adrenergic positive inotropic influence. In contrast, in PLB-ablated mice, the inotropic effect of isoprenaline is attenuated (21). Under several chronic situations, however, the altered PLB level may be matched by an altered level of phosphorylation to adjust SERCA activity. For example, after chronic β-adrenergic stimulation leading to cardiac hypertrophy, augmented PLB dephosphorylation compensates, at least in part, for reduced PLB occurring during this situation (34) to coordinate SERCA activity. It is thus reasonable to hypothesize that β-adrenergic receptor affinity and/or tissue catecholamine levels are associated with PLB phosphorylation and, in turn, with PLB/SERCA-lusitropic state relationships. Whereas the sarcolemma adrenergic components (receptors and G proteins) have control over sensitivity, the SR mediators (calcium regulatory proteins) control maximal responsiveness of the adrenergic inotropic response (33).

In this investigation, adrenergic signaling was examined by measuring the density and affinity of the β-ARs and by the magnitude of the norepinephrine inotropic response. Receptor density was almost unchanged throughout the entire acclimation period. Their affinity, however, largely decreased. Accelerated catecholamine release at the initial acclimation phase and markedly higher tissue catecholamine level stemming from lowered turnover on LTHA (16, 17) are the likely reasons for the decreased affinity observed.

Cardiac inotropic response to norepinephrine, in contrast, varied temporally with progression of acclimation. Among our experimental groups, the STHA-AC₂ hearts showed almost blunted β-adrenergic responsiveness, whereas the LTHA hearts showed the greatest β-adrenergic responsiveness. In view of the concept that SR controls maximal responsiveness of the adrenergic inotropic response (33), it is conceivable that the marked PLB upregulation observed on LTHA allows the significantly augmented norepinephrine-induced inotropic response. Concomitantly, the LTHA hearts showed negative lusitropy. This combination of PLB upregulation together with negative lusitropy resembles the overexpressed PLB mice model or hypothyroid state (19, 21). In the LTHA hearts, these features correspond to the sustained low thyroxin level developed in this acclimation phase (Table 2). Indeed, maintenance of a euthyroid state during acclimation abolishes the development of these features (3, 13).

In the STHA phase, PLB maintains its preacclimation level. At that phase, marked decreased inotropic response to norepinephrine (Fig. 3) and marked abolition of the norepinephrine inotropic response after propranolol administration were observed. Therefore, it is likely that control over cardiac contractility in this acclimation phase is largely dominated by the membranous contribution to β-adrenergic signaling compared with the nonacclimated hearts. STHA is characterized by accelerated autonomic excitability. This (together with the global stress response) elevates catecholamine release (25), ultimately leading to signaling desensitization via decreased receptor affinity and, possibly, decreased ratio of the stimulatory to inhibitory G proteins (33). Despite increased PLB/SERCA ratio on day 5 of the acclimation, the preacclimation velocities of contraction and relaxation were maintained in both AC₂ and AC₅. This favors the assumption that the accelerated autonomic excitability compensates for the receptor desensitization and PLB phosphorylation level with respect to the decreased SERCA levels from day 2 to 5 of the acclimation in a dynamic manner. PLB phosphorylation was not measured in the present investigation.

The short- and long-term effects of heat acclimation are also displayed by the alterations in the steady-state profile of PLB and SR-Ca²⁺-ATPase and PLB transcripts and the consequent protein expression. On STHA, both SR-Ca²⁺-ATPase and PLB transcripts showed initial upregulation. This was not accompanied by upregulation of the encoded proteins. Furthermore, the AC₅ hearts showed SERCA downregulation. On LTHA, both transcript levels decreased compared with STHA. However, whereas SR-Ca²⁺-ATPase mRNA decreased to below the preacclimation level, the PLB transcript remained significantly higher than that in the C hearts. It was only during LTHA that PLB translation followed the change in mRNA, whereas SERCA matched SR-Ca²⁺-ATPase transcript downregulation, as observed for several long-term processes, e.g., hyperthyroid and hypothyroid states, heat acclimation as shown in our own observations, and several other pathophysiological situations (31). The conservation of the preacclimation PLB, SERCA, and V₃ profile by the maintenance of a euthyroid state during acclimation indicates unequivocally that the sustained lowered thyroxin level on LTHA mediates the changes observed. Hence, upregulation of SR-Ca²⁺-ATPase and PLB transcripts
on STHA must be mediated by triggers other than altered thyroxin level. Interestingly, in another study on acclimated hearts, we showed on 2 days of STHA, marked upregulation of heat shock protein (HSP) 72-kDa mRNA, unaccompanied by protein synthesis (28). This situation resembles that observed for PLB and SR-Ca\(^{2+}\)-ATPase in the present investigation: a rapid transcription response with attenuation of posttranscriptional processes. Heat stress halts protein synthesis. We hypothesize that the initial acclimatory strain has a similar effect. This might explain the mismatching observed between the changes in mRNA and protein expression. In the case of HSP mRNA transcription, accelerated sympathetic activity is a likely candidate for triggering transcription (A. Maloyan and M. Horowitz, unpublished observations and Ref. 29). As PLB mediates adrenergic signaling (22), we would like to hypothesize that accelerated sympathetic activity during STHA (9, 12) induces PLB and SR-Ca\(^{2+}\)-ATPase transcript upregulation as well. Despite numerous studies on \(\beta\)-adrenergic signal mediation by PLB, the reverse cross talk has not yet been largely documented for the heart. In the canine skeletal muscles latissimus dorsi and vastus intermedius, however, chronic \(\beta\)-adrenostimulation affects PLB and Ca\(^{2+}\)-ATPase expression (36).

Collectively it can be concluded that although the message for the SR-calcium regulatory proteins is altered rather early in the heat-acclimating heart, this is not displayed phenotypically.

In summary, the findings of the present investigation indicate that the initial phase of cardiac adaptation to chronic heat constitutes temporally changing responses at both the molecular and integrative levels. The greater pressure with preacclimation velocities of contraction and relaxation observed in the STHA phenotype does not express changes occurring in calcium regulatory proteins at the translation level. Temporally varying expression of \(\beta\)-adrenergic signaling and enhanced sympathetic activity might induce the changes observed. These are replaced by the sustained changes observed on LTHA. On LTHA, greater pressure was accompanied by a drop in the velocity of contraction and relaxation. These changes are the outcome of the influence of sustained low thyroxin levels on transcription of the genes encoding calcium regulatory proteins and myosin isofoms. This feature of temporally varying changes with the course of adaptation is in accord with the biphasic acclimation model proposed by Horowitz (8, 9).

Collectively, the physiological manifestation in vivo of cardiac adaptation to chronic heat is the elevated stroke volume (15). A consensus explanation of this adaptive response was an increased venous return due to vasodilatation and augmented blood volume, both occurring on heat acclimation. Our data suggest that intrinsic cardiac adaptations develop to accommodate the peripheral load. These enhance cardiac work efficiency and cardiac reserves.

**Perspectives**

To date, our understanding of heat acclimation in mammalian species is confined mostly to studies at the integrative level. The results of this investigation expand our understanding of molecularly driven mechanisms, which, at least in part, dominate the integrative adaptive responses. This may promote future studies on cross talk between molecular and integrative processes in heat adaptation in mammals.

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