Rapid thyroid-hormone effect on mitochondrial and cytosolic ATP/ADP ratios in the intact liver cell

Hans J. SEITZ,* Manfred J. MÜLLER† and Sibylle SOBOLL‡

*Institut für Physiologische Chemie, Universitäts-Krankenhaus Eppendorf, 2000 Hamburg 20, †Abteilung Klinische Endokrinologie, Medizinische Hochschule Hannover, 3000 Hannover 61, and ‡Institut für Physiologische Chemie I, Universität Düsseldorf, 4000 Düsseldorf, Federal Republic of Germany

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The effect of thyroid-hormone application on cytosolic and mitochondrial ATP/ADP ratio was investigated in rat liver *in vivo* and in the isolated perfused organ. *In vivo* the ATP/ADP ratio in livers from hypothyroid rats was 0.84 ± 0.08 in the mitochondrial matrix and 5.6 ± 0.9 in the cytosol, as was observed in euthyroid controls. In contrast, hyperthyroidism was followed by a significant decrease in the mitochondrial and by an increase in the cytosolic ATP/ADP ratio (to 0.34 ± 0.06 and 11.3 ± 2.8 respectively). In the perfused liver from hypothyroid animals, addition of L-3,3',5-tri-iodothyronine in the perfusate also provoked, within 2 h, a significant decrease in the mitochondrial ATP/ADP ratio, whereas the cytosolic ratio was unaffected. From these and previous data in the isolated perfused liver and in isolated mitochondria from hypothyroid and tri-iodothyronine-treated rats it is concluded that thyroid hormones increase mitochondrial respiration and ATP regeneration, which is associated with an acceleration of mitochondrial adenine nucleotide transport and significant alterations in the mitochondrial and cytosolic ATP/ADP ratios.

Application of thyroid hormones *in vivo* stimulates cellular oxygen consumption as well as ATP synthesis in several organs, including the liver. Accordingly, the turnover, number, size and inner surface area of mitochondria as well as the numbers of functional respiratory-chain units are all increased in liver cells in hyperthyroidism (for reviews see Sterling, 1979; Müller & Seitz, 1984). As a consequence of increased cellular oxidative capacity, steady-state concentrations of adenine nucleotides are altered in hyperthyroidism, resulting in an enhanced cellular ATP/ADP ratio (Müller & Seitz, 1980). However, others reported decreased ATP/ADP ratios in experimental hyperthyroidism (Ismail-Beigi & Edelman, 1973). With respect to subcellular concentrations, only calculated data are available, indicating a thyroid-hormone effect on increasing cytosolic ATP/ADP (Müller & Seitz, 1980). As subcellular adenine nucleotide distribution depends on mitochondrial adenine nucleotide translocation, it is noteworthy that thyroid-hormone application leads to an acceleration of mitochondrial ADP uptake, as measured in isolated

mitochondria (Babior *et al.*, 1973; Hoch, 1977; Palacios-Romero & Mowbray, 1979).

The present study sets out to investigate (i) whether the observed effect of T_3 , in stimulating the mitochondrial energy metabolism, is followed by corresponding alterations in mitochondrial and/or cytosolic ATP/ADP ratio in the intact liver cell *in vivo*, and (ii) whether these changes can be induced by T_3 itself in the isolated perfused liver.

Methods

In male Wistar (specific-pathogen-free) rats (180–200 g) hypothyroidism was induced by intraperitoneal injection of $Na^{131}I$ (250 μ Ci/100 g body wt.) 21–28 days before the experiments. Hyperthyroidism was produced by daily intraperitoneal injections of thyroxine (Sigma, St. Louis, MO, U.S.A.; 50 μ g/100 g body wt.). The hypo- and hyper-thyroid states were monitored by serum thyroxine (<10 and >350 ng/ml of serum) and serum cholesterol (>350 and <100 mg%) concentrations and hepatic malic enzyme activity (<6 and >170 munits/mg of protein). In order to

Abbreviation used: T_3 , L-3,3',5-tri-iodothyronine.

deplete hepatic glycogen, experiments were performed in 48 h-starved rats. Rapid liver sampling was performed on unanaesthetized unrestrained rats by the double-hatchet method (Faupel *et al.*, 1972).

Livers were isolated and perfused without gas loss by the technique of Schimassek (1963) as described elsewhere (Müller & Seitz, 1980), by using a Fluorocarbon FC 43 medium (without serum) made up in the non-ionic detergent Pluronic F 68 by sonication and diluted with saline buffer (Krone *et al.*, 1974). Flow rates were about 3–4 ml/min per g of liver; liver weights were about 4.5 g. The functional state of the liver was determined by continuous measurement of pH in the medium (7.35 ± 0.04), hepatic O_2 consumption and glutamate-oxaloacetate transaminase (EC 2.6.1.1) release (less than 1.5 units/g of liver over 120 min perfusion time in the perfusion medium; cf. Krone *et al.*, 1974). After an equilibrium period of 15 min, the following substances were added to the perfusate (initial concn.): 2.8 mM-glucose, 10 mM-alanine, 4 μ Ci of [U - 14 C]alanine. After a further 15 min T_3 was injected (over a period of about 20 s) into the portal vein. It was shown previously that the injection time was sufficient for a single circulation of the bolus through the liver (Pardridge & Mietus, 1980). Hepatic T_3 uptake amounts for about 30% of the initial dose in our perfusion system (Köhrle *et al.*, 1982). Fractionation of tissues in non-aqueous solvents and determination of mitochondrial and cytosolic contents of ATP and ADP were performed on freeze-clamped livers as described previously (Soboll *et al.*, 1980, 1984; Schwenke *et al.*, 1981).

Hepatic oxygen consumption, hepatic malic enzyme activity, conversion of [14 C]alanine into [14 C]glucose and $^{14}CO_2$, glucose, alanine, lactate, pyruvate, β -hydroxybutyrate, acetoacetate, long-chain acyl-CoA and acetyl-CoA in the perfusate

and/or liver tissue were measured by standard enzymic procedures as described previously (Krone *et al.*, 1974; Müller & Seitz, 1980, 1981).

Statistically significant differences were examined by Student's *t* test. Data are given as means $\pm$ S.E.M.

Results

Effects of different thyroid states on subcellular ATP/ADP ratios in vivo

In Table 1 the subcellular concentrations of ATP and ADP and the ATP/ADP ratios in livers from hypo-, eu- and hyper-thyroid 48 h-starved rats *in vivo* are shown. Compared with euthyroid controls, hypothyroidism caused no alteration in either the mitochondrial or the cytosolic ATP/ADP ratio. Also, for all other metabolites measured no pronounced alterations were observed compared with the euthyroid control (euthyroid values: acetyl-CoA 82 ± 2 nmol/g of liver; long-chain acyl-CoA 41 ± 2 nmol/g of liver; β -hydroxybutyrate/acetoacetate ratio 2.10 ± 0.10 ; lactate/pyruvate ratio 17.8 ± 1.2 ; $n = 8$ –17). In contrast, hyperthyroidism provoked a significant fall in the mitochondrial ATP/ADP ratio and a pronounced increase in the cytosolic ATP/ADP ratio (Table 1). In addition, statistically significant decreases in the cellular contents of long-chain acyl-CoA (35 ± 3 nmol/g of liver), acetyl-CoA (62 ± 2 nmol/g of liver), acetoacetate and β -hydroxybutyrate were observed, whereas the ratios of β -hydroxybutyrate/acetoacetate (2.6 ± 0.13) and lactate/pyruvate (19.1 ± 1.1) were not affected. Oxygen consumption with endogenous substrates, as measured in the isolated perfused organ, varied with the thyroid state: it was enhanced in hyperthyroidism (2.97 ± 0.04 μ mol/min per g of liver) and decreased in the hypothyroid state (0.94 ± 0.12 μ mol/min per

Table 1. *Effect of different thyroid states on subcellular ratios of ATP and ADP in the liver from 48 h-starved rats*
For details see the text ($n = 5$).

	Thyroid state		
	Hypo-	Eu-	Hyper-
Concn. in mitochondrial matrix (μ mol/ml of mitochondrial water)			
ATP	10.4 ± 1.0	7.92 ± 0.93	4.6 ± 0.7
ADP	12.5 ± 0.9	7.62 ± 0.84	13.9 ± 1.1
ATP/ADP	0.84 ± 0.08	1.04 ± 0.03	0.34 ± 0.06
Concn. in cytosol (μ mol/ml of cytosolic water)			
ATP	3.7 ± 0.2	5.33 ± 0.25	4.1 ± 0.2
ADP	0.73 ± 0.13	0.93 ± 0.08	0.48 ± 0.13
ATP/ADP	5.6 ± 0.9	5.91 ± 0.65	11.3 ± 2.8

g of liver), (euthyroid control: $1.81 \pm 0.08 \mu\text{mol/min per g of liver}$).

Effect of T₃ on subcellular ATP/ADP ratios in the isolated perfused liver of hypothyroid rats

Addition of T₃ ($1 \mu\text{M}$) to the perfusion medium of livers from hypothyroid 48 h-starved rats resulted

in a continuous increase in oligomycin-sensitive oxygen consumption, reaching a new steady state at about 60–80 min [basal value in the presence of 10 mM-alanine 2.1 ± 0.2 , increased to 3.3 ± 0.3 ($\mu\text{mol/min per g of liver}$); $P < 0.01$]. This hormone effect is dose-dependent and significant for a hormone concentration of 10 pM (Müller & Seitz, 1981). Measurements of the mitochondrial ATP/ADP ratio at $1 \mu\text{M-T}_3$ revealed a significant fall to 0.28, whereas for the cytosol value no alteration was observed (Table 2). For all other tissue metabolites measured, no significant alterations were observed (values for perfused liver of hypothyroid rats: long-chain acyl-CoA $16 \pm 2 \text{ nmol/g of liver}$, acetyl-CoA $46 \pm 9 \text{ nmol/g of liver}$, ratio of lactate/pyruvate 19 ± 2 and of β -hydroxybutyrate/acetoacetate 0.27 ± 0.04). In addition, a dose-dependent stimulation of [^{14}C]alanine conversion into [^{14}C]glucose and $^{14}\text{CO}_2$ was observed (Fig. 1).

Discussion

Different thyroid states were associated with alterations in hepatic oxygen consumption, which was decreased in hypo- and increased in hyperthyroid rats (see the Results section). The essential finding of the present study is that, in addition, hyperthyroidism was associated with a significant decrease in the mitochondrial and an increase in the cytosolic ATP/ADP ratio (Table 1), whereas both the cytosolic and mitochondrial redox states were unaffected (see the Results section). These data show that the thyroid-hormone-stimulated mitochondrial respiration is not due to uncoupling of oxidative phosphorylation, yet reflects stimulation of mitochondrial ATP synthesis. This fact is further confirmed by the finding that the degree of coupling, which can be calculated as described by Stucki (1980) from the steady-state concentrations

Fig. 1. Dose-dependent stimulation of [^{14}C]alanine conversion into $^{14}\text{CO}_2$ and [^{14}C]glucose by T₃ in the isolated perfused liver of hypothyroid starved rats. Measurements were performed between 60 and 120 min ($n = 5-6$). * $P < 0.025$.

Table 2. Effect of addition of T₃ ($1 \mu\text{M}$) on subcellular ratios of ATP and ADP in the isolated perfused liver of hypothyroid 48 h-starved rats

Measurements were performed at 120 min perfusion time ($n = 5$).

	-T ₃	+T ₃
Concn. in mitochondrial matrix ($\mu\text{mol/ml of mitochondrial water}$)		
ATP	8.1 ± 1.2	3.0 ± 0.8
ADP	12.0 ± 1.4	10.8 ± 0.3
AMP	2.5 ± 0.4	3.2 ± 0.8
ATP/ADP	0.72 ± 0.12	0.28 ± 0.07
Concn. in cytosol ($\mu\text{mol/ml of cytosolic water}$)		
ATP	3.3 ± 0.2	2.6 ± 0.1
ADP	0.54 ± 0.08	0.41 ± 0.11
AMP	0.26 ± 0.13	0.42 ± 0.14
ATP/ADP	6.9 ± 1.5	6.8 ± 1.4

of cytosolic adenine nucleotides, was identical ($q = 0.96$) in livers of both hypo- and hyper-thyroid rats. Thus hyperthyroidism enhances the mitochondrial oxidative capacity, and it is furthermore evident that the decreased mitochondrial phosphorylation state of ATP reflects not decreased mitochondrial ATP synthesis but rather stimulated ATP export. This is further supported by the enhanced cytosolic ATP/ADP ratio *in vivo* (Table 1), despite enhanced ATP utilization for increased ureagenesis, gluconeogenesis, futile-cycle activity (Okajima & Ui, 1979; Rognstad, 1977; Müller & Seitz, 1980) and ($\text{Na}^+ + \text{K}^+ + \text{Mg}^{2+}$)-dependent ATPase activity (Guernsey & Edelman, 1983). Thus, obviously thyroid-hormone-induced cellular energy supply exceeds cytosolic ATP consumption under conditions *in vivo*. Furthermore our data provide evidence for the physiological significance of the finding by Babior *et al.* (1973), demonstrating an accelerated rate of ADP/ATP exchange across the mitochondrial membrane in hyperthyroidism in experiments *in vitro*. These effects are generally ascribed to increased synthesis of mitochondrial proteins and/or alterations in the mitochondrial membrane lipid composition (see Müller & Seitz, 1984).

When T_3 was added to the perfusion medium of perfused liver of hypothyroid rats, (i) an increase in oligomycin-sensitive (Müller & Seitz, 1980) oxygen consumption (see the Results section), (ii) a fall in the mitochondrial ATP/ADP ratio (Table 2) and (iii) simultaneously unaltered mitochondrial and cytosolic redox states were observed (see the Results section). These data indicate that part of the hyperthyroid-induced alteration in adenine nucleotide metabolism in the intact liver cell is due to a rapid action of T_3 itself. However, the cytosolic ATP/ADP ratio was not affected (Table 2). This may be due to the differences in experimental conditions: (i) the artificial conditions of the isolated perfused liver system, (ii) the short-term effect of T_3 , and/or (iii) the concomitant increases in ATP-utilizing processes [e.g. increased rates of gluconeogenesis (Fig. 1) and ureagenesis (see above)] especially in the presence of unphysiological high concentrations of alanine (see the Methods section).

It could be argued that the T_3 dose applied in our experiments is supra-physiological. However, T_3 in its physiological range (i.e. 10 pM) already increased oligomycin-sensitive O_2 consumption (Müller & Seitz, 1980) as well as alanine conversion into CO_2 and glucose (Fig. 1). As the rapid T_3 effect in stimulating hepatocyte oxygen consumption was also observed in the presence of cycloheximide, an inhibitor of protein synthesis (Kaminski, 1980), protein biosynthesis is not involved in the process. With respect to the mechanism,

there has been increasing evidence for a mitochondrial pathway of thyroid-hormone action (for reviews see Sterling, 1979; Müller & Seitz, 1984). Specific binding sites on the mitochondrion have been demonstrated in several organs, including the liver (Sterling & Milch, 1975; Goglia *et al.*, 1981; Hashizume & Ichikawa, 1982), although others have failed to demonstrate a specific mitochondrial binding protein for T_3 (Greif & Sloane, 1978). Nevertheless, it is now generally accepted that the mitochondria are a target for thyroid-hormone action, which is demonstrated by thyroid-hormone-induced (i) modifications of mitochondrial proteins (Richter *et al.*, 1983), (ii) alterations in mitochondrial ion (e.g. Ca^{2+} ; Greif *et al.*, 1982) and substrate (e.g. long-chain fatty acyl-CoA) fluxes (Müller *et al.*, 1981), and/or (iii) elevation of the protonic electrochemical potential difference across the mitochondrial inner membrane (Shears, 1980).

The metabolic significance of shifting the mitochondrial ATP/ADP ratio has been emphasized for the regulation of pyruvate carboxylase and pyruvate dehydrogenase activity, thereby controlling pyruvate oxidation as well as carboxylation and consequently glucose production (Walter *et al.*, 1974). Yet, in liver of hyperthyroid rats *in vivo* (Okajima & Ui, 1979), and after addition of T_3 to the perfusate of the isolated perfused liver of hypothyroid rats, gluconeogenesis was increased (Fig. 1) when the mitochondrial ATP/ADP ratio was decreased (Tables 1 and 2). This observation strongly argues against a predominant role of the mitochondrial ATP/ADP ratio in regulating hepatic glucose production by controlling pyruvate carboxylase activity. On the other hand, a decrease in the mitochondrial ATP/ADP ratio (Table 2) should favour pyruvate oxidation (Siess & Wieland, 1975). In fact, addition of T_3 resulting in a circulating concentration of 10 pM rapidly stimulated $^{14}\text{CO}_2$ production from [^{14}C]alanine (Fig. 1).

Taken together, our results favour the hypothesis that mitochondria are possibly a target of early thyroid-hormone action. T_3 by itself rapidly stimulates hepatic oligomycin-sensitive oxygen consumption and, since at decreased mitochondrial ATP/ADP ratios oxidative phosphorylation and mitochondrial adenine nucleotide translocation are co-ordinately enhanced, ATP supply for numerous energy-consuming reactions in the cytosol is favoured.

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