Thyroid hormone regulation of cardiac bioenergetics: role of intracellular creatine

Marcia Silva Queiroz,1 Yvonne Shao,1 Deborah A. Berkich,2 Kathryn F. LaNoe, and Faramarz Ismail-Beigi1
1Department of Medicine and Physiology and Biophysics, Case Western Reserve University, Cleveland, Ohio 44106-4951; and 2Cellular and Molecular Physiology, Pennsylvania State University College of Medicine, Hershey, Pennsylvania 17033

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Queiroz, Marcia Silva, Yvonne Shao, Deborah A. Berkich, Kathryn F. LaNoe, and Faramarz Ismail-Beigi. Thyroid hormone regulation of cardiac bioenergetics: role of intracellular creatine. Am J Physiol Heart Circ Physiol 283: H2527–H2533, 2002; 10.1152/ajpheart.00426.2002.—The effect of thyroid hormone (T3) on the content of myocardial creatine (Cr), Cr phosphate (CrP), and high-energy adenine nucleotides and on cardiac function was examined. In the hearts of control and T3-treated rats perfused in vitro, while “low” and “high” contractile work was performed, T3 treatment resulted in a ~50% reduction in CrP, Cr, total Cr content (Cr + CrP), and in the CrP-to-Cr ratio. In addition, there was a slight fall in myocardial content of ATP and a large rise in calculated free ADP (fADP), resulting in a significant decrease in the ATP-to-fADP ratio in the hearts of hyperthyroid compared with euthyroid rats. Moreover, there was a substantial decrease in the level of ATP in hearts of T3-treated rats under high work conditions. Importantly, the ratio of cardiac work to oxygen consumption was not altered by thyroid status. Treatment with T3 also resulted in an almost threefold reduction in the content of Na+/Cr transporter mRNA in the ventricular myocardium and skeletal muscle but not in the brain. We conclude with the following: 1) changes in the expression of the Na+/Cr transporter mRNA correlate with Cr + CrP in the myocardium; 2) hearts of hyperthyroid rats contain lower levels of ATP and higher levels of fADP under both low and high work conditions but no reduction in efficiency of work output; and 3) the reduction in Cr and ATP in hearts of hyperthyroid rats may be the basis for the reduced maximal work capacity of the hyperthyroid heart.

free ADP; ATP; creatine phosphate; cardiac work output; creatine transporter mRNA

The calorigenic action of thyroid hormone in the heart is manifested by an increased rate of cardiac metabolism and oxygen consumption. This results from a dual action of the hormone: a direct one, which is due to stimulation of cardiomyocyte metabolism, and a second, indirect one, which occurs in response to increased demand of peripheral tissues for oxygen and substrates and for the removal of waste products (5, 10). The combination of these effects, at least during the early phase of hyperthyroidism, leads to increased “contractility” of the hyperthyroid heart, which is manifested by positive changes in virtually all measurable parameters of cardiac muscle function (1, 3, 5, 6, 10, 21, 29, 30, 46). Excess thyroid hormone is associated with an increase in the number and volume of mitochondria in cardiac myocytes and skeletal muscle, and the resultant stimulation of oxidative phosphorylation is associated with increased numbers of respiratory units per milligram mitochondrial protein with little or no change in the phosphorylation-to-oxygenation ratio (5, 10, 30, 46).

These thyroid hormone (T3)-induced alterations are associated with increased expression and activity of adenine nucleotide translocase (isoform 2) without a consistent change in cytosolic or mitochondrial creatine (Cr) kinase protein (5, 10, 13). Despite the positive inotropic and chronotropic effects of thyroid hormone on the heart that are accompanied with increased expression of mitochondrial and contractile proteins and membrane-bound enzymes, including Na-K+-ATPase and Ca-ATPase, the hyperthyroid heart exhibits a decrease (not an increase) in its capacity to perform work at a maximal level (5, 10, 30, 46). Moreover, the excessive workload imposed on the hyperthyroid heart can ultimately lead to myopathy and organ failure (10, 28, 39, 46). However, the mechanism leading to the limitation in the maximal work capacity of the heart in hyperthyroidism remains unknown.

One of the striking alterations in the hyperthyroid heart and skeletal muscle is a profound ~50% reduction in the cellular concentrations of Cr and Cr phosphate (CrP) (9, 17, 35) with minimal change in their ratio (39); the mechanism underlying the decrease in the Cr pool is unknown. It is of interest that a similar decrease in the myocardial Cr pool (Cr + CrP) has been described in various forms of congestive heart failure in both humans and experimental animals (17, 25, 40). Given the importance of oxidative phosphorylation in the heart (9, 18, 20, 31, 35, 39, 41), it is plausible that the decrease in myocardial CrP and/or Cr is instrumen-

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tinal in the observed decrease in the “reserve” and “maximal” work capacity of the heart in the hyperthyroid state.

It is well known that hyperthyroidism is associated with elevated levels of Cr in the circulation (38, 42, 43). Because Cr is not synthesized in cardiac or skeletal muscle, these and other tissues (except for the liver and kidney, which synthesize Cr) rely on the uptake of Cr from the circulation to maintain intracellular free Cr levels at ~100 times that in plasma (43). An investigation (35) was undertaken to study the dramatic ~50% decline in total Cr content (Cr + CrP pool) in cardiac muscle induced by the hyperthyroid state (35). These researchers studied the effect of thyroid hormone on Cr uptake in the isolated perfused heart and found an increase in the rate of uptake, suggesting that the reduction in Cr pool results from an even greater increase in the cellular loss of Cr. This observation, however, has not been confirmed. Results of other studies (23, 26, 38) have shown that Cr uptake is an “active” transport process requiring Na+ (and chloride) cotransport. The Na+/Cr cotransporter mediating the uptake of Cr has been cloned in several species within the past few years and its transport properties have been partially characterized in transfected cells (11, 34).

The present study was undertaken to evaluate the functional importance of the reported T3-induced diminution of Cr + CrP levels in the heart and to identify more clearly the molecular basis for the reduction in Cr levels. To accomplish these goals, we determined the effect of thyroid hormone on levels of high-energy adenine phosphates, CrP, Cr, Cr + CrP, the CrP-to-Cr (CrP/Cr) ratio, oxygen consumption, and work output in hearts of control and T3-treated rats perfused in vitro under low pressure (low work) and high pressure (high work) conditions. In addition, the effect of T3 on mRNA levels encoding the Na+/Cr transporter was determined in the heart, skeletal muscle, and brain. The results are consistent with the proposal that the low Cr + CrP of the myocardium, secondary to diminished expression of the Na+/Cr transporter, might limit the maximal work output of the hyperthyroid heart.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats (~250 g) provided with free access to food and water were treated with a receptor-saturating dose of T3 (100 μg subcutaneously/100 g body wt) every other day for 1 wk (2, 27, 37). This treatment regimen has been shown to result in a “steady-state” hyperthyroid condition (2, 37). Controls were similarly injected with diluent. T3 solution was prepared by dissolving 20 mg of the hormone in 2.0 ml of 50 mM NaOH, followed by dilution to 5.0 mM NaOH in saline.

Isolated heart perfusions. T3-treated and untreated male rats (~400 g) were anesthetized by an intraperitoneal injection of pentobarbital sodium. Hearts were perfused isovolumically, as described previously (12, 44, 45), with medium containing (in mM) 118 NaCl, 4.7 KC1, 1.75 CaCl2, 0.5 EDTA, 1.2 MgSO4, 25 NaHCO3, 11 glucose, and 5 pyruvate. Ventricular pressure and heart rate were recorded via a balloon inserted into the left ventricle and connected to a pressure transducer and a Digitimed Heart Performance Analyzer (Micromed; Louisville, KY). Cardiac oxygen consumption was measured as described previously (12, 44, 45). Hearts were perfused and all parameters were measured under two conditions: 1) a low work condition, where the heart was perfused at 60 mmHg with an unfilled balloon in the left ventricle, and 2) a high work condition, in which a fluid-filled balloon (15 mmHg) was placed in the left ventricle and the heart was perfused at 120 mmHg with 10 nM isoproterenol in the perfusate. In some instances, hearts were perfused at a “moderate” work level. In this case, hearts were perfused as described for high work but with no isoproterenol in the perfusate medium. Data were obtained from hearts perfused for 90 min. Work output, the product of heart rate multiplied by peak systolic pressure (RPP), and O2 consumption were obtained by averaging values over the final 30 min of perfusion. At the end of the perfusions, hearts were rapidly frozen with clamps precooled in liquid N2. It should be noted that in this model the heart is performing only pressure work, which is known to be more energy costly than volume work.

Assay of tissue metabolites. In some experiments, after anesthesia, ventricular myocardium were freeze clamped in situ, and the cerebrum was isolated as quickly as possible and frozen in liquid N2. In other experiments, hearts were freeze clamped after the perfusion studies in vitro. ATP, CrP, and Cr (4) were measured in neutralized perchloric extracts of freeze-clamped hearts with the use of enzymatic techniques linked to spectrophotometric changes in NADH. Free ADP ([ADP]CrP) in heart cytosol was calculated assuming that the Cr kinase reaction is in equilibrium. The equilibrium constant (Keq) of Cr kinase was taken to be 122 × 109 M−1 = [ATP][Cr]/[ADP][CrP] [H+], (12). An aqueous volume of 3.23 ml/g dry wt was used (12) to convert metabolite values as assessed (μmol/g dry wt) to concentrations. The Cr kinase Keq varies with free Mg2+, and the value used here is the one reported by Lawson and Veech (see Ref. 12) for a free Mg2+ concentration of 0.4 mM. This value of free Mg2+ and the value of [H+] = 10−7.05 was obtained in the laboratory of K. F. LaNoue, by performing experiments on rat hearts under conditions almost identical to those employed here (12).

Isolation of RNA and Northern blot. Total RNA was isolated from in situ freeze-clamped qudriceps and heart ventricles or from rapidly isolated and frozen cerebrum, diaphragm, kidney cortex, and liver by the method of Chirgwin et al. (8). After quantitation, equivalent amounts of RNA (~30 μg) from tissues of euthyroid and hyperthyroid rats were loaded per lane of agarose-formaldehyde gels (37). RNA bands were transferred to nitrocellulose and monitored for equivalent loading of the lanes and for completeness of transfer by measurement of ribosomal 28S RNA. The resulting blot was probed with a radiolabeled 2,100-bp DNA fragment encoding the rat Na+/Cr transporter prepared by RT-PCR of rat heart RNA (and verified by sequencing) (34). The intensity of the mRNA band was adjusted against the intensity of ethidium bromide staining of 28S rRNA of the same blot.

To determine the effect of thyroid status on tissue Na+/Cr mRNA expression, the resulting X-ray films were scanned, and the adjusted densities of appropriate bands in lanes containing RNA from euthyroid rats were averaged and normalized to 1.0 for each tissue. The density of the bands of rRNA derived from hyperthyroid rat tissues were divided by the mean value of euthyroid group for each tissue and averaged.

Statistical analysis. All data are presented as means ± SE. An unpaired Student’s t-test was used, and P < 0.05 was considered significant.
Table 1. Effect of thyroid status on body weight, heart weight, and heart weight-to-body weight ratio

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<tr>
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<th>Euthyroid</th>
<th>Hyperthyroid</th>
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<tr>
<td>Body weight, g</td>
<td>301 ± 5</td>
<td>252 ± 6*</td>
</tr>
<tr>
<td>Heart weight, g</td>
<td>0.86 ± 0.03</td>
<td>1.06 ± 0.04*</td>
</tr>
<tr>
<td>Heart weight/body weight</td>
<td>0.0029 ± 0.0001</td>
<td>0.0042 ± 0.0001†</td>
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Values are means ± SE. Hyperthyroidism was produced by injection of euthyroid rats with 100 μg thyroid hormone (T3)/100 g body wt three times on alternate days (n = 8). Euthyroid rats (n = 8) injected with vehicle were used as control. Animals were anesthetized, weighed, and then euthanized by decapitation. After complete exsanguination, the hearts were removed and weighted. *P < 0.05; †P < 0.01; ‡P < 0.001 compared with the euthyroid state.

RESULTS

Table 1 summarizes the effect of T3 on body weight, heart weight, and heart weight-to-body weight ratio, with the latter parameter being a highly reliable indicator of thyroid status (5–7, 10, 14, 27, 36); the T3-induced hypertrophy of the heart is associated with significant increases in myocardial protein-to-DNA and RNA-to-protein ratios (7, 14). As can be seen in Table 1, treatment with T3 resulted in a dramatic increase in the heart weight-to-body weight ratio.

We then determined the effects of T3 treatment and workload on the contents of CrP, Cr, and high-energy adenine nucleotide phosphates in perfused rat hearts. Figure 1, A–D, summarizes the contents of CrP and Cr, values of the CrP/Cr ratio, and Cr + CrP, respectively, measured after perfusion of euthyroid and hyperthyroid hearts for 90 min at low work and high workloads. In the perfused heart model employed herein, only pressure work is being performed. The contents of CrP, Cr, the CrP/Cr ratio, and Cr + CrP were lower in hearts of T3-treated rats compared with hearts of control animals, irrespective of the workload (P < 0.05).

The above changes in the contents of myocardial CrP and Cr were verified in a second series of experiments in which hearts were perfused for only 5–10 min before being freeze clamped (data not shown). Finally, in a third series of experiments, in hearts freeze clamped in situ, Cr + CrP was reduced from 64.8 ± 1.3 μmol/g dry wt in control rats to 41.2 ± 1.9 μmol/g dry wt in T3-treated rats (n = 6, P < 0.05).

Figure 2, A–C, summarizes the levels of ATP and fADP and values of the ATP-to-fADP (ATP/fADP) ratios in the same hearts studied above. ATP was lower in hearts of hyperthyroid animals at both workloads, with the difference in ATP levels between hyperthyroid and euthyroid hearts being greater at higher workload (Fig. 2A). Of special note was the decrease in the content of ATP in hearts of T3-treated rats at high workload. Although ATP levels of both euthyroid and hyperthyroid hearts decreased significantly when work output increased, the effect of work was larger and more significant in the case of hyperthyroid hearts. Knowing ATP and the CrP/Cr ratio in hearts of control and T3-treated rats, it is possible to calculate the concentration of unbound fADP in the cytosol of euthyroid and hyperthyroid hearts at both workloads (Fig. 2B). The fADP was increased and the ATP/fADP ratio was significantly reduced in hearts of T3-treated animals, irrespective of the workload (Fig. 2C). We also noted a ~2-fold rise in myocardial AMP levels in T3-treated rats under low work conditions (from 0.38 ± 0.02 to 0.64 ± 0.07 μmol/g dry wt; P < 0.05).

The effect of T3 treatment on the rate of oxygen consumption at different levels of work performed by the heart was also determined (Fig. 3). In addition to the two work levels employed above, in some instances isoproterenol was omitted from the perfusate to provide an intermediate level of work. Work output (cal-
culated as RPP) was plotted as a function of O₂ consumption (Fig. 3). Of note is the finding that at the low work level, hearts of T₃-treated animals had higher work output and rates of O₂ consumption compared with hearts of euthyroid animals, whereas at the high work level, the work performed and rates of O₂ consumption were similar in hearts of euthyroid and hypothyroid rats. Moreover, and importantly, it is evident that all of the values fall on the same straight line, strongly suggesting a constant coupling efficiency between O₂ consumption and work output, irrespective of the thyroid state of the animal.

The observed decrease in the Cr + CrP pool in the heart can result from decreased Cr uptake, increased loss of Cr, or both. To explore these possibilities, we determined the effect of thyroid hormone on the expression of Na⁺/Cr transporter mRNA in three tissues of the rat. In initial experiments we verified the presence of the mRNA in the heart, skeletal muscle (quad-riiceps and diaphragm), kidney cortex, and cerebrum, and its virtual absence in the liver (Fig. 4) (38). Previous results (34) have documented that the Na⁺/Cr transporter mRNA is expressed as two mRNA species, reflecting usage of two poly-A signals. Treatment of euthyroid rats with T₃ to induce hyperthyroidism was associated with an appreciable decrease in the content of Na⁺/Cr transporter mRNA in ventricular myocardium and skeletal muscle but not in the brain (Fig. 5, Table 2); the abundance of both mRNA species was decreased in hearts of T₃-treated animals. In keeping with the findings obtained in the brain, treatment with T₃ resulted in no change in the Cr + CrP pool in the brain (47.9 ± 0.5 and 49.8 ± 0.6 μmol/g dry wt in control and T₃-treated rats, respectively; n = 6; P > 0.5).

DISCUSSION

The studies described in this report provide novel information concerning the bioenergetic properties of the hyperthyroid heart. Our goal was first to verify the

Fig. 2. The combined influence of hyperthyroidism and workload on ATP (A), free ADP (fADP) (B), and ATP-to-fADP (ATP/fADP) ratios (C) of isolated perfused hearts. The experimental procedures are described in Fig. 1. Cytosolic fADP was calculated assuming that the substrates and products of Cr kinase are in near equilibrium and that the equilibration constant (Kₐ) of Cr kinase is 122. The calculation also assumes that the intracellular aqueous volume is 3.23 μl/mg dry wt. ○, Euthyroid; ●, T₃ treated. All values are means ± SE; n = 7–17. *P < 0.05; **P < 0.01 comparing values for hyperthyroid vs. euthyroid hearts at low and high RPP values.

Fig. 3. Myocardial O₂ consumption as a function of work output in control and hyperthyroid rat hearts. Oxygen consumption was assessed by the product of coronary flow times the difference between the O₂ content of the cardiac influent and coronary venous effluent. Work output was monitored as RPP. ○, Euthyroid; ●, T₃ treated. Values shown are means ± SE, n = 6–12 for each data point.

Fig. 4. Expression of Na⁺/Cr transporter mRNA in different tissues of the rat. Total RNA was isolated from euthyroid rat tissues (heart ventricle, skeletal muscle or quadriceps femoris, diaphragm, liver, kidney cortex, and cerebrum). Equal amounts (40 μg) of total RNA from each tissue was fractionated and transferred to nitrocellulose. The resulting blot was probed with rat Na⁺/Cr transporter cDNA. Positions of 28S and 18S rRNA (open arrows) and the two Na⁺/Cr transporter mRNA bands (solid arrows) are shown.
reduction of Cr in hyperthyroid hearts and explore the potential mechanism underlying this reduction, and second to examine whether the lower Cr + CrP is associated with changes in cardiac performance. Recent data (20) from transgenic mice lacking mitochondrial Cr kinase show that a marked reduction in Cr kinase activity can limit rates of ATP synthesis, especially under high work conditions. Previous data (32, 33) from other types of animal models also suggest that flux through mitochondrial Cr kinase is an important determinant of maximal rates of ATP synthesis.

The results of this study show for the first time that expression of the mRNA encoding the Na\(^+\)/Cr transporter is decreased about threefold in ventricular myocardium and skeletal muscle of hyperthyroid rats. This is consistent with, and may provide an explanation for, the lowered levels of Cr + CrP in hyperthyroid compared with euthyroid rat hearts. In keeping with this inference, T\(_3\) treatment resulted in no change in Cr + CrP nor in any change in the expression of Na\(^+\)/Cr transporter mRNA in the brain, a tissue that does not respond thermogenically to thyroid hormone (19, 27). The positive correlation between the expression of the transporter mRNA and the content of Cr + CrP in the heart strongly suggests that the Cr content is influenced by the expression of the transporter.

The lowered level of Cr observed in hyperthyroid hearts is likely to be responsible for the elevated fADP levels and low ratios of ATP/fADP both at high and low workloads. The rise in fADP (and lowered ATP/fADP ratio) would be expected to maintain higher rates of O\(_2\) consumption and ATP synthesis manifested in the hyperthyroid myocardium. However, it is possible that a limitation in Cr availability in a mitochondrial microcompartment might be present, especially under conditions of high workload (see below). It should also be noted that the higher levels of AMP in hearts of T\(_3\)-treated animals (and the resulting elevation of the AMP-to-ATP ratio) is apt to cause a stimulation of AMP-activated protein kinase activity (AMPK) (16). Whether AMPK activity is indeed stimulated in hyperthyroid myocardium deserves investigation.

There has been controversy concerning the relative efficiency of hyperthyroid compared with euthyroid hearts (6, 15, 24). Efficiency, defined as work output per unit O\(_2\) consumed, was found to be similar in euthyroid and hyperthyroid hearts in one study (24) but lower in hyperthyroid hearts in another study (15). In the present study, we plotted O\(_2\) consumption as a function of work output over a much wider range of work output than employed by previous studies (more than fourfold compared with less than twofold) (15, 24). Nevertheless, we found no evidence of an alteration in the efficiency of the hyperthyroid heart, irrespective of the workload. Although other studies (24) have reported the constancy of coupling efficiency in hearts of control and hyperthyroid rats, this is the first study to demonstrate normal coupling efficiency in hyperthyroid hearts at such high O\(_2\) consumption rates.

Table 2. Effect of thyroid hormone on content of Na\(^+\)/Cr transporter mRNA in rat heart, skeletal muscle, and brain

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<tr>
<th>Tissue</th>
<th>Euthyroid</th>
<th>Hyperthyroid</th>
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<tr>
<td>Heart</td>
<td>1.00 ± 0.07</td>
<td>0.35 ± 0.09*</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>1.00 ± 0.02</td>
<td>0.30 ± 0.12*</td>
</tr>
<tr>
<td>Brain</td>
<td>1.00 ± 0.08</td>
<td>0.80 ± 0.05</td>
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Values are means ± SE; n = 4–6 rats. Cr, creatine. Total RNA was isolated and probed for Na\(^+\)/Cr transporter mRNA. The resulting X-ray film for each tissue was scanned and the densities of the two mRNA bands (corrected against 28S rRNA) in lanes containing RNA from euthyroid rats were added, averaged, and normalized to 1.0. For each tissue, the density of mRNA bands derived from each hyperthyroid rat tissue was divided by the mean value of the euthyroid group and averaged. *P < 0.01 compared with euthyroid tissue.
An important finding of the present study was that hearts of T₃-treated animals not only had lowered levels of Cr and CrP and ATP/fADP ratios, but that ATP levels declined significantly at high work levels in the hyperthyroid heart. The decrease in the ATP level at high workload might explain the limitation in the maximal work capacity of the heart in the hyperthyroid state (5, 22, 30, 46). The exact mechanism for this limitation is not entirely clear. However, we speculate that the low levels of free Cr in a microcompartment adjacent to mitochondrial inner membrane may limit the capacity of mitochondrial Cr kinase to generate ADP near the mitochondrial inner membrane (fADP+, microcompartmented fADP), and it may be precisely fADP+ that is necessary to maximally stimulate mitochondrial ATP synthesis. Hence, the reduction in free Cr may limit the rise in oxygen consumption and ATP synthesis in the hyperthyroid heart by lowering fADP+, especially under high work conditions. Further studies are necessary to delineate the maximum level of work that can be performed by the hyperthyroid heart and to identify the mechanisms underlying the limitation in its maximal work capacity.

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