Correlation between myocardial malate/aspartate shuttle activity and EAAT1 protein expression in hyper- and hypothyroidism

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Correlation between myocardial malate/aspartate shuttle activity and EAAT1 protein expression in hyper- and hypothyroidism. Am J Physiol Heart Circ Physiol 288: H2521–H2526, 2005. First published December 22, 2004; doi:10.1152/ajpheart.00991.2004.—In the heart, elevated thyroid hormone leads to upregulation of metabolic pathways associated with energy production and development of hypertrophy. The malate/aspartate shuttle, which transfers cytosolic-reducing equivalents into the cardiac mitochondria, is increased 33% in hyperthyroid rats. Within the shuttle, the aspartate-glutamate carrier is rate limiting. The excitatory amino acid transporter type 1 (EAAT1) functions as a glutamate carrier in the malate/aspartate shuttle. In this study, we hypothesize that EAAT1 is regulated by thyroid hormone. Adult rats were injected with triiodothyronine (T3) or saline over a period of 8–9 days or provided with propylthiouracil (PTU) in their drinking water for 2 mo. Steady-state mRNA levels of EAAT1 and aralar1 and citrin (both cardiac mitochondrial aspartate-glutamate transporters) were determined by Northern blot analysis and normalized to 18S rRNA. A spectrophotometric assay of maximal malate/aspartate shuttle activity was performed on isolated cardiac mitochondria from PTU-treated and control animals. Protein lysates from mitochondria were separated by SDS-PAGE and probed with a human anti-EAAT1 IgG. Compared with control, EAAT1 mRNA levels (arbitrary units) were increased nearly threefold in T3-treated (3.1 ± 0.5 vs. 1.1 ± 0.2; P < 0.05) and decreased in PTU-treated (2.0 ± 0.3 vs. 5.2 ± 1; P < 0.05) rats. Ara1 mRNA levels were unchanged in T3-treated and somewhat decreased in PTU-treated (7.1 ± 1.0 vs. 9.3 ± 0.1; P < 0.05) rats. Citrin mRNA levels were decreased in T3-treated and unchanged in PTU-treated rats. EAAT1 protein levels (arbitrary units) in T3-treated cardiac mitochondria were increased compared with controls (8.9 ± 0.4 vs. 5.9 ± 0.6; P < 0.005) and unchanged in PTU-treated mitochondria. No difference in malate/aspartate shuttle capacity was found between PTU-treated and control cardiac mitochondria. Hyperthyroidism in rats is related to an increase in cardiac expression of EAAT1 mRNA and protein. The 49% increase in EAAT1 mitochondrial protein level shows that malate/aspartate shuttle activity increased in hyperthyroid rat cardiac mitochondria. Although hyperthyroidism resulted in a decrease in EAAT1 mRNA, neither the EAAT1 protein level nor shuttle activity was affected. EAAT1 regulation by thyroid hormone may facilitate increased metabolic demands of the cardiomyocyte during hyperthyroidism and impact cardiac function in hyperthyroidism.

thyroid hormone; myocardial hypertrophy

Given the central role of the heart in responding to physiological changes and the high energy requirements of the myocardium, it is not surprising that cardiomyocytes are sensitive to the effects of thyroid hormone. Thyroid hormone acts on the heart through mechanisms that are both independent of and dependent on gene transcription. Acute effects seen early after exposure to the bioactive form of thyroid hormone, 3,5,3’-triiodothyronine (T3), include increases in heart rate, ejection fraction, and cardiac output, as well as increases in blood volume (14). Important myocyte genes responsive to thyroid hormone that impact mechanical function have been identified, including α-myosin heavy chain, sarcoplasmic reticulum Ca2+/ATPase, Na+–K+–ATPase, and several voltage-gated potassium channels (22).

Beyond its effects on cardiac mechanical function, thyroid hormone also directly impacts cardiac energetics and mitochondrial function. Thyroid hormone actions on mitochondria result from direct regulation of both nuclear and mitochondrial DNA transcription as well as from hemodynamic changes that indirectly alter gene expression (9). Several nuclear-encoded respiratory chain genes have been identified that are induced after administration of T3, including cytochrome c1, cytochrome-c oxidase, F1-ATP synthase subunit, and glycerol-3-phosphate dehydrogenase (8). Potential direct effects on the mitochondria include transcriptional activation of all the mitochondrial-encoded genes, which have been shown to increase two- to eightfold after 1–3 days of T3 treatment (19). This global mitochondrial transcriptional activation supports the increase in mitochondrial ATP production but is likely also needed to enable the observed increase in mitochondrial biogenesis induced by T3, a process closely coordinated with activity in the nucleus (8, 18).

We have previously evaluated the effect of hyperthyroid-induced hypertrophy on the expression of the components of two important cardiac NADH shuttles: the malate/aspartate shuttle and the α-glycerophosphate shuttle (28). Of the two, the malate/aspartate shuttle is the dominant pathway in the immature heart that allows NADH to gain access to the mitochondrial matrix across the NADH-impermeable inner mitochondrial membrane (3, 27).

As depicted in Fig. 1, the malate/aspartate shuttle consists of complementary cytosolic and mitochondrial reactions, with two nuclear-encoded carrier proteins spanning the inner mitochondrial membrane: the oxoglutarate-malate carrier and the aspartate-glutamate carrier (AGC). The AGC is electrogenically driven and thought to represent the rate-limiting step of the malate/aspartate shuttle (15, 16).

The malate/aspartate shuttle has the potential to provide an important pathway for optimal substrate utilization under conditions of increased metabolic demand. Hyperthyroid-induced left ventricular (LV) hypertrophy is known to have an associated increase in NADH shuttle capacity that may be due in part
to hypertrophy-related increased cardiac workload (28). Indeed cardiac hypertrophy is associated with an increased reliance on the glycolytic pathway for energy production, an increase that would also be expected to necessitate an increased flux through the malate/aspartate shuttle (1). What is less understood is the direct regulatory role, if any, exerted by thyroid hormone on the genes coding the AGC of the malate/aspartate shuttle.

Candidate genes for the AGC have only recently been described (20, 23). Elegant studies by Palmieri et al. (20) demonstrated that aralar1 and its isoform citrin function in the role of the AGC in mitochondria, with aralar1 showing wide tissue distribution with preponderance in heart, skeletal muscle, and brain. Citrin is the only isoform expressed in liver tissue and with individual members widely characterized in the brain but with individual members widely distributed throughout other tissues. In brain, EAAT1 is localized on the astroglial cell surface where it removes glutamate from cellular membranes and other organelles by differential centrifugation. Mitochondria were separated from cellular membranes and other organelles by differential centrifugation.

Recently, we (23) have also shown that the excitatory amino acid transporter type 1 (EAAT1) is localized to the inner mitochondrial membrane in cardiac cells and functions in the malate/aspartate shuttle. EAAT1 is a member of a family of high-affinity Na+/K+- and K+-coupled glutamate transporters characterized in the brain but with individual members widely distributed throughout other tissues. In brain, EAAT1 is localized on the astroglial cell surface where it removes glutamate from the synaptic space (30). In the heart, this protein localizes to the mitochondrial inner membrane, where it functions as an AGC. Its mechanism of function in cardiac mitochondria, including requirements for ion cotransport, remains to be determined.

Given the increase in malate/aspartate shuttle capacity in hyperthyroidism and the rate-limiting role of the AGC in the malate/aspartate shuttle, we hypothesized that, in cardiac mitochondria, expression of EAAT1 or the isoforms aralar1 and citrin would be altered in response to thyroid hormone. These changes would correlate with measured changes in the malate/aspartate shuttle capacity. To define the relationship between thyroid hormone and the malate/aspartate shuttle, we evaluated expression patterns of the candidate AGC genes in myocardium from hyper- and hypothyroid rats.

METHODS

Animal preparation. Adult Sprague-Dawley rats were prepared previously as described (28). Tissues from the hyperthyroid animals used in the present study were used in an earlier study that determined T3-induced effects on NADH shuttle capacity (28). That study was performed before the identification and characterization of the AGC proteins aralar1, citrin, and EAAT1. The hyperthyroid model was created using animals weighing 300–400 g that were given subcutaneous injections daily for 9 days of either sterile T3 (300 μg/100 g; Sigma, St. Louis, MO) or 0.09% sodium chloride. The model of hyperthyroidism was generated by providing propylthiouracil (PTU; 0.05%) in drinking water daily over a period of 2 mo. The animals were weighed and anesthetized, a blood sample was drawn from the abdominal aorta, and the heart and liver were excised. Heart and liver tissues for Northern and immunoblot studies were rapidly frozen. The hearts used for mitochondrial isolation were excised and immediately perfused as described below. Free T3 was measured from the plasma of all animals with the EZ bead enzyme immunoassay test kit (Diaitect Diagnostics, Boston, MA) as described previously (28).

This study was reviewed and approved by the Institutional Animal Care and Use Committee of the University of Iowa. All animals were handled in accordance with the University’s Animal Care and Use Guidelines.

Isolation of mitochondria and NADH shuttle assays. Mitochondria from heart and liver were isolated, and shuttle assays were performed as described in detail previously (28) based on methods described by McKee et al. (17). Briefly, hearts were retrograde-perfused with ice-cold isolation buffer containing (in mM) 220 mannitol, 70 sucrose, and 2 EGTA at pH 7.0 (MSE) followed by perfusion with MSE plus 0.4 mg/ml Nagarse (Sigma protease XXVII). The LV free wall and septum were isolated and minced in Nagarse-containing MSE on ice followed by blade homogenization. Liver tissue was placed in MSE and subjected to blade homogenization. Mitochondria were separated from cellular membranes and other organelles by differential centrifugation.

Mitochondrial pellets were resuspended in MSE, protein concentration was measured, and respiratory control ratios (state 3 oxygen consumption divided by state 4 oxygen consumption) were determined for all mitochondria in the presence of the substrates glutamate and malate (5 mM each) (28).

Capacity of the malate/aspartate shuttle was determined as described previously (25, 26, 28). Cytosolic components were added in saturating concentrations to 50 μg of intact cardiac mitochondria, and the rate of oxidation of NADH was monitored at 340 nm at 37°C. The rate of change of absorbance with and without substrate was normalized to the amount of added mitochondrial protein. Shuttle data from the hyperthyroid cardiac mitochondria were reported previously (28).

Preparation of cDNA clones. A partial cDNA clone of the EAAT1 (SLC1A3) was prepared from rat myocardial total RNA as described previously (23). Partial cDNA clones for aralar1 (SLC25A12) and citrin (SLC25A13) were prepared based on Del Arco et al. (4), using portions of a cDNA clone generously provided by Dr. Ferdinando Palmieri (University of Bari, Bari, Italy). Using published rat aralar1 cDNA sequence (GenBank XM-230015), we generated the following PCR primers using the computer software Primer3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi): 5’-GATAGGGGTGTC-AACCAGAAA-3’ and 5’-TCTCATCACCCAGGGAGGT-3’. To gen-
erate a partial cDNA for citrin, primers to human citrin (GenBank NM-014251) were generated corresponding to sequence beginning with nucleotide 355 (5’-ATCTGTCCCTGCTGCTCCGG-3’) and 1430 (5’-TCCATGGGTGTAACCTGACC-3’). RNA from adult rat heart LV free wall was prepared as described below (Northern blot studies) and treated with DNase to remove genomic DNA. Reverse transcription was performed with 1 μg of total RNA using avian myeloblastoma virus reverse transcriptase (Boehringer Mannheim, Indianapolis, IN) followed by PCR under standard conditions. The PCR products were purified using QIAquick kit (Qiagen, Chatsworth, CA), quantitated spectrophotometrically, and ligated into PCR4 (Clonetech, San Francisco, CA). The University of Iowa Core DNA Facility sequenced both strands.

Northern blot studies. We isolated RNA from 100 mg of frozen tissue using the RNeasy kit (Qiagen); this was quantitated spectrophotometrically and stored at −80°C as described previously (2). We performed probe preparation, gel electrophoresis, and hybridization as described elsewhere (23) using either the 390-bp partial cDNA clone unique to EAAT1 (23), the 349-bp partial cDNA clone of rat aralar1 described using a well-characterized polyclonal antibody to the amino-terminal region of EAAT1 (1:100 dilution; Santa Cruz Biotechnology, Santa Cruz, CA). Protein lysates were prepared from isolated cardiac mitochondria, and 20-μg samples were separated by SDS-PAGE as previously described (24, 26). Primary antibody labeling was identified using IRdye 700DX-conjugated donkey anti-goat IgG (Rockland Immunochemicals, Gilbertsville, PA). Protein labeling was visualized and quantitated with the Odyssey infrared imaging system (Li-Cor Biosciences, Lincoln, NE). Ponceau S staining verified even sample loading.

Immunoblotting. We performed immunoblotting as previously described using a well-characterized polyclonal antibody to the amino-terminal region of EAAT1 (1:100 dilution; Santa Cruz Biotechnology, Santa Cruz, CA). Protein lysates were prepared from isolated cardiac mitochondria, and 20-μg samples were separated by SDS-PAGE as previously described (24, 26). Primary antibody labeling was identified using IRdye 700DX-conjugated donkey anti-goat IgG (Rockland Immunochemicals, Gilbertsville, PA). Protein labeling was visualized and quantitated with the Odyssey infrared imaging system (Li-Cor Biosciences, Lincoln, NE). Ponceau S staining verified even sample loading.

Statistical analysis. All data are expressed as means ± SE. Comparisons between the thyroid hormone-treated (T3) and PTU-exposed animals and their respective controls were done with an unpaired, two-tailed t-test (P < 0.05 considered significant). For the malate/aspartate shuttle assay, a two-way ANOVA was performed [pair-wise comparisons between treatment groups (T3 and PTU) and controls], with a P value of < 0.05 considered significant. We used SigmaStat3.0 for all analyses.

RESULTS

The 9-day course of T3 effectively induced the hyperthyroid state in the treated animals as determined by total T3 level and ratio of LV mass to total body weight (LVM/BW) measurements, as has been previously presented (28). The PTU-treated animals demonstrated a significant decrease in total T3 compared with controls (PTU-treated: 0.58 ± 0.07 ng/ml, control: 0.71 ± 0.04 ng/ml; P < 0.05) and a decrease in both BW (PTU-treated: 308 ± 9 g, control: 449 ± 6 g; P < 0.0001) and LVM (PTU-treated: 0.81 ± 0.04 g, control: 1.18 ± 0.05 g; P < 0.0001). LVM/BW was preserved in the PTU-treated animals compared with the control group. This reduction in both BW and LVM with preservation of the LVM-to-BW ratio during hypothyroidism is consistent with the observations of others (21).

Malate/aspartate shuttle activity. Capacity of the mitochondrial malate/aspartate shuttle in hyperthyroid rat cardiac tissue has been shown previously to be increased 33% compared with normal controls (28). In this study, mitochondria were isolated from the LV free wall and septum from PTU-treated and control rats. State 3 and state 4 oxygen consumptions were determined, and respiratory control ratios were calculated to ensure similar mitochondrial coupling between the mitochondria derived from PTU-treated and control animals before the malate/aspartate shuttle assay was started. No significant difference was demonstrated between mitochondria from PTU-treated animals and controls for either state 3 or state 4 oxygen consumption. The mitochondria were well coupled with similar respiratory control ratios from both groups (PTU-treated: 5.99 ± 0.41, control: 5.85 ± 0.78; P = 0.81).

Figure 2 shows results from the malate/aspartate shuttle assay performed with cardiac mitochondria isolated from PTU-treated and control animals. There was no significant difference in cardiac malate/aspartate shuttle capacity between PTU-treated and controls (PTU-treated: 168.5 ± 4.5 nmol·min⁻¹·mg protein⁻¹, control: 160.4 ± 5.1 nmol·min⁻¹·mg protein⁻¹; P = 0.26). For comparison, malate/aspartate shuttle capacity in T3-treated animals was 201.6 ± 6.9 nmol·min⁻¹·mg protein⁻¹ (28).

mRNA studies. Since publication of our previous study (28) evaluating the effect of thyroid hormone on the expression of known genes involved in the cardiac NADH shuttles, candidate genes have been identified for the AGC of the malate/aspartate shuttle. Rat-specific partial cDNA clones of EAAT1, aralar1, and citrin were prepared as described above and used to determine steady-state mRNA levels of the genes. A single band of ~4 kb was found with the EAAT1 riboprobe. Steady-state mRNA levels for EAAT1 were significantly increased in T3-treated animals compared with control animals (Fig. 3). The riboprobe specific to rat aralar1 identified bands of ~2.7, 3.5, and 4 kb, with the 2.7-kb product being the dominant band in heart. None of the bands was significantly increased in the hyperthyroid animals compared with controls. The analysis of the 2.7-kb band is presented in Fig. 3. Our cDNA probe for citrin identified a single 3-kb product in rat heart. Steady-state levels for citrin in heart were significantly decreased in the hyperthyroid animals (data not shown). In the hypothyroid model, both cardiac EAAT1 and aralar1 steady-state mRNA levels were decreased (Fig. 4), whereas citrin was unchanged compared with their controls.

To evaluate load-independent effects of thyroid hormone on the three genes of interest, we measured their steady-state mRNA levels in liver. EAAT1 levels were undetectable in liver...
at baseline and did not change in T3-treated animals (data not shown). Aralar1 levels did not change significantly in liver. Citrin levels decreased in the PTU-treated animals (PTU-treated: 1.44 ± 0.10, control: 1.91 ± 0.14; P < 0.05).

**Immunoblot studies.** Quantitative immunoblots were performed to correlate changes in EAAT1 mRNA with protein levels in cardiac mitochondria from T3- and PTU-treated animals. As seen in Fig. 5, a single dominant 60-kDa band was identified in both T3-infused and control mitochondria samples, with a significant increase in signal observed in the T3 animals (T3-treated: 8.90 ± 0.42, control: 5.98 ± 0.61; P < 0.01). This change reflects a 49% increase in the EAAT1 protein level in cardiac mitochondria. No significant change in mitochondrial EAAT1 was demonstrated in the PTU-treated animals compared with control (PTU-treated: 27.94 ± 3.03, control: 31.12 ± 2.47)

**DISCUSSION**

In this study, we evaluated the effect of thyroid hormone on expression of the three AGCs that have been identified (EAAT1, aralar1, and citrin), which are the rate-limiting step of the cardiac mitochondrial malate/aspartate shuttle. Our hypothesis that thyroid hormone status would impact the AGC is based on the wide-ranging effects of thyroid hormone on the mitochondria, from the stimulation of mitochondrial biogenesis to upregulation of key enzymes of the respiratory chain. These effects rely on transcriptional activation of both nuclear- and mitochondrial-encoded genes. As nuclear-transcribed amino acid carriers involved in an important metabolic pathway in mitochondria, EAAT1 or the isoforms aralar1 and citrin represent likely targets for thyroid hormone action. Understanding the role of thyroid hormone in cardiac energetics is increasingly relevant as ongoing research seeks to define the impact of supplemental thyroid hormone administration on improving myocardial functional recovery after ischemic injury.

**Hyperthyroidism and cardiac mitochondria.** Although the effect of T3 on mitochondrial gene expression remains to be fully characterized, the role of T3 on the nuclear genome is well accepted, and several genes directly involved in cardiac energy metabolism are regulated by T3, including succinic dehydrogenase, NADH dehydrogenase, and α-glycerophosphate dehydrogenase (see Ref. 8 for review). This study demonstrated that, in hyperthyroid rat myocardium, steady-state mRNA levels of the nuclear-transcribed glutamate carrier EAAT1 are increased 65%, with a corresponding 49% increase in the mitochondrial EAAT1 protein. The change in EAAT1 expression correlates closely with the measured changes in the malate/aspartate shuttle capacity of the hyperthyroid myocardium (28). No significant changes were seen in the steady-state mRNA levels for the cardiac mitochondrial AGC aralar1, whereas citrin was found to decrease. The decrease in citrin mRNA levels may reflect increased message degradation rather than a decrease in transcriptional rate. On the other hand, the observed decrease may be in relation to an overall increase in RNA content in the thyroid hormone-stimulated tissue. In

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**Fig. 3.** Steady-state excitatory amino acid transporter type 1 (EAAT1) and aralar1 mRNA in myocardium from T3-treated rats. Standard Northern blot analysis was performed with total RNA isolated from the left ventricular free wall of adult rats treated with either T3 or saline as detailed in METHODS. 32P-labeled probe to either EAAT1, aralar1, or citrin was applied and quantitated, followed by 32P-labeled probe to the 18S ribosomal RNA. Representative data of 2 animals from each group are shown. Lower: 2.7-kb aralar1 band was used for analysis. Bar graphs represent specific probe signals normalized to the 18S rRNA signal to correct for variable RNA loading. Error bars indicate SE; n = 4 for each group. *P < 0.05 vs. control by unpaired t-test.

**Fig. 4.** Steady-state EAAT1 and aralar1 mRNA in myocardium from PTU-treated rats. Data were derived using the same riboprobes as in Fig. 3. Shown are Northern blot data from 2 representative animals from each group (n = 4 for each group). Error bars indicate SE. *P < 0.05 vs. control by unpaired t-test.
either case, we conclude that neither aralar1 nor citrin appears to play an important role in the cardiac response to hyperthyroidism.

When mRNA levels of these carriers were evaluated in liver from the hyperthyroid animals, EAAT1 was not detectable, and aralar1 and citrin were unchanged (data not shown). To our knowledge, EAAT1 expression has not been identified in liver. Consistent with the absence of EAAT1 and the stable levels of aralar1 and citrin mRNA in liver, our previous study (28) did not find a change in the malate/aspartate shuttle capacity in the liver mitochondria isolated from hyperthyroid animals. Together, these data support the adaptive role of EAAT1 in the malate/aspartate shuttle as the heart responds to the increased energy requirements of the hyperthyroid state.

Myocardial response to hypothyroidism. The effects of hypothyroidism on the heart have recently begun to receive greater attention, particularly as a potential role has emerged for hypothyroidism in cardioprotection (21, 22). Although a great deal of information is known about the impact of hypothyroidism on the cellular level, relatively little is known about the effects of hypothyroidism on the mitochondria. This study found a decrease in the mRNA levels of both EAAT1 and aralar1 in myocardium from hypothyroid animals; however, for EAAT1, this difference was not demonstrated at the protein level. Most importantly no significant change was seen in the capacity of the malate/aspartate shuttle in the hypothyroid animals. These data would support that the malate/aspartate shuttle is not involved in the cellular response to hypothyroidism, possibly reflecting that the malate/aspartate shuttle is not rapidly downregulated when myocardial energy demands are reduced. This may not be unexpected given the limited reliance of the adult myocardium on the glycolytic pathways during normal, baseline conditions. Further reduction in capacity may be of little physiological significance. The persistence of EAAT1 protein may reflect the rate of protein degradation or mitochondria turnover.

EAAT1 regulation by thyroid hormone. The transcriptional regulation of EAAT1 is only partially characterized, and no evidence to our knowledge has been presented that EAAT1 is regulated by thyroid hormone. The promoter region for human EAAT1 has been cloned from 293T cells (a human embryonic kidney cell line) and found to be free of the classic T3 response element (TRE) that would indicate a binding site for the T3-thyroid receptor complex (11, 13). The mouse EAAT1 analog GLUT-1/GLAST promoter is likewise deficient in any TRE sequence (10).

Human EAAT1 is under control of the Sp1 promoter and also contains a binding site for the transcription factor NF-kB. Both of these promoter sites have been shown to interact with T3 receptor complexes and may function as atypical TREs (6). Although this mechanism has not received much attention in the literature since postulated, it nevertheless represents a potential mechanism for T3 to influence expression of EAAT1 in a tissue-specific pattern. Characterization of the EAAT1 promoter region and transcriptional activation in cardiac cells would help further our understanding of tissue-specific patterns of transcriptional regulation of EAAT1.

In our experiments, the steady-state levels of mRNA do not discriminate between the rates of message production vs. the rates of destruction. Therefore, it is possible that the increase in EAAT1 message reflects increased message stability rather than an increase in transcription. Furthermore, given the wide range of gene products that are increased in the presence of T3, a global increase in expression of all important mitochondrial genes (whether nuclear or mitochondrial) could explain the increase in EAAT1 mRNA levels. However, because we did not find the same increase in the other AGC genes, we conclude that the increase in EAAT1 message reflects a specific response rather than a general increase in mitochondrial-related gene transcription.

Thyroid hormone is known to increase mitochondrial biogenesis, a response that is likely to enhance the ability of the cell to generate energy to meet associated increased metabolic demands (12). An increase in mitochondrial number per cell requires an increase in the rate of transcription of both nuclear and mitochondrial-encoded genes as well as an increase in their subsequent translation. We did not verify the effect of thyroid hormone on mitochondrial number or mass in our model of hyperthyroidism. The increase in expression of EAAT1 protein was measured per milligram of mitochondrial protein, which compared with controls reflects a change in the relative amount of EAAT1 on a per mitochondrion basis. Furthermore, the lack of increase in the oxoglutarate-malate carrier (28) and the other AGC genes indicates that the change in EAAT1 does not
simply reflect a global increase in quantity of mitochondria or protein content in response to thyroid hormone or appreciable mitochondrial biogenesis during the study period.

This study is limited by the absence of protein data for aralar1 and citrin. In our hypothyroid animals, this deficiency is lessened by the lack of change in the malate/aspartate shuttle activity. As a result, the relative contribution of the individual AGCs to aspartate-glutamate exchange in the heart remains to be defined. Presently, the lack of specific inhibitors for these transporters makes these studies technically challenging. The apparent disconnect between the mRNA levels and protein levels for EAAT1 in the hypothyroid animals and the decrease in citrin mRNA in the hyperthyroid animals underscores the limitations of relying solely on steady-state mRNA levels to draw important physiological conclusions about changes in gene expression.

Conclusion. Thyroid hormone regulates both mRNA and protein levels of the cardiac mitochondrial EAAT1. In hyperthyroid animals, increased steady-state EAAT1 protein levels correlated with an increase in malate/aspartate shuttle capacity. No change in AGC expression or shuttle capacity was found in thyroid animals, increased steady-state EAAT1 protein levels of the cardiac mitochondrial EAAT1. In hyperthyroid animals, increased steady-state EAAT1 protein levels of the cardiac mitochondrial EAAT1. In hypothyroid animals, this deficiency is lessened by the lack of change in the malate/aspartate shuttle activity. As a result, the relative contribution of the individual AGCs to aspartate-glutamate exchange in the heart remains to be defined. Presently, the lack of specific inhibitors for these transporters makes these studies technically challenging. The apparent disconnect between the mRNA levels and protein levels for EAAT1 in the hypothyroid animals and the decrease in citrin mRNA in the hyperthyroid animals underscores the limitations of relying solely on steady-state mRNA levels to draw important physiological conclusions about changes in gene expression.

GRANTS

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