Anaerobic Performance and Metabolism of the Hyperthyroid Heart

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ABSTRACT Anaerobically perfused hearts from rats with experimentally induced hyperthyroidism exhibited accelerated deterioration of pacemaker activity and ventricular performance. The diminished anaerobic performance of hyperthyroid hearts was associated with decreased adenosine triphosphate (ATP) levels and a reduced rate of anaerobic glycolysis as reflected in decreased lactic acid production during 30 min of anoxic perfusion.

Studies on whole heart homogenates demonstrated inhibition at the phosphofructokinase (PFK) step of the glycolytic pathway. Such inhibition was not demonstrated in the hyperthyroid heart cytosol. It is postulated that an inhibitor of PFK which resides dominantly in the particulate fraction is probably responsible for the diminished anaerobic glycolysis and performance of the hyperthyroid heart.

INTRODUCTION

The effect of thyroxine administration on oxidative metabolism has received considerable attention in recent years. Many metabolic consequences of hyperthyroidism have been attributed to such alterations in oxidative metabolism as reduced mitochondrial respiratory control (1, 2) and increased levels of several oxidative enzymes (3, 4). Less attention has been paid to the effects of hyperthyroidism on anaerobic glycolytic metabolism. Glock, McLean, and Whitehead have reported that liver glycolysis is stimulated by thyroxine treatment (5), while Bressler and Wittles have reported decreased aerobic glycolysis in homogenates of hyperthyroid guinea pig hearts (6).

The mammalian heart is extremely sensitive to thyroxine administration. Myocardial hypertrophy, increased heart rate, and increased myocardial contractility are all consequences of hyperthyroidism (7–9). These changes are accompanied by increased cardiac oxygen consumption and free fatty acid utilization, whereas glucose uptake and oxidation are decreased (10, 11).

In view of the recent demonstration in our laboratory of the importance of glucose metabolism in preserving structure and function of the anoxic heart (12) and the reported inhibition of glucose metabolism in hyperthyroid hearts (6), it seemed desirable to determine whether anaerobic metabolism is altered in the intact hearts from hyperthyroid animals and to ascertain what effects these metabolic alterations have on anoxic performance of the heart.

METHODS

Male Wistar rats fed ad lib. with Purina laboratory chow were selected for study. The experiments were so designed to permit matching of both heart weight and body weight of euthyroid and hyperthyroid animals. In one series (matched heart weight) rats weighing 160–180 g were made hyperthyroid with seven daily injections of 0.2 mg of sodium L-thyroxine dissolved in 0.25 ml of 0.01 N NaOH. At the time of sacrifice, mean body weight was 180 g (range 149–205 g), while the mean body weight of the euthyroid control animals was 250 g (220–280 g). The heart weights of the two groups did not differ significantly. Mean wet heart weight of the control rats was 1.20 ±0.15 g (range 0.94–1.56 g) compared with 1.11 ±0.15 g (0.82–1.47 g) for the hyperthyroid animals. These hearts were used in both perfusion and homogenate studies.

In a second series (body weight matched) rats weighing 125–150 g were injected daily for 7 days with 0.25 ml of 0.01 N NaOH, while rats weighing 135–160 g were injected daily with 0.2 mg of sodium L-thyroxine dissolved in 0.25 ml of 0.01 N NaOH. At the time of sacrifice mean body weight of the sodium hydroxide-injected group was 154 ±5.1 g (SEM) compared with 155 ±5.8 g for the thyroxine injected group. Mean heart weight of the sodium hydroxide-injected group was 0.73 ±0.025 g (SEM) compared with 0.99 ±0.029 g for the thyroxine injected animals. These hearts were used only in the studies on homogenate glycolysis.

Perfused hearts. In the studies on the perfused hearts, rats from the first series (matched heart weights) were

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used. In this manner, constancy of perfusion per unit weight of tissue was maintained. Hearts were perfused at constant rate in a modified Langendorf apparatus which permitted constant monitoring of the electrocardiogram, left ventricular pressure (LVP), the first derivative of left ventricular pressure (LV dp/dt), and oxygen consumption (QO_2) (12). The maximum rate of left ventricular rise was calculated from the recording of LV dp/dt. The hearts performed isovolumically. The perfusion fluid was 5% bovine serum albumin (BSA) which had been dialyzed against and diluted with Krebs-Ringer bicarbonate buffer (KRB) (13). The KRB was modified to contain 5.0 mg of calcium per 100 ml. Coronary flow was maintained at 10 ml/min, and the temperature was 32°C.

Aerobic control hearts. The first group of hearts (eight euthyroid and seven hyperthyroid) was perfused for 60 min with 5% BSA in KRB which had been gassed with 96% O_2, 4% CO_2 (pH 7.40). The aerobic perfusion fluid contained no glucose. These hearts were rinsed for 1 min with oxygenated KRB after the 60 min perfusion period and were clamped between liquid nitrogen–cooled Wollenberger tongs (14).

Anoxic hearts. The second group of hearts (six euthyroid and six hyperthyroid) was perfused aerobically without glucose for 1 hr and then anaerobically with fluid containing 200 mg of glucose per 100 ml (11.1 mM glucose) for 30 min. The anaerobic perfusion fluid was gassed with 96% N_2, 4% CO_2. After the anaerobic perfusion period the hearts were rinsed for 1 min with anaerobic KRB containing 200 mg of glucose per 100 ml and were clamped between liquid nitrogen–cooled tongs.

Recovery hearts. The third group of hearts (six euthyroid and six hyperthyroid) was perfused aerobically without glucose for 1 hr, anaerobically with glucose for 30 min, and then aerobically without glucose for an additional 30 min. The hearts were rinsed for 1 min with oxygenated KRB and clamped between liquid nitrogen–cooled tongs. The frozen hearts were stored in liquid nitrogen until analysis of heart metabolic intermediates.

Lactic acid content of the perfusing fluid was determined by the enzymatic technique of Scholz, Schmidt, Bücher, and Lampen (15). Total lactate production was calculated from the concentration and volume of the perfusing medium.

Homogenate studies. Three hyperthyroid and three euthyroid rats were used for each of the 25 heart homogenate studies. The animals were killed by decapitation, and the thoracic cavity was opened and immediately flooded with iced 0.154 M KCl. The hearts were removed to iced KCl and flushed via the aortic stump with KCl to remove blood. The hearts were then blotted, weighed and forced through a stainless steel tissue press. The tissue mince was homogenized with 9 volumes of 0.154 M KCl by 15 strokes of a motor-driven Teflon pestle in a Potter-Elvehjem homogenizer. Cytosol was prepared by centrifuging the 10% heart homogenate at 100,000 *g* for 1 hr in an International model B-60 centrifuge.

Glycolytic activity was measured by injecting 1.5 ml of homogenate or cytosol into stoppered bottles containing 6 ml of modified LePage buffer, pH 7.6 (16), which had been equilibrated with 95% N_2, 5% CO_2 for 30 min. The bottles were incubated at 37°C in a Dubnoff metabolic shaker under a stream of 95% N_2, 5% CO_2. Final concentration of the incubation mixture was 3 mM K_2HPO_4, 50 mM nicotinamide, 8.3 mM $MgCl_2$, 0.25 mM nicotinamide adenine dinucleotide (NAD), 60 mM KCl, 4.2 mM adenosine diphosphate (ADP), and 12.5 mM glycolytic substrate (glucose, glucose-6-phosphate, or fructose-1,6-diphosphate). The incubation mixture contained 2% heart tissue (wet weight) or approximately 1.9 mg of homogenate protein or 0.5 mg of cytosol protein per ml.

Each tissue preparation was incubated in duplicate with four different substrate preparations; glucose, glucose plus 0.1 mg/ml of type III yeast hexokinase, glucose-6-phosphate, and fructose-1,6-diphosphate.

Samples were withdrawn at 0, 15, and 30 min of incubation and immediately deproteinized. Samples for determination of lactic acid or phosphorylated compounds were added to an equal volume of chilled perchloric acid (0.6 mole/liter), mixed, and centrifuged at 3000 *g* for 15 min in a refrigerated centrifuge.

Homogenate ATPase activity was measured continuously with a Gilford recorder attached to a Beckman DU spectrophotometer. The composition of the assay mixture was 0.5 M Tris-acetate, pH 7.4, 3 mM $MgCl_2$, 5 mM phosphoenolpyruvic acid, 5 mM adenosine triphosphate (ATP), and 0.15 mM nicotinamide adenine dinucleotide, reduced form (NADH) (17). The assay mixture also contained 2 μg/ml of lactic dehydrogenase and 10 μg/ml of pyruvic kinase. The reaction was started by adding 50 μl of homogenate. Hydrolysis of ATP generated ADP which was then available to react with phosphoenolpyruvic acid in the presence of pyruvic kinase regenerating ATP and liberating pyruvic acid. The pyruvate was converted to lactate, and the NADH consumed was proportional to the amount of ATP hydrolyzed. The disappearance of NADH was measured by recording the change in optical density at 340 mμ.

Analysis of metabolic intermediates. Each frozen heart was ground to a fine powder under liquid nitrogen in a nitrogen-cooled mortar and pestle. The frozen powder was gradually added to a tared tube containing 2.5 ml of 0.6 M perchloric acid. The tubes were well mixed after each addition of approximately 50 mg of heart so that each particle was in contact with the perchloric acid as it thawed. The tubes were then weighed, and sufficient perchloric acid was added to give a ratio of volume of extract to tissue weight of 4:1. (Total perchloric acid = 3.25 × tissue weight in grams, assuming the water content of heart tissue is 75% (18). The samples were well mixed and centrifuged at 5000 *g* for 10 min at 2°C.

Equal volumes of perchloric acid extract (whole heart or homogenate) and 0.4 M triethanolamine buffer, pH 7.6, to which sufficient K_2CO_3 had been added to bring the final concentration to 0.55 M K_2CO_3 were combined, mixed and allowed to stand in the cold for 10 min. Aliquots of the clear supernatant fluid were used immediately for the determination of metabolic intermediates. Glucose-6-phosphate and fructose-6-phosphate were measured enzymatically according to the method of Hohorst (19). Fructose-1,6-diphosphate and triose phosphates were determined by the method of Bücher and Hohorst (20). ATP was measured enzymatically using ATP Calsuls (Calbiochem, Los Angeles, Calif. [21]). In this procedure ATP reacts with glucose in the presence of hexokinase to form glucose-6-phosphate. The glucose-6-phosphate is then oxidized by glucose-6-phosphate dehydrogenase in the presence of NAD phosphate (NADP). An amount of NADPH proportional to the concentration of ATP in the sample is produced. Since any glucose-6-phosphate present in the sample also reacts with glucose-6-phosphate dehydrogenase, the glucose-6-phosphate content of the sample is subtracted from the apparent ATP value. Creatine phosphate was measured by adding 0.5 mg of creatine phosphokinase to the completed ATP test cuvette and recording the further increment in optical density (22). ADP and AMP were determined enzymatically using ADP/AMP kits (C. F. Boehringer & Soehne Gmbh., Mannheim, Germany) (23).

TABLE I

Performance of Isolated Perfused Euthyroid (E) and Hyperthyroid (H) Rat Hearts

		Aerobic, 60 min	Anoxia				Recovery					
			5 min		10 min	20 min	30 min	10 min	20 min		30 min	
Ventricular rate, *beats/min*	E	209	±3*	75	±3	71 ±6	56 ±10	34 ±11	147 ±44	205	±10	201 ±7
	H	280	±6‡	68	±10	0‡	0‡	0‡	94 ±17	137	±15‡	165 ±19
Pulse pressure, *mm Hg*	E	66	±4	5.7	±0.9§	3 ±0.4	14 ±3	16 ±4	24 ±6	32	±5	43 ±4
	H	72	±5	3.6	±1.3	0‡	0‡	0‡	14 ±4	43	±9	54 ±13
End diastolic pressure, *mm Hg*	E	0.6	±0.2	1	±0.3	4 ±2	20 ±7	23 ±9	4 ±3	0.4	±0.6	0.4 ±0.5
	H	1.0	±0.5	15	±6.0§	—	—	—	18 ±8	26	±17	42 ±18
Maximum LV dp/dt, *mm Hg/sec*	E	1405	±174	218	±21	108 ±21	310 ±78	344 ±101	526 ±134	752	±125	1066 ±94
	H	1846	±160	143	±50	0‡	0‡	0‡	246 ±61	680	±160	1185 ±311
Oxygen consumption, *μl/mg protein per hr*	E	53.1	±3.4							46.1	±3.7	
	H	73.9	±4.9‡							51.3	±2.7	

Hemodynamic data represent average of all hearts including 0 levels for those in arrest at the time of determination. In arrested hearts the end diastolic pressure is the intraluminal pressure of the nonbeating left ventricle. LVdp/dt, first derivative of left ventricular pressure.

* Standard error of mean.

‡ $p < 0.001$.

§ $p < 0.05$.

The NADH was freed of contaminating AMP according to the procedure described by Williamson (24).

Glucose was determined by a glucose oxidase method (Glucostat, Worthington Biochemical Corp., Freehold, N. J.).

Protein content of the perfused hearts and of the heart homogenates was determined by the biuret method (Technicon Co., Chauncey, N. Y.). Precipitated heart protein was dispersed with 10% sodium deoxycholate in ethanol and dissolved in 2.5 N NaOH. An aliquot was added to the biuret reagent and read at 550 mμ after 10 min.

Statistical analyses were performed by the method of Snedecor (25).

RESULTS

Performance of perfused hearts. After 60 min of aerobic perfusion the isolated perfused hearts of both euthyroid and hyperthyroid rats reached a steady state of performance (Table I). The mean heart rate and QO_2 among hyperthyroid rats were significantly elevated ($P < 0.01$), while pulse pressure and end diastolic pressure were not significantly different in the two groups. The mean maximum LV dp/dt was elevated among the hyperthyroid hearts. This difference was not significant ($P < 0.10$).

Upon exposure to anoxic perfusate both euthyroid and hyperthyroid hearts rapidly evidenced a slowing in sinus rate followed by a transient period (1–2 min) of varying degrees of atrioventricular block and then complete heart block with nodal or idioventricular rhythm. Of the 12 euthyroid hearts exposed to anoxia, eight hearts maintained nodal or idioventricular rhythm for 30 min of anoxia. Complete electrical arrest developed at 11 and 18 min in two and at 25 min in the remaining two hearts. Among the 12 hyperthyroid hearts exposed to anoxia, complete electrical arrest occurred during the first 10 min of anoxic perfusion in all hearts. Anoxia induced a gradual and progressive elevation in end diastolic pressure associated with diminution in pulse pressure, maximum LV dp/dt, and ventricular rate in the euthyroid hearts. In the hyperthyroid hearts, anoxia caused an immediate elevation in end diastolic pressure which was significantly higher at 5 min than the end diastolic pressure of the euthyroid group. At 5 min of anoxia the LVP, maximum LV dp/dt, and ventricular rate of the hyperthyroid hearts were slightly but not significantly lower than in the euthyroid hearts. Thus, anoxic perfusion resulted in a more accelerated deterioration in pacemaker activity associated with diminished left ventricular function during the period of spontaneous activity in the hyperthyroid heart.

TABLE II

Metabolite Content of the Perfused Rat Heart

		Aerobic	Anoxia	Recovery
		μmoles/g heart protein		
Creatine phosphate	Euthryoid	41.1 ±1.8	6.0 ±1.2	42.5 ±5.0
	Hyperthyroid	29.4 ±3.3	5.2 ±1.7	10.2 ±4.8
ATP	Euthyroid	27.6 ±0.6	15.7 ±3.1	22.0 ±4.8
	Hyperthyroid	28.6 ±3.0	6.9 ±0.3	14.7 ±1.0
ADP	Euthyroid	5.23 ±0.40	7.8 ±0.70	4.11 ±0.40
	Hyperthyroid	5.10 ±0.58	4.2 ±0.33	3.82 ±0.28
AMP	Euthyroid	0.83 ±0.18	3.77 ±1.14	0.91 ±0.21
	Hyperthyroid	1.14 ±0.09	5.21 ±1.91	2.73 ±0.81
Lactic acid	Euthyroid	3.91 ±0.50	20.94 ±3.61	5.69 ±0.74
	Hyperthyroid	3.03 ±0.98	11.50 ±1.53	4.46 ±0.86

ATP, adenosine triphosphate; ADP, adenosine diphosphate; AMP, adenosine monophosphate.

Upon reexposure to aerobic perfusate (96% O_2, 4% CO_2), the euthyroid hearts demonstrated prompt return to sinus rhythm (within 2 min); control levels of ventricular rate were reached by 20 min. This reversion to normal pacemaker activity was accompanied by progressive increases in pulse pressure and maximum LV dp/dt, while end diastolic pressure diminished toward control levels. In contrast, aerobic reperfusion of hyperthyroid hearts was accompanied by a more variable and delayed recovery of sinus rhythm and ventricular performance. End diastolic pressure remained at elevated levels throughout the recovery period in contrast to the return to normal levels in the euthyroid hearts.

Metabolism of perfused hearts. Analysis of the high energy phosphate stores after 60 min of aerobic perfusion is summarized in Table II. There was no difference between euthyroid and hyperthyroid hearts with respect to ATP or ADP content. Creatine phosphate stores were significantly lower ($P < 0.05$), and AMP concentration was slightly but not significantly higher in the hyperthyroid hearts.

During anoxia the hyperthyroid hearts produced significantly less lactic acid than did the euthyroid hearts ($P < 0.01$) (see Fig. 1). Decreased lactic acid production was associated with significantly lower heart concentrations of ATP ($P < 0.05$), ADP ($P < 0.001$), and lactic acid ($P < 0.05$) (see Table II). At the end of the 30 min aerobic recovery period creatine phosphate, ATP, and total adenine nucleotide concentrations were significantly lower ($P < 0.01$) in the hyperthyroid hearts, whereas AMP was markedly elevated ($P < 0.05$). These data are summarized in Table II.

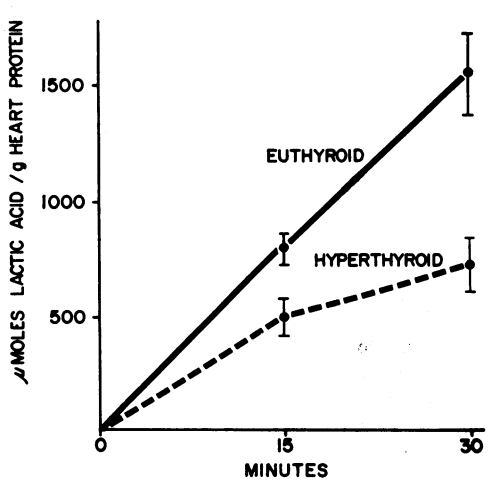

FIGURE 1 Lactic acid production by isolated perfused euthyroid and hyperthyroid hearts during 30 min of anoxia. The differences at 15 and 30 min are significant ($P < 0.05$ and $P < 0.01$, respectively). Vertical bars represent ±1 SEM.

Homogenate glycolysis.[1] The results obtained by incubating euthyroid and hyperthyroid heart homogenates and cytosol for 30 min with modified LePage buffer containing 4.2 mM ADP and no pyruvate are shown in Table III. Hyperthyroid heart homogenates produced significantly less lactic acid from glucose and hexokinase and from glucose-6-phosphate than did homogenates of euthyroid heart, whereas there was no difference between euthyroid and hyperthyroid heart homogenate lactate production from fructose-1,6-diphosphate. There were no significant differences between euthyroid and hyperthyroid heart cytosol lactate production with any of the substrate preparations studied.

As shown in Table IV, the rate of disappearance of glucose from hyperthyroid heart homogenates incubated with glucose and hexokinase was less than half that observed in euthyroid heart homogenates ($P < 0.01$). The total content of glucose, plus major phosphorylated intermediates, plus lactic acid, expressed in terms of triose equivalents, was determined at 0 and 30 min of incubation. Virtually all of the added glucose in both euthyroid and hyperthyroid preparations could be accounted for as nonmetabolized substrate plus glucose-6-phosphate, fructose-6-phosphate, fructose-1,6-diphosphate, triose phosphate, and lactic acid (Table IV). Other glycolytic intermediates were present in extremely low concentrations and could not be measured accurately.

Since the relatively slow rate of lactate production from fructose-1,6-diphosphate in euthyroid heart homogenates and cytosol is probably due to an accumulation of ATP which inhibits phosphoglycerate kinase (26), 12.5 mM deoxyglucose and hexokinase as an ATP sink were added to the incubation mixture (deoxyglucose + ATP $\xrightarrow{\text{hexokinase}}$ deoxyglucose-6-phosphate + ADP) (27) (Table III). A catalytic amount (0.5 mmole/liter) of pyruvate was added as well to prevent the accumulation of NADH which inhibits glyceraldehyde phosphate dehydrogenase (28). The complete conversion of this pyruvate to lactic acid could account for only about 10% of the total lactic acid production. Under these conditions there was an acceleration in lactate production from fructose-1,6-diphosphate, with no significant difference between euthyroid and hyperthyroid heart homogenates.

[1] Similar results were obtained whether euthyroid and hyperthyroid animals were matched on the basis of heart weight or body weight. Since animals with similar heart weights were used in the perfusion studies, only data from the matched heart weight series will be discussed. When lactic acid production from glucose and hexokinase by homogenates of hearts in the matched body weight series was compared, the hyperthyroid hearts produced significantly less lactic acid than did the euthyroid hearts ($P < 0.05$). Euthyroid heart homogenates produced 1.31 ±0.27 (SEM) µmoles/mg of heart protein during 30 min of incubation compared with 0.71 ±0.173 in the hyperthyroid group.

TABLE III

*Lactic Acid Production by Heart Homogenates and Cytosol**

	Modifications	12.5 mM glucose	12.5 mM glucose plus 0.1 mg/ml hexokinase	12.5 mM glucose-6-phosphate	12.5 mM fructose-1,6-diphosphate
Euthyroid heart homogenate (7)		0.22 ±0.03	1.95 ±0.15	1.24 ±0.12	0.77 ±0.12
Hyperthyroid heart homogenate (7)		0.15 ±0.03	0.78 ±0.23‡	0.81 ±0.12§	0.62 ±0.05
Euthyroid heart homogenate (6)	0.5 mM pyruvate, 12.5 mM deoxyglucose, and 0.1 mg/ml of hexokinase		2.04 ±0.21	2.76 ±0.38	2.19 ±0.24
Hyperthyroid heart homogenate (6)	0.5 mM pyruvate, 12.5 mM deoxyglucose, and 0.1 mg/ml of hexokinase		1.04 ±0.18‡	1.81 ±0.35‖	2.67 ±0.39
Euthyroid heart cytosol (6)		0.16 ±0.02	0.95 ±0.16	0.39 ±0.08	0.35 ±0.08
Hyperthyroid heart cytosol (6)		0.17 ±0.05	0.79 ±0.14	0.35 ±0.11	0.29 ±0.08

Data in parentheses indicate number of experiments.

* Lactic acid production equals micro-moles of lactate produced during 30 min of incubation per milligram of homogenate of protein.

‡ $P < 0.01$.

§ $P < 0.05$.

‖ $P = 0.05$.

Steady-state concentrations of the glycolytic intermediates generated during incubation of euthyroid and hyperthyroid heart homogenates with glucose and hexokinase or glucose-6-phosphate were expressed as ratios between the concentrations obtained in hyperthyroid homogenates and the concentrations in euthyroid homogenates. These ratios were then plotted in sequence according to their occurrence in the glycolytic pathway in order to locate any crossover points. According to the crossover theorem, if steady-state flux is decreased and there is a relative accumulation of one intermediate followed by a relative depletion of the next, the accumulation-depletion pair constitutes a crossover point and can only occur at a site of interaction (29).

In hyperthyroid homogenates incubated with glucose and hexokinase or glucose-6-phosphate there was an accumulation of glucose-6-phosphate and fructose-6-phosphate and a depletion of fructose-1,6-diphosphate, triose phosphates, and lactate relative to the euthyroid homogenate. As shown in Fig. 2, a distinct crossover occurs between fructose-6-phosphate and fructose-1,6-diphosphate. These observations indicate the existence of less phosphofructokinase activity in the hyperthyroid homogenates. No crossover is obtained when the data from cytosol experiments are plotted in a similar manner (Fig. 3).

Addition of potassium citrate to euthyroid heart homogenates had no effect on lactic acid production from glucose and hexokinase as shown in Fig. 4.

TABLE IV

Glucose Disappearance during Incubation of Heart Homogenates and Cytosol with Glucose and Hexokinase and Recovery of Added Glucose as Major Glycolytic Intermediates, Lactic Acid, and Nonmetabolized Glucose*

	Glucose disappearance	Recovery‡
	μmoles/30 min ×mg homogenate protein	%
Euthroid heart homogenate (6)	1.7 ±0.26	102 ±2.6
Hyperthyroid heart homogenate (6)	0.7 ±0.12§	101 ±3.5
Euthyroid heart cytosol (7)	1.8 ±0.22	109 ±2.1
Hyperthyroid heart cytosol (7)	1.9 ±0.29	100 ±4.0

* Major glycolytic intermediates included glucose-6-phosphate, fructose-6-phosphate, fructose-1,6-diphosphate, and triose phosphates.

‡ Total lactate equivalents after 30 min of incubation are expressed as a per cent of the initial concentration.

§ $P < 0.01$.

There were no differences in Mg^{++}-activated ATPase activity between the euthyroid and hyperthyroid heart homogenates. The euthyroid heart homogenates hydrolyzed 4.77 ±0.42 μmoles of ATP per min × g of heart compared with 4.86 ±0.30 for the hyperthyroid group.

DISCUSSION

It is apparent from the present studies that upon exposure to anoxia, the isolated, perfused beating heart of the hyperthyroid rat demonstrates accelerated deterioration of electrical and mechanical activity and diminished recoverability of function upon reexposure to aerobic perfusate. The diminished performance characteristics during anoxia are accompanied by a decreased rate of lactate generation and a diminution in ATP content of the myocardium. Among possible explanations for these findings are: (*a*) a primary inhibition of anaerobic glycolysis in the hyperthyroid heart resulting in diminished ATP generation with resultant functional deterioration, (*b*) a diminution in anaerobic glycolysis secondary to diminished energy demand by the hypodynamic heart, and (*c*) a combination of these mechanisms. With exposure to an anoxic environment, the ATP content of the myocardium reflects the balance between the ATP production from glycolysis and creatine phosphate stores and the hydrolysis of ATP. If diminished ATP content of the myocardium reflected primarily a decreased utilization of ATP secondary to a suppression of contractile performance in the anaerobic hyperthyroid heart, one would expect to find a relative preservation of ATP and creatine phosphate levels. In fact, in the hyperthyroid hearts the ATP content was less than half that of the anaerobically perfused euthyroid heart, while creatine phosphate content was equivalently reduced in both groups. These data suggested that inhibition of anaerobic metabolism was primarily responsible for the low ATP and diminished performance of the anoxic perfused heart. To test further the thesis that inhibition of anaerobic glycolysis accounted for the changes observed in the intact heart, anaerobic glycolysis was studied in homogenates from euthyroid and hyperthyroid rat hearts. These studies demonstrated that lactic acid production by the hyperthyroid heart homogenate incubated with glucose plus hexokinase or glucose-6-phosphate was reduced. Lactic acid production from fructose-1,6-diphosphate was not altered. When the crossover pattern of glycolytic intermediates from the homogenates of euthyroid and hyperthyroid hearts was examined, a decrease in phosphofructokinase (PFK) activity of the hyperthyroid heart became apparent. These data support the hypothesis that inhibition of anaerobic glycolysis at the PFK step is responsible for the diminished glycolytic flux in the hyperthyroid heart.

Bressler and Wittles demonstrated elevated levels of citrate in hyperthyroid myocardium and suggested that

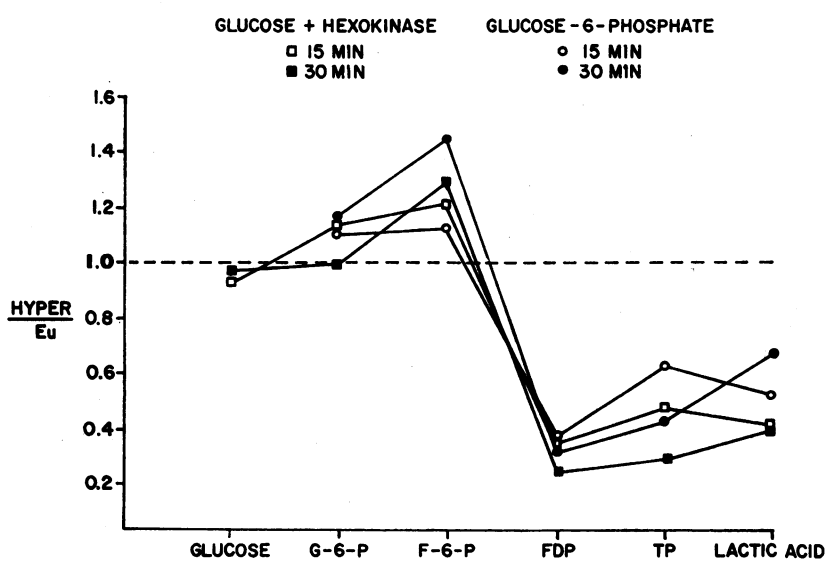

FIGURE 2 The ratio of hyperthyroid to euthyroid heart homogenate glycolytic intermediate levels. The concentration of fructose-1,6-diphosphate (FDP) and triose phosphates (TP) in hyperthyroid heart homogenates was significantly lower than in euthyroid homogenates ($P < 0.01$).

citrate inhibition of PFK is responsible for decreased aerobic glycolysis in hearts from hyperthyroid guinea pigs (6). In the present studies we were unable to induce inhibition of anaerobic lactic acid production in heart homogenates by additions of potassium citrate equal to and up to 10 times as great as those found by Bressler and Wittles in hyperthyroid hearts. It is possible that the concentrations of AMP and fructose-6-phosphate in our system were great enough to prevent citrate inhibition of PFK, since both of these compounds are known to deinhibit PFK (28). The ADP present in our incubation mixture (4.2 mmoles/liter) would be expected to provide approximately 1.2 mM AMP, 1.2 mM ATP, and 1.8 mM ADP if the myokinase reaction attained equilibrium ($[ATP]\ [AMP]/[ADP]^2 = 0.44$) (30). Inhibition of glycolysis by citrate therefore cannot explain the observed changes in the anaerobic glycolytic rate of hyperthyroid heart homogenates in this study.

Mitochondria prepared from the livers of hyperthyroid animals exhibit increased ATPase activity (31, 32). While ATP strongly inhibits the PFK reaction (33), ATP is also a reactant in the conversion of fructose-6-phosphate to fructose-1,6-diphosphate. In addition, the hexokinase reaction requires ATP as substrate. We therefore investigated the possibility that excessive ATPase activity, with marked diminution in ATP concentrations, might be responsible for diminished anaerobic glycolysis in the hyperthyroid heart homogenates. Measurement of Mg^{++}-activated ATPase activity of the heart homogenates used in this study revealed no significant difference between the euthyroid and hyperthyroid preparations. Racker has shown that the addition of apyrase (ATPase) in low concentrations stimulates rather than inhibits homogenate lactic acid production (34). Thus it seems unlikely that differences in mitochondrial ATPase activity are responsible for the inhibition of glycolysis in hyperthyroid hearts.

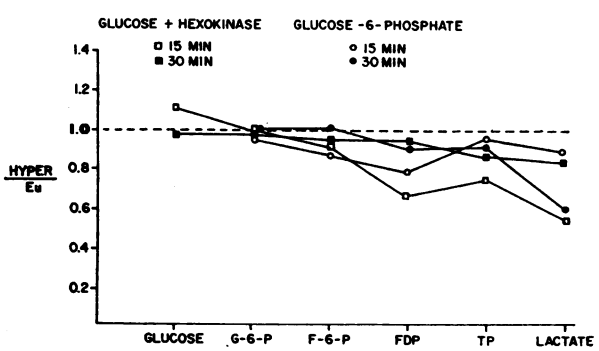

FIGURE 3 The ratio of hyperthyroid to euthyroid cytosol glycolytic intermediate levels.

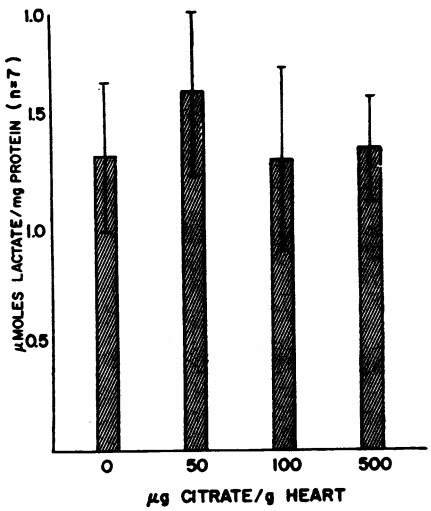

FIGURE 4 Effect of citrate on lactic acid production by heart homogenates incubated for 30 min with glucose and hexokinase. Bars represent ±1 SEM.

Free fatty acids have been shown to inhibit several of the key glycolytic enzymes (hexokinase, pyruvic kinase, and PFK) of liver (35), and Bressler and Wittels have reported that hyperthyroid hearts contain elevated concentrations of free fatty acids (6). The free fatty acids are largely found in the cellular particulate fraction. Hence it is possible that the elevated free fatty acids may be responsible for the inhibition of anaerobic glycolysis in anoxic hyperthyroid hearts.

In the present study, inhibition of the glycolytic pathway at the PFK step occurred in whole heart homogenates but not in the cytosol fraction of the hyperthyroid heart. It thus appears that the primary inhibitor of anaerobic glycolysis resides in the particulate fraction of the myocardium. Among possible mechanisms for the inhibition of PFK in anaerobic hyperthyroid heart homogenates, increased myocardial citrate concentrations and increased mitochondrial ATPase activity would appear to be negligible, while the possible contribution of increased free fatty acid concentrations is not known.

Recent studies by Pool, Skelton, Seagren, and Braunwald (36) on the isolated right ventricular papillary muscle treated with iodoacetate and nitrogen have demonstrated decreased efficiency of conversion of chemical energy to mechanical work in hyperthyroidism. When considered in light of the present investigation it would hence appear that hyperthyroidism imposes metabolic defects in myocardial energy generation and utilization in the anaerobic state.

The present study demonstrates that the metabolic effects of hyperthyroidism involve the glycolytic pathway

as well as oxidative metabolism. In the aerobic state excess thyroid hormone induces a state of hyperdynamic cardiac performance associated with enhanced aerobic metabolism. In contrast, in the anaerobic state the effects of excess thyroid hormone are diminished performance and metabolism. Any explanation of the fundamental metabolic effects of hyperthyroidism must reconcile these apparently disparate effects of thyroid hormone.

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