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Biochemical and morphological study of cardiac hypertrophy. Effects of thyroxine on enzyme activities in the rat myocardium*

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Summary

Experimental hyperthyroidism induced in rats by daily injections of 3,3',5,5'-tetraiode-L-thyroxine (0.5 mg/kg i.p.) for 14 days resulted in a significant increase in heart weight and heart weight/body weight ratio. Hemodynamic and morphological studies were performed in one group. Thyroxine-treated rats showed a characteristic cardiovascular hyperdynamic state, such as tachycardia and augmented rate of contraction, but no evidence of heart failure such as elevated end-diastolic pressures. The cardiac cells in hyperthyroid rats had a significantly larger diameter and more mitochondria than did those of the control rats. In another group the activities of cardiac enzymes involved in energy utilization and liberation were measured biochemically and compared with those of normal controls. Hyperthyroidism resulted in increased specific activity of cytochrome C oxidase and actomyosin ATPase in the myocardium. The specific activity of long-chain acyl-CoA synthetase, carnitine palmityltransferase, carnitine acetyltransferase, malate dehydrogenase and citrate synthase showed a moderate to marked increment, whereas the specific activity of lactate dehydrogenase and pyruvate kinase remained at the control values. These results suggest that in hyperthyroid rat hearts the functions of both energy liberation and utilization systems are enhanced to meet the added workload. Moreover, the increased activity of the enzymes participating in fatty acid metabolism suggest that in thyroxine-induced hypertrophic and hyperdynamic rat hearts, fatty acids contribute more to the energy supply than do carbohydrates.

Key words: cardiac hypertrophy, thyroxine, cardiac enzyme activity, energy metabolism

Introduction

Thyroid hormones are well known as regulators of the metabolism of most adult tissues, including the heart, and changes in the thyroid state are associated with marked alterations of the intrinsic properties of cardiac muscle (5, 12). Hyperthyroidism is characterized by a cardiovascular hyperdynamic state with reduction in the duration of the active state and an augmentation in the rate of tension development and rate of relaxation (9, 20, 25, 28). In hyperthyroidism, the increased energy demand on the heart has been demonstrated in terms of increased oxygen consumption, and cardiac hypertrophy eventually develops (18). Therefore, the increased energy demand together with alterations in the cellular architecture, such as those induced during the process of cardiac hypertrophy, are likely to cause

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derangements of energy metabolism in the cardiac muscle. Myocardial hypertrophy may be considered the interface between the normal and the failing heart. An understanding of the circumstances which oblige adaptive cardiac growth to become pathological is the boundary where clinical medicine and basic science must meet. It therefore seemed important to study the effect of thyroid hormones on energy metabolism in cardiac muscle. However, there have been only a few studies on the changes in enzyme activities which account fully for changes in the energy demand of the hypertrophied heart.

This investigation was designed to determine whether cardiac enzyme activities were increased commensurate with the increased energy demand and hypertrophy of the heart induced by thyroid hormone.

Materials and methods

Reagents and preparations

Coenzyme A, NADH, phospho*enol*pyruvate, rabbit muscle pyruvate kinase and rabbit muscle lactate dehydrogenase were obtained from Boehringer (Mannheim, F.R.G.). 3,3',5,5'-Tetraiode-L-thyroxine, ATP, ADP, cytochrome C and crystalline bovine serum albumin were purchased from Sigma (St. Louis, U.S.A.). Triton X-100, a product of Rom and Haas (Philadelphia, U.S.A.), had an average molecular weight of 628. [U-^{14}C]palmitic acid, D,L-[methyl-^{14}C]carnitine hydrochloride and [1-^{14}C]acetyl-CoA were products of the Radiochemical Centre (Amersham, England). All other reagents were analytical grade.

Palmityl-CoA and acetyl-CoA were prepared by the procedures of Young and Lynen (50) and Simon and Shemain (41), respectively.

Animals and preparations

Male Wistar rats, weighing 270–320 g, were fed laboratory rat chow and tap water *ad libitum* and divided randomly into two groups; 1) rats injected with vehicle and 2) rats treated with thyroxine to cause hyperthyroidism. The rats in the hyperthyroid group were given intraperitoneal injections of 50 μg of thyroxine dissolved in 0.01 N NaOH containing 0.8 % NaCl per 100 g body weight daily for 14 days. The rats in the control group were given injections of the same volume of 0.01 N NaOH containing 0.8 % NaCl.

Preparation of cardiac homogenate and cardiac actomyosin

Cardiac homogenate and cardiac actomyosin were prepared according to the method of Solaro et al. (42) modified as described below. All operations were carried out at 0–4 °C. Rat hearts, quickly removed from the animals killed by a sharp blow on the head, were immediately washed with 0.8 % NaCl. Atrium, connective tissue and fat were trimmed away and the ventricular muscle was cut into small pieces for homogenization. The muscle was homogenized in 5 volumes of 0.25 M sucrose containing 20 mM Tris-HCl buffer at pH 6.8, 2 mM $MgCl_2$, 2 mM EGTA and 2 mM $Na_4P_2O_7$ using an Ultrax homogenizer for 25 sec three times at 5-sec intervals. The whole homogenate was suspended in a mixture containing Triton X-100, Tris-HCl buffer at pH 6.8, sucrose, $MgCl_2$, EGTA and $Na_4P_2O_7$, the final concentrations of which were 16 mM, 20 mM, 0.25 M, 2 mM, 2 mM and 2 mM, respectively, to give a volume 10 times that of the heart specimen. The mixture was further homogenized by three down-and-up strokes of a Teflon pestle in the glass tube and then centrifuged at 17,000 × g for 15 minutes. The resulting supernatant was collected and used for the enzyme assay. The resulting pellet was suspended in 20 mM Tris-HCl buffer at pH 6.8 containing 0.6 M KCl and 2 mM dithiothreitol to give a volume 10 times that of the heart specimen. The mixture was allowed to stand overnight and then centrifuged at 17,000 × g for 30 minutes. The resulting supernatants was collected and used to determine actomyosin ATPase activity.

Assay of enzymes

Enzyme activity and protein content were determined in the Triton X-100 treated homogenates. All assays were carried out within the range at which the reaction proceeded lineally with time, and the

initial rate of the reaction was proportional to the amount of enzyme added. For spectrophotometric assays a recording spectrophotometer equipped with a circulating water cell chamber (Hitachi, model 320) was used.

Long-chain acyl-CoA synthetase activity was determined at 35 °C by the isotope method described previously (43). The reaction mixture (total volume, 0.2 ml) contained 100 mM Tris-HCl buffer at pH 8.0, 1.6 mM Triton X-100, 5 mM dithiothreitol, 150 mM KCl, 15 mM $MgCl_2$, 10 mM ATP, 1 mM potassium [U-^{14}C]palmitate (0.2 Ci/mol), 0.1 mM CoA and enzyme; the time of reaction was 5 minutes. One unit (U) of acyl-CoA synthetase activity is defined as that amount which catalyzes the formation of 1 µmole palmityl-CoA per minute under the conditions of the assay.

Carnitine palmityltransferase activity was measured by a modification of the original method of Bremer and Norum (8) as described by Pande and Blanchear (37). The reaction mixture (total volume, 0.2 ml) contained 125 mM Tris-HCl buffer at pH 7.4, 5 mM D,L-[methyl-^{14}C]carnitine (0.2 Ci/mol), 0.2 mM palmityl-CoA and enzyme; the reaction was initiated by the addition of palmityl-CoA and was carried out for 5 minutes at 35 °C. One unit (U) of carnitine palmityltransferase activity is defined as that amount which catalyzes the formation of 1 µmole palmitylcarnitine per minute under the conditions of the assay.

Carnitine acetyltransferase activity was determined according to the method of Cederblad and Lindstedt (11) with the modifications described below. The reaction mixture (total volume, 0.2 ml) containing 150 mM HEPES buffer at pH 7.4, 5 mM [1-^{14}C]acetyl-CoA (0.25 Ci/mol), 2 mM D,L-carnitine and enzyme; the reaction was initiated by the addition of D,L-carnitine and was carried out for 5 minutes at 35 °C. The reaction was terminated by freezing the test-tube with dry ice-acetone, and then the reaction mixture was thawed and transferred to columns of Dowex 2-X8 in acetate form which were made in disposable insulin syringes. The columns were washed with 1 ml of water which was collected together with the original effluent. The isotope count in the eluate was determined in a liquid scintillation spectrometer. One unit (U) of carnitine acetyltransferase activity is defined as the amount which catalyzes the formation of 1 µmole acetylcarnitine per minute under the conditions of the assay.

Lactate dehydrogenase, pyruvate kinase and malate dehydrogenase activities were measured spectrophotometrically by monitoring the oxidation of NADH at 340 nm by the methods of Schweitzer et al. (38), Valentine and Tanaka (46) and Bergmeyer and Bernt (4), respectively. The reaction was carried out at 35 °C, and one unit (U) of enzyme activity is defined as the amount which catalyzes the oxidation of 1 µmole of NADH per minute under the conditions of the assay.

Citrate synthase activity was determined by measuring the rate of initial reaction at 405 nm by the method of Shepherd and Garland (39). The reaction mixture (total volume, 1 ml) contained 100 mM Tris-HCl buffer at pH 8.0, 0.2 mM 5,5'-dithiobis (2-nitrobenzoate), 50 µM acetyl-CoA, 0.1 mM oxaloacetate and enzyme. The reaction was carried out at 35 °C and initiated by the addition of oxaloacetate. One unit (U) of citrate synthase activity is defined as the amount which catalyzes the liberation of 1 µmole of CoA per minute under the conditions of the assay.

Cytochrome C oxidase activity was measured by the rate of oxidation of ferrocytochrome C at 550 nm, as described by Wharton and Tzagoloff (48). One unit (U) of cytochrome C oxidase activity is defined as the amount which catalyzes the oxidation of 1 µmole of ferrocytochrome C per minute at 35 °C under the conditions of the assay.

Actomyosin ATPase activity was measured spectrophotometrically. The rate of ADP formation was determined by coupling the reaction of actomyosin ATPase with those of pyruvate kinase and lactate dehydrogenase and following the oxidation of NADH at 340 nm with a recording spectrophotometer. Actomyosin preparations contained no interfering enzymes, such as NADH-oxidizing enzymes. The reaction mixture contained 50 mM Tris-HCl buffer at pH 7.5, 150 mM KCl, 15 mM $MgCl_2$, 50 µM $CaCl_2$, 10 mM ATP, 0.2 mM phospho*enol*-pyruvate, 0.16 mM NADH, 15 µg/ml pyruvate kinase, 30 µg/ml lactate dehydrogenase and enzyme in a total volume of 1 ml. The reaction was initiated by the addition of enzyme and carried out at 35 °C. Under the assay conditions described, the initial rate of reaction is proportional to the enzyme concentration. In this assay, the formation of 1 mole of ADP corresponds to the oxidation of 1 mole of NADH. So one unit (U) of actomyosin ATPase activity is defined as the amount which catalyzes the oxidation of 1 µmole NADH per minute under the conditions of the assay.

Physiological analysis

Rats were anesthetized with sodium pentobarbital, 45 mg/kg body weight, injected intraperitoneally, a 1-cm-long parasternal incision was made and the pectoral muscle was retracted to the left side. The electrocardiogram was recorded with needle electrodes applied to the four extremities. Left ventricular pressure was obtained transthoracically by direct puncture of the ventricular apex with a Millar micromanometer tipped catheter (PC-350) mounted with a disposabel 23-gauge needle. Left ventricular pressure and the maximal value of the first derivative (dp/dt) of the ventricular pressure were recorded on a multichannel photographic recorder (HP-4588D, Hewlett-Packard Co.) at slow and fast paper speeds from 2.5 to 200 mm/sec. The $dp/dt/DP_{40}$ and time constant T of left ventricular pressure fall were measured as described by Davidson et al. (16) and Weiss et al. (47), respectively.

Morphological analysis

After the physiological measurements, the hearts were excised. Small tissue specimens were cut from the left ventricle for ordinary electron microscopy and the rest of the heart was fixed in toto in neutral formalin and embedded in paraffin for conventional light microscopy. In order to assess the hypertrophy of myocardial cells by light microscopy, the shortest transverse diameter of fibers crossing through the nuclei was measured with a calibrated eye reticule at 600 times magnification in 100 consecutive muscle cells in sections stained with periodic acid-Schiff (PAS) reagent. Quantitative assessment of volume fractions of mitochondria in myocardial cells was performed in electron micrographs, as described previously (24).

Determinations

Protein was determined by the method of Lowry et al (29) with bovine serum albumin as the standard, as described previously (43). DNA was determined by the method of Ceriotti (12) with calf thymus DNA as the standard.

Statistical evaluation

All quantitative data were evaluated statistically with Student's t-test. Significance was defined for probability values of less than 5 %.

Results

Effects of thyroxine treatment of the physiological and morphological properties of the rat heart

Table 1 represents the physiological and biochemical effects of thyroxine on rats. The body weight of thyroxine-treated rats at the time of sacrifice was significantly smaller than

Table 1. Effects of thyroxine on body weight, heart weight and protein, DNA and actomyosin in rat hearts.

		Control (n = 5)	Thyroxine (n = 6)	% Change
Body weight	(g)	333.0 ± 11.3	300.0 ± 8.0*	− 9.8
Heart weight	(g)	0.844 ± 0.051	1.082 ± 0.053*	+ 28.2
Heart wt./Body wt	($\times 10^{-3}$)	2.54 ± 0.18	3.61 ± 0.23*	+ 42.1
Total protein	(mg/heart)	139.52 ± 9.23	182.83 ± 7.59*	+ 31.0
Total DNA	(mg/heart)	2.603 ± 0.172	2.769 ± 0.139**	+ 6.4
Protein/DNA	(mg/mg)	52.11 ± 2.19	62.30 ± 0.98*	+ 19.6
Total actomyosin	(mg/heart)	51.10 ± 3.05	64.64 ± 2.59*	+ 25.3
Actomyosin/DNA	(mg/mg)	19.19 ± 1.39	22.05 ± 1.04*	+ 14.1

* $p < 0.001$
** N. S.
All values are mean ± standard deviation.

Table 2. Diameter of cardiocytes and volume fractions of mitochondria in cardiocytes of left ventricle in thyroxine-treated and control rats.

		Control	Thyroxine
Diameter	(μm)	18.46 ± 4.42	20.86 ±4.36*
Volume fraction of mitochondria in cardiocytes	(%)	37.49 ± 4.49	45.40 ±10.7*

* $p < 0.001$
All values are mean ± standard deviation.

that of the control rats. The heart weight of the thyroxine-treated rats was significantly greater than that of the controls. So the heart weight(g)/body weight(g) ratio was significantly increased in the thyroxine-treated rats. Total protein in the hearts of thyroxine-treated rats was about 1.3 times that of the controls. There was no difference in the total DNA content of the heart between the two groups.

Table 2 represents morphological data on hearts of two groups. The diameter of cardiac cells in the thyroxine-treated group was significantly larger than in the controls. There was no significant difference between these two groups except for the greater abundance of mitochondria in the thyroxine-treated rats. A more detailed study on the fine structure of the myocardium will be published elsewhere.

These results signify that the increase in heart weight in thyroxine-treated rats was due mainly to an increase in cell size and protein content, so that hypertrophy developed eventually.

Table 3 represents hemodynamic measurements. The heart rate in the thyroxine-treated rats was significantly greater than in the controls. There was no significant difference in left ventricular systolic pressure between the two groups, but a tendency toward an increase in left ventricular systolic pressure was noted in the thyroxine-treated rats. The $dp/dt/DP_{40}$, which represents the rate of tension development in the left ventricle (16), was significantly higher in thyroxine-treated rats than in the controls. The time constant T of left ventricular

Table 3. Effects of thyroxine on various hemodynamic parameters.

		Control (n = 5)	Thyroxine (n = 6)	% Change
Heart rate	(min^{-1})	458 ± 32	564 ± 32*	+ 23.1
LVSP	(mm Hg)	98 ± 20	110 ± 18**	+ 12.2
LVDP	(mm Hg)	− 0.4 ± 0.5	0 ± 0**	0
LVEDP	(mm Hg)	1.2 ± 1.2	1.2 ± 0.9**	0
+dp/dt	(mm Hg/sec)	4507 ± 909	5304 ± 1082*	+ 17.7
−dp/dt	(mm Hg/sec)	3147 ± 900	3999 ± 1017**	+ 27.1
$dp/dt/DP_{40}$	(sec^{-1})	55.7 ± 2.0	69.9 ± 3.7*	+ 25.5
Time constant T	(msec)	6.4 ± 1.3	5.9 ± 0.8**	− 7.8

* $p < 0.001$
** N. S.

All values are mean ± standard deviation.
LVSP; left ventricular systolic pressures, LVDP; left ventricular diastolic pressures, LVEDP; left ventricular end-diastolic pressures.

pressure fall, which is the appropriate index of the rate of relaxation (47), was not different in the two groups, but there was a tendency toward a decrease of the time constant T in the thyroxine-treated rats.

Effects of thyroxine treatment on the actomyosin ATPase activity

The changes in weight and physiological properties in hyperthyroid rat hearts prompted us to investigate the actomyosin ATPase activity. The total actomyosin content in the hearts of thyroxine-treated rats was significantly greater than in the controls (table 1). The specific activity of actomyosin ATPase was also significantly higher in the hearts of thyroxine-treated rats than in the controls. Thus, total actomyosin ATPase activity per heart was almost 1.6 times that of the controls (table 4).

Effects of thyroxine treatment on the activities of enzymes involved in the energy producing process

Cardiac enzyme activities expressed as specific activity and total activity are shown in table 4. The specific activity, expressed per mg of protein, of the enzymes involved in carbohydrate metabolism, i.e. lactate dehydrogenase and pyruvate kinase, was essentially the same in the two groups. The specific activity of the enzymes which participate in fatty acid metabolism, i.e. acyl-CoA synthetase, carnitine palmityltransferase and carnitine acetyltransferase, was somewhat higher in the hearts of thyroxine-treated rats than in the controls. The activity of enzymes involved in TCA cycle or the respiratory chain, such as

Table 4. Effects of thyroxine on enzyme activities in myocardium.

		Control (n = 5)	Thyroxin (n = 6)	% Change
Lactate	(U/mg prot.)	5.893 ± 0.118	5.628 ± 0.106$	− 4.5
dehydrogenase	(U/heart)	819.36 ± 55.10	1021.96 ± 48.54*	+ 24.7
Pyruvate	(U/mg prot.)	1.107 ± 0.034	1.132 ± 0.044**	+ 2.3
kinase	(U/heart)	154.50 ± 11.88	205.46 ± 9.84	+ 33.0
Acyl-CoA	(mU/mg prot.)	86.27 ± 1.26	95.12 ± 1.65*	+ 10.3
synthetase	(mU/heart)	12034.68 ± 788.2	17258.08 ± 657.72*	+ 43.4
Carnitine	(mU/mg prot.)	9.39 ± 0.27	10.46 ± 0.31*	+ 11.4
palmityltransferase	(mU/heart)	1308.7 ± 68.8	1898.8 ± 109.5*	+ 45.1
Carnitine	(mU/mg prot.)	55.45 ± 2.87	66.32 ± 3.61*	+ 19.6
acetyltransferase	(mU/heart)	7741.3 ± 720.8	12381.9 ± 1403.1*	+ 59.9
Citrate	(U/mg prot.)	1.623 ± 0.052	1.891 ± 0.057*	+ 16.5
synthase	(U/heart)	226.83 ± 21.14	343.03 ± 14.57*	+ 51.2
Malate	(U/mg prot.)	16.29 ± 0.29	20.12 ± 0.48*	+ 23.5
dehydrogenase	(U/heart)	2271.9 ± 139.2	3649.6 ± 131.9*	+ 60.6
Cytochrome C	(U/mg prot.)	0.296 ± 0.012	0.400 ± 0.011*	+ 35.1
oxidase	(U/heart)	41.26 ± 2.77	73.44 ± 4.26*	+ 78.0
Actomyosin	(mU/mg prot.)	13.78 ± 0.55	17.30 ± 0.61*	+ 25.5
ATPase	(mU/heart)	710.8 ± 43.4	1119.2 ± 71.4*	+ 57.5

$ $p < 0.01$
* $p < 0.001$
** N. S.
All values are mean ± standard deviation.

citrate synthase, malate dehydrogenase and cytochrome C oxidase, was much higher in the hearts of thyroxine-treated rats than in the controls (table 4).

Discussion

It is well-known that thyroxine increase the metabolism of all organs and tissues, including the heart, and leads to cardiac hypertrophy (18). This study was designed to show how the carbohydrate and lipid metabolic pathways are used for energy production in the hearts of rats treated with thyroxine and how the respiratory chain is affected and energy utilization proceeds in such hearts.

Both the heart weight and heart weight/body weight ratio were significantly increased following thyroxine treatment. Similar results have been well documented by many other investigators (19, 23, 25). Except for the increased diameter of the myocytes and mitochondriosis in the myocytes, however, there were no significant morphological differences from the hearts of control rats (details will be published elsewhere). The DNA content of the heart was the same in thyroxine-treated rats and controls. Therefore, the enlargement of the heart induced by thyroxine appears to be due to hypertrophy of the cardiocytes rather than hyperplasia.

Hemodynamic data showed an increase in heart rate and $dp/dt/DP_{40}$ of the left ventricle in thyroxine-treated rats, indicating a hyperdynamic cardiovascular state. There was no evidence of cardiac failure, such as increased end-diastolic pressure in the left ventricle.

We analyzed enzyme activities in the myocardium of these hypertrophic and hyperdynamic rat hearts.

First, to determine the energy utilization step, we measured actomyosin ATPase activity in the myocardium. It has been well established that myosin ATPase activity is closely related to the intrinsic speed of muscle contraction (3). In the present study the specific activity of actomyosin ATPase was significantly higher in the hearts of thyroxine-treated rats than in the controls. In hyperthyroidism, increased myofibrillar and/or myosin ATPase activity has been seen in rats (49), guinea-pigs (21), rabbits (1, 2, 49) and dogs (14). Our results agree with theirs. The increase in enzymatic activity of actomyosin is propably related to the increased velocity of contraction, as demonstrated in hemodynamic investigations. Thus, it appears that in thyroxine-induced hypertrophic and hyperdynamic rat hearts energy liberation is enhanced in proportion to the increased ATP demand.

Secondly, we determined the activity of cytochrome C oxidase in the myocardium.

This enzyme is the terminal component of the chain of respiratory carriers in mitochondria and is responsible for the reaction whereby electrons resulting from the oxidation of substrate molecules are transferred to the final acceptor, oxygen. In hyperthyroidism, oxygen consumption is increased to meet the increased oxygen demand (25). So cytochrome C oxidase activity is expected to be elevated in thyroxine-treated rat hearts. Our results showed that the specific activity of cytochrome C oxidase was significantly higher in the hearts of thyroxine-treated rats than in the controls, and its total activity was almost 1.8 times that of the controls. Cytochemical studies of the myocardium of thyroxine-treated animals showed that the higher cytochrome C oxidase activity was due mainly to the proliferation of mitochondria (data will be published elsewhere). These findings are in agreement with those of other investigators (15, 30, 31, 36). In regard to the enzymes related to energy production in the myocardium, the tricarboxylic acid cycle is the common central pathway for the metabolism of the two-carbon acetyl residues derived not only from carbohydrates but also fatty acids and amino acids. In the hearts of thyroxine-treated rats there were enhanced activities of citrate synthase and malate dehydrogenase involved in this cycle might be activated to meet the increased energy demand in the hyperthyroid hearts.

Two-carbon acetyl residues are derived from carbohydrate and fatty acids as mentioned above. It is of interest to know which pathway, carbohydrate or fatty acid metabolism, contributes more to energy liberation in thyroxine-induced hypertrophic and hyperdynamic rat hearts. It is well established that fatty acids are major substrates for oxidative metabolism in cardiac muscle (6, 17, 35), and indeed the oxidation of fatty acids tends to take precedence over the oxidation of glucose or pyruvate (7, 32, 34, 40). We have focused our attention upon the enzymes involved in fatty acid metabolism rather than those involved in carbohydrate metabolism.

Long-chain acyl-CoA synthetase plays a central and important role in fatty acid metabolism, and carnitine palmityltransferase mediates the transfer of activated fatty acids from cytosol to mitochondrial β-oxidation. β-oxidation of long-chain fatty acids is important for energy production. It was considered that the reaction catalyzed by acyl-CoA synthetase, acyl-CoA dehydrogenase and carnitine palmityltransferase might be a rate-determining step (34). In the present study there was a significant increase in the specific activity of long-chain acyl-CoA synthetase and carnitine palmityltransferase in the hearts of thyroxine-treated rats. The total activity of these enzymes was almost 1.4 and 1.5 times, respectively that of the controls. On the other hand, the specific activity of pyruvate kinase and lactate dehydrogenase which are both involved in carbohydrate metabolism, showed essentially no difference between thyroxine-treated and control rats, so their total activities rose in parallel with the increase in heart weight. These results suggest that in thyroxine-induced hypertrophic and hyperdynamic, but non-failing, rat hearts, functions of both energy liberation and utilization systems are enhanced to meet the added workload.

Cells of higher animals have a highly developed capacity for enzyme induction and positive regulation of the synthesis of specific protein by specific hormones. Thyroid hormones are of particular interest in enzyme induction and have been reported to induce various enzyme activities (10, 22, 26, 27, 45). In cardiac cells they seem to have at least two effects, one through their action on genes in controlling development and differentiation and the other through their effect on the hemodynamic state. In this study prominent activation was seen in cytochrome C oxidase and actomyosin ATPase. The specific activities of long-chain acyl-CoA synthetase, carnitine palmityltransferase, carnitine acetyltransferase, malate dehydrogenase and citrate synthase showed a moderate to marked increase, whereas the specific activities of lactate dehydrogenase and pyruvate kinase remained at the control levels. Cytochrome C oxidase, long-chain acyl-CoA synthetase, carnitine palmityltransferase, carnitine acetyltransferase, malate dehydrogenase and citrate synthase are all located in or on mitochondria, while lactate dehydrogenase and pyruvate kinase are in cytosol. Both biochemical and morphological findings suggest that in thyroxine-induced hypertrophic and hyperdynamic rat hearts the greatest change in cellular organelles is the proliferation of mitochondria. These findings are comparable to the results reported by other investigators (36, 44). The enzymes participating in fatty acid metabolism were activated much more than those involved in carbohydrate metabolism. These findings suggest that in thyroxine-induced hypertrophic and hyperdynamic rat hearts, fatty acids contribute more to the energy supply than do carbohydrates and that fatty acids are a major substrate for oxidative metabolism.

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