Rapid stimulation of hepatic oxygen consumption by 3,5-di-iodo-L-thyronine

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Tri-iodothyronine (T_3) and thyroxine (T_4) as well as 3,5-di-iodothyronine (T_2) stimulated O_2 consumption by isolated perfused livers from hypothyroid rats at a concentration as low as 1 pM by about 30 % within 90 min. Application of T_2 resulted in a faster stimulation than with application of T_3 or T_4 . Inhibition of iodothyronine monodeiodinase by propylthiouracil, thereby blocking the degradation of T_4 to T_3 and of T_3 to T_2 , demonstrated that only T_2 is the active hormone for the rapid stimulation of hepatic O_2 consumption: T_3 and T_4 lost all of their stimulative activity, whereas T_2 was as potent as in the absence of propylthiouracil. Perfusion experiments with thyroid-hormone analogues confirmed the specificity of the T_2 effect. The nucleus is unlikely to contribute to the rapid T_2 effect, as can be deduced from perfusion experiments with cycloheximide and lack of induction of malic enzyme by T_2 . In conclusion, a new scheme of regulation of mitochondrial activity is proposed: T_2 acts rapidly and directly via a mitochondrial pathway, whereas T_3 exerts its long-term action indirectly by induction of specific enzymes.

INTRODUCTION

Mitochondria are a target of thyroid-hormone action. *In vivo*, application of thyroid hormones for several days provokes an increase in their number and respiratory capacity, alterations in their size and inner surface area (for reviews see [1,2]) as well as in a set of proteins [3]. Whether thyroid hormones exert an additional rapid action on increasing mitochondrial activity is a matter of debate.

T_3 (1 μ M) significantly increased O_2 consumption (Q_{O_2}) in the isolated perfused liver of hypothyroid and euthyroid rats [4]. Q_{O_2} of mitochondria isolated 20 or 30 min after an intravenous injection of T_3 was likewise increased [5,6]. When mitochondria were isolated 15 min after the injection, no effect on Q_{O_2} was detectable, whereas other parameters (ADP/ O_2 , H^+ / O , pH gradient, adenine nucleotide translocation) were significantly altered [7–9]. The alterations of ADP/ O and H^+ / O ratios, however, were not reproduced by others [5,6] and were recently suggested to be an experimental artefact [10]. The enhanced adenine nucleotide translocation, on the other hand, was also detected in the isolated perfused liver [11].

With respect to the mechanism(s) involved, opinions are also controversial. Sterling [12] claimed to have evidence that these effects are due to T_3 binding at the adenine nucleotide translocase. Several observations argue against this hypothesis, however (for a review see [13]). A direct stimulation of oxidative phosphorylation in submitochondrial particles by T_3 was reported [14], whereas others failed to obtain any T_3 effect on intact mitochondria [9].

The present study was carried out to investigate the rapid effect of iodothyronines on Q_{O_2} in the isolated

perfused liver, to verify its specificity by use of thyroid-hormone analogues, and to approach the underlying mechanism(s).

MATERIALS AND METHODS

Chemicals

3'-Acetyl- T_2 , Dibit, L-94901 and the ether-bridge analogues were from Smith Kline and French Research (Welwyn, Herts., U.K.), and 3,3',5-tri-iodopropionic acid was from Sigma Chemie G.m.b.H. (Munich, Germany). All other iodothyronines and thyroid-hormone analogues were from Henning Berlin G.m.b.H. All tested compounds were > 99 % pure. For application, they were dissolved in a minimum of 1 M-NaOH before dilution with water, and tested at 1 pM except where otherwise stated.

6-n-Propyl-2-thiouracil was from Sigma (Munich, Germany), and enzymes were from Boehringer Mannheim (Mannheim, Germany). All other chemicals were of analytical grade and purchased from Merck (Darmstadt, Germany) or Boehringer Mannheim.

Animals

Male Wistar rats (specific-pathogen-free) from Wiga (Charles River, Sulzfeld, Germany) were used throughout this study. Over the entire experimental period, veterinary supervision was provided. Hypothyroidism was provoked by an intraperitoneal injection of 0.25 mCi of $Na^{131}I$ 28 days before the experiments. The hypothyroid state was monitored by measurement of serum T_4 (< 10 ng/ml) and hepatic malic enzyme activity (< 2 units/mg of protein). Experiments were performed in rats fasted overnight.

Abbreviations used: T_2 , 3,5-di-iodo-L-thyronine; T_3 , 3,3',5-tri-iodo-L-thyronine; T_4 , thyroxine; Dimit, 3,5-dimethyl-3'-isopropyl-L-thyronine; Dibit, 3,5-dibromo-3'-isopropyl-L-thyronine; L-94901, 3,5-dibromo-3'-pyridazinone-L-thyronine; Q_{O_2} , O_2 consumption.

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Perfusion system

Rats were anaesthetized with pentobarbital, and isolated livers were perfused in a recirculating system with Krebs/Henseleit bicarbonate buffer saturated with O_2/CO_2 (19:1), pH 7.4, at 37 °C, with 10 mM-alanine as substrate. Q_{O_2} was continuously monitored by a Clark-type electrode. Q_{O_2} of controls ($n = 7$) increased linearly during perfusion, from $1.90 \pm 0.20 \mu\text{mol/min per g}$ after 30 min of preperfusion to 2.16 ± 0.09 after 30 min perfusion time, 2.35 ± 0.12 after 60 min, and 2.48 ± 0.13 after 90 min. Hepatic function was monitored by alanine uptake ($210 \pm 30 \mu\text{mol/2 h per g}$), glucose production ($30 \pm 10 \mu\text{mol/2 h per g}$), urea production ($60 \pm 15 \mu\text{mol/2 h per g}$) and the release rate of glutamate–oxaloacetate transaminase ($< 1.5 \text{ units/2 h per g}$).

Spectrophotometric analysis

Substrate concentrations and enzyme activities were determined as described in Bergmeyer [15], except for malic enzyme [16]. A unit of enzyme activity is defined as turnover of $1 \mu\text{mol}$ of substrate/min. Protein was measured by the Bio-Rad method.

Statistical analysis

Statistical studies were performed with a computer program using Student's t test; $P < 0.01$ was considered significant.

RESULTS

Effect of iodothyronines in the isolated perfused liver

In order to examine the rapid effect of iodothyronines on hepatic Q_{O_2} , a series of compounds was tested in the isolated perfused liver of hypothyroid rats. T_2 , T_3 and T_4 stimulated Q_{O_2} at a concentration as low as 1 pM (Fig. 1). Stimulation by T_2 was statistically significant after only 30 min, whereas stimulation by T_3 and T_4 was slower, being significant after 60 min. Q_{O_2} was roughly similar in all three cases 90 min after addition of iodothyronines to the perfusate. Other iodothyronines tested (3-monoiodothyronine, 3,3'- T_2 , 3',5'- T_2 , rT_3 ; nos. 2, 4, 5, 10) as well as 3,5-di-iodotyrosine (no. 1) had no effect (Table 1).

In dose/response experiments, T_2 and T_3 (Fig. 2) showed a maximal response at 1 pM . No further effects were detectable at higher concentrations, whereas they were diminished at lower concentrations.

T_3 is produced from T_4 in the liver and kidney by type I iodothyronine monodeiodinase [17]. The enzyme also rapidly degrades iodothyronines to thyronines including the production of T_2 from T_3 . The observed different time courses of the Q_{O_2} stimulation by T_4 , T_3 and T_2 led to the suggestion that T_4 and T_3 are deiodinated to T_2 to become active for this rapid stimulation. Therefore, perfusion experiments were carried out in the presence of 6-n-propyl-2-thiouracil, a potent inhibitor of deiodinase [17]. Propylthiouracil was applied at $1 \mu\text{M}$, in accordance

Table 1. Effect of thyroid-hormone analogues (1 pM) on the O_2 consumption of isolated perfused livers of hypothyroid rats in comparison with 'anti-goitre activity' and induction of hepatic mitochondrial glycerol-3-phosphate dehydrogenase activity (from refs. [19,23,44,45])

Data given are ΔO_2 above controls ($n = 7$) at 90 min perfusion time (O_2 consumption at 0 min was $1.9 \pm 0.2 \mu\text{mol/min per g}$) or % of T_3 action ($T_3 = 100\%$); $n = 2$ (single values given), except for nos. 3, 9, 11 ($n = 3$; mean $\pm$ S.E.M.). All compounds are L-isomers, except no. 21, which is DL. Key: * 4'- CH_3T_3 ; ^bDL; ^c full agonist (refs. [23,44]); Ac, $-COCH_3$; IP, $-CH(CH_3)_2$; Py, pyridazinone; AG, anti-goitre test (ref. [19]); GPDH, induction of hepatic mitochondrial glycerol-3-phosphate dehydrogenase activity (refs. [23,44,45]).

No.	Name	R ₃	R ₅	X	R _{2'}	R _{3'}	R _{4'}	R _{5'}	ΔO_2 ($\mu\text{mol/min per g}$)	ΔO_2 (% T_3)	AG (% T_3)	GPDH (% T_3)
1	DIT	I	I	OH	—	—	—	—	0, 0	0		
2	T_1	I	H	O	H	H	OH	H	0.33, 0.13	18	< 0.1	
3	T_2	I	I	O	H	H	OH	H	0.82 ± 0.07	108	0.8	1.1
4	3,3'- T_2	I	H	O	H	I	OH	H	0, 0	0	0 ^b	
5	3',5'- T_2	H	H	O	H	I	OH	I	0, 0	0	< 0.1	
6	3',5'- CH_3T_2	I	I	O	H	CH_3	OH	CH_3	0.97, 0.79	117	9	
7	3'-Br T_2	I	I	O	H	Br	OH	H	0.55, 0.52	69	24	20
8	3',5'-Br T_2	I	I	O	H	Br	OH	Br	0.97, 0.91	125		
9	T_3	I	I	O	H	I	OH	H	0.76 ± 0.10	100	100	100
10	rT_3	I	H	O	H	I	OH	I	0, 0	0	< 0.1	
11	T_4	I	I	O	H	I	OH	I	0.73 ± 0.10	96	18	
12	3'-Ac T_2	I	I	O	H	Ac	OH	H	0.22, 0.32	31		121
13	3'-IP T_2	I	I	O	H	IP	OH	H	0.12, 0.24	18	142	290
14	Dibit	Br	Br	O	H	IP	OH	H	0.11, 0.33	24	30	
15	Dimit	CH_3	CH_3	O	H	IP	OH	H	0.46, 0.58	66	3.6	16
16	L-94901	Br	Br	O	H	Py	OH	H	0, 0	0		18
17	2'- CH_3T_2	I	I	O	CH_3	H	OH	H	0.85, 0.78	108	< 0.1 ^b	
18	2'- CH_3T_3	I	I	O	CH_3	H	OH	I	0.20, 0.12	15	< 0.1 ^b	
19	4'- CH_3T_2	I	I	O	H	H	OCH_3	H	0.26, 0.32	34	11.3 ^a	
20	S-bridge T_3	I	I	S	H	I	OH	H	0.72, 0.56	83	26.4 ^b	4.5
21	CH_2 -bridge T_3 ^b	I	I	CH_2	H	I	OH	H	0, 0	0	54.2 ^b	^{b,c}

Fig. 1. Effect of iodothyronines (T_2 ; 1 pM) on the O_2 consumption of isolated perfused livers of hypothyroid rats in the absence or presence of propylthiouracil (PTU; 1 μ M)

Data given are ΔO_2 above controls ($n = 7$); O_2 consumption at 0 min was $1.9 \pm 0.2 \mu\text{mol/min per g}$ ($n = 3$, mean $\pm$ S.E.M.). $\bullet$, T_2 ; $\blacksquare$, T_3 ; $\blacktriangle$, T_4 . * $P < 0.01$ versus T_3 , T_4 and controls; ** $P < 0.01$ versus perfusion without PTU.

Fig. 2. Effect of different concentrations of T_2 and T_3 on the O_2 consumption of isolated perfused livers of hypothyroid rats

Data given are ΔO_2 above controls ($n = 7$) at 90 min perfusion time; O_2 consumption at 0 min was $1.9 \pm 0.2 \mu\text{mol/min per g}$. Results are means $\pm$ S.E.M. (for $n = 3-6$), or single values are depicted for $n = 2$.

with [18]. Control perfusions with propylthiouracil alone revealed no influence of the inhibitor on Q_{O_2} . T_4 and T_3 lost all their stimulative activity, whereas T_2 was as potent as in the absence of propylthiouracil (Fig. 1). Thus T_2 can be perceived to be the sole thyroid hormone to stimulate hepatic Q_{O_2} rapidly and significantly.

Effect of thyroid-hormone analogues in the isolated perfused liver

A series of perfusion experiments with thyroid-hormone analogues was performed to confirm the specific T_2 effect (Table 1).

Those of the 3',5'-substituted 3,5-di-iodothyronine analogues with smaller substituents (CH_3 , Br; nos. 6-8) gave rise to a stimulation of Q_{O_2} comparable with that by T_2 , whereas larger substituents (I, acetyl, isopropyl; nos.

9, 12, 13) diminished the effect. Among the 3,3',5-substituted analogues, Dibit (no. 14) and Dimit (no. 15) stimulated Q_{O_2} to a considerable extent; L-94901 (no. 16), with its large pyridazinone substituent in the 3' position, was ineffective.

When rotation of the phenolic ring was prevented by 2'-methylation [19], the T_2 analogue (no. 17) was as active as T_2 itself, whereas the T_3 analogue (no. 18) hardly stimulated Q_{O_2} at all. 4'-Methylation (no. 19) also led to a remarkable loss of effectiveness. Replacement of the ether oxygen atom by a sulphur bridge (no. 20) slightly decreased the stimulative activity. The DL-methylene bridge analogue (no. 21) was ineffective.

No analogue with alterations in the side chain was as potent as the iodothyronines with an intact L-alanine side chain (Table 2). Among the acetic acid and ethylamine side-chain analogues, only di-iodothyroacetic acid (no.

Table 2. Effect of side-chain analogues (1 pM) of thyroid hormones on the O_2 consumption of isolated perfused livers of hypothyroid rats in comparison with 'anti-goitre activity' and induction of hepatic mitochondrial glycerol-3-phosphate dehydrogenase activity (from refs. [19,23,44])

Data given are ΔO_2 above controls ($n = 7$) at 90 min perfusion time (O_2 consumption at 0 min was $1.9 \pm 0.2 \mu\text{mol/min per g}$) or % of T_3 action ($T_3 = 100\%$); $n = 2$ (single values given). Nos. 29 and 30 are L-isomers. Key: *Triam; ^bDL; ^cfull agonist (refs. [23,44]); Ac, $-\text{COCH}_3$; AG, anti-goitre test (ref. [19]); GPDH, induction of hepatic mitochondrial glycerol-3-phosphate dehydrogenase activity (refs. [23,44]).

No.	Name	R ₁	R ₃	R ₅	ΔO_2 ($\mu\text{mol/min per g}$)	ΔO_2 (% T_3)	AG (% T_3)	GPDH (% T_3)
22	D- T_2	D-Alanine	H	H	0.14, 0.17	23		
23	D- T_3	D-Alanine	I	H	0, 0	0	7.5	44
24	Triprop	$\text{CH}_2\text{CH}_2\text{CO}_2\text{H}$	I	H	0.40, 0.46	54	4.5	1.9
25	Diac	$\text{CH}_2\text{CO}_2\text{H}$	H	H	0.17, 0.25	23	< 0.5	
26	Triac	$\text{CH}_2\text{CO}_2\text{H}$	I	H	0.14, 0	0	6.5	^c
27	Tetrac	$\text{CH}_2\text{CO}_2\text{H}$	I	I	0.23, 0	8	9	
28	Diam	$\text{CH}_2\text{CH}_2\text{NH}_2$	H	H	0.06, 0.12	6	2 ^a	
29	N-Ac T_2	$\text{CH}_2\text{CH}(\text{NHAc})\text{CO}_2\text{H}$	H	H	0.28, 0.24	30		
30	N-Ac T_4	$\text{CH}_2\text{CH}(\text{NHAc})\text{CO}_2\text{OH}$	I	I	0.24, 0.32	32	4.3 ^b

Fig. 3. Effect of T_3 ($1 \mu\text{M}$) on the O_2 consumption of isolated perfused livers of hypothyroid rats in the presence of cycloheximide ($20 \mu\text{g/ml}$)

Data give are ΔO_2 during perfusion; O_2 consumption at 0 min was $1.9 \pm 0.2 \mu\text{mol/min per g}$. Single values of four experiments are given. $\circ$, Control; $\bullet$, T_3 .

25) showed a noteworthy effect. Alterations at the amino acid group (nos. 24, 29, 30) led to a loss, but not to abolition, of stimulation. The isomeric D- T_2 (no. 22) and D- T_3 (no. 23) were barely active or inactive.

Effect of cycloheximide in the isolated perfused liver

It is generally accepted that iodothyronines exert their action on intermediary metabolism by increasing the synthesis of specific proteins via the nuclear pathway [20]. In order to show that ongoing protein synthesis is not involved in the rapid T_3 effect on hepatic Q_{O_2} , protein synthesis was inhibited by the addition of cycloheximide [21,22]. The effect of T_3 was still apparent (Fig. 3).

Effect of T_2 on malic enzyme induction *in vivo*

Only a little information is available on T_2 effects on increasing the synthesis of specific enzymes by the nuclear pathway [19,23]. Therefore, the T_2 effect on the induction of hepatic malic enzyme activity as a very sensitive parameter of thyroid-hormone action was measured. T_2 was injected intraperitoneally into hypothyroid rats fed on a carbohydrate-rich diet, at a daily dose of $50 \mu\text{g}/100 \text{ g}$ body wt. for 3 days. This led to a minimal increase in malic enzyme activity (0.29 ± 0.04 munit/g of liver above control values of 0.32 ± 0.05 munit/g). In comparison, a similar dose of T_3 increased malic enzyme activity by 2.6 ± 0.2 munits/g above controls, as could be expected from data in the literature [24].

DISCUSSION

The essential finding of the present study is the demonstration that T_2 is an additional metabolically active thyroid hormone that rapidly increases mitochondrial Q_{O_2} at a concentration as low as 1 pM . T_2 appears to act on mitochondria via a direct pathway.

Besides T_2 , application of T_3 and T_4 resulted in an increased Q_{O_2} . The different time courses and the

propylthiouracil experiments (Fig. 1) demonstrate that T_4 and T_3 must be considered as 'preprohormone' and 'prohormone' for the rapidly acting hormone T_2 . This conclusion is ascertained by the experimental series with thyroid-hormone analogues (Tables 1 and 2). The volumes [23] of the phenolic-ring substituents of T_2 analogues can easily be correlated with the rapid mitochondrial and long-term nuclear effects: H ($0.03 \text{ cm}^3/\text{mol}$; no. 3), CH_3 (0.14 ; no. 6) and Br (0.15 ; nos. 7, 8) rapidly stimulate hepatic Q_{O_2} , but are less effective or ineffective on nuclear-mediated effects. Conversely, the larger substituents I (0.20 ; no. 9), acetyl (0.25 ; no. 12), and isopropyl (0.34 ; no. 13) are less effective at the mitochondrial, but quite effective at the nuclear pathway. The charges of the substituents will also play a considerable role in the effectiveness, as they lead to alterations in the conformation of the molecules. The effect of Dimit (no. 15: 66%) in comparison to 3'-isopropyl- T_2 (no. 13: 18%) and Dibit (no. 14: 24%) might result from this phenomenon.

2'-Methylation leads to a fixed proximal 5' position [19]. As 2'- CH_3T_3 (no. 18) is a poor inhibitor of T_4 5'-deiodination [25], it might be suggested that it is hardly deiodinated. In fact, this compound failed to stimulate hepatic Q_{O_2} as did T_3 in the presence of propylthiouracil (Fig. 1).

The sulphur-bridge analogue (no. 20) had little effect, and the methylene-bridge analogue (no. 21) was found to be a full agonist, on the nuclear pathway, whereas the contrary was discovered for the rapid stimulation of hepatic Q_{O_2} . The inhibitory concentration for T_4 5'-deiodination of methylene-bridged T_4 is very high, but that of sulphur-bridged T_4 is comparable with that of T_4 itself [25]. It can be concluded from these data that in the present study the sulphur-bridged T_3 was deiodinated and thus able to stimulate Q_{O_2} , whereas the methylene-bridged T_3 was not deiodinated, and consequently was without effect.

The results of the experiments with the side-chain analogues (Table 2) also demonstrate potential differences in their mechanism of action: whereas D- T_3 (no. 23) and tri-iodothyroacetic acid (no. 26) stimulate mitochondrial glycerol-3-phosphate dehydrogenase activity, they are inactive in increasing O_2 consumption. Hepatic Q_{O_2} is, on the other hand, rapidly stimulated by tri-iodopropionate (no. 24), di-iodothyroacetic acid (no. 25) and the *N*-acetyl derivatives (nos. 29 and 30), compounds with very low nuclear activity.

In summary, the experiments with thyroid-hormone analogues confirm the specificity of the T_2 effect on hepatic Q_{O_2} . The data also suggest that the mechanism of the T_2 effect may be distinct from that mediating enzyme induction in response to T_3 .

Target cell organelle

Thyroid hormones exert an effect not only on nuclei and mitochondria, but also at the cell membrane; they rapidly ($< 1 \text{ min}$) stimulate the uptake of sugar and Ca^{2+} into rat thymocytes [26,27] and of amino acids and Ca^{2+} into the liver [4,28]. In isolated erythrocyte membranes, iodothyronines and thyroid-hormone analogues (0.1 nM) stimulated Ca^{2+} -ATPase activity within 60 min [29]. This stimulation seems to be rather unspecific, because not only T_2 , T_3 and T_4 , but also 3,5-di-iodotyrosine, was effective. An increase in cytosolic Ca^{2+} should in turn increase the activities of some mitochondrial enzymes

(e.g. pyruvate dehydrogenase, isocitrate dehydrogenase and 2-oxoglutarate dehydrogenase) [30] and thus lead to an enhanced Q_{O_2} . Ca^{2+} -mediated effects on mitochondria, however, were found to be associated with an elevation of the intramitochondrial ATP/ADP ratio [31–33], whereas a significant decrease was observed after application of T_3 [11]. There is also a considerable lag period between the onset of Ca^{2+} influx (approx. 2 min) [26–28] and Q_{O_2} stimulation (approx. 20 min) ([28], and cf. Fig. 1). Ca^{2+} can thus be considered unlikely to be a messenger for the rapid T_2 effect on hepatic Q_{O_2} .

In order to exclude the participation of the nuclear pathway in the rapid Q_{O_2} effect, the following experiments were carried out. (i) T_3 was added to the perfusion system at 1 μM , a concentration known to induce, for example, phosphoenolpyruvate carboxykinase in isolated liver systems [34]. A rapid action on Q_{O_2} could still be detected in the presence of the inhibitor of protein biosynthesis, cycloheximide [21,22] (Fig. 3). (ii) Application of T_2 *in vivo* hardly induced hepatic malic enzyme activity (cf. the Results section). Furthermore, the fastest known nuclear effect described in the literature is the induction of the spot 14 mRNA, coding for a protein with so far unknown function [20]. The induction of this mRNA, however, is not apparent 30 min after application of T_3 [20], when the stimulation of Q_{O_2} in the isolated perfused liver is already significant (Fig. 1). Thus a participation of the nuclear pathway in the rapid stimulation of hepatic Q_{O_2} seems rather unlikely.

Preliminary findings with isolated liver mitochondria suggest that T_2 at 1 pM can produce a rapid stimulation of Q_{O_2} . It is noteworthy that this stimulation is very rapid, as there was no preincubation. It was reported previously that T_3 directly enhanced oxidative phosphorylation in submitochondrial particles [14] and increased ADP/O ratios in intact mitochondria [35]. These T_3 effects are presumably due to microsomal contaminations containing deiodinase and/or to T_2 impurities in the T_3 used. Palacios-Romero & Mowbray [9] found a direct T_3 effect on mitochondria only after incubation with liver homogenate, indicating that deiodination of T_3 to T_2 might be involved.

It can be assumed from the literature cited above and the data presented here that T_2 stimulates hepatic Q_{O_2} directly and specifically at the mitochondrial level. Uptake and transport of T_2 in the liver as well as deiodination of T_4 and T_3 to T_2 seem to take time, as can be deduced from the time differences between the effects in isolated mitochondria and the isolated perfused liver on one hand, and between the three effective iodothyronines on the other. It should be investigated whether other cell organelles (e.g. plasma membrane, cytosol [36]) contribute to the effect observed in the isolated 'perfused' liver.

Conclusions

T_2 has so far been considered as an intermediate product in the degradative pathways of T_3 . 5'-Deiodination of T_3 to T_2 contributes to only about 5% of T_3 disposal [17]. Consequently serum T_2 concentrations in humans are low (0.4–5.3 ng/dl) compared with T_3 (116–147 ng/dl) [17,37]. Serum T_2 concentrations were inversely correlated with age [38] and were higher in men than in women, whose metabolic clearance and production rates of T_2 , however, are higher than those of

men [37]. In hyperthyroidism, a 4-fold increase in serum T_2 was observed [17,37].

In the present paper, iodothyronines at a concentration of 1 pM stimulated only Q_{O_2} of isolated perfused livers. At higher concentrations (10 nM–10 μM), T_3 was found to stimulate rapidly ATP-consuming processes such as lipogenesis [39], gluconeogenesis [4,28,40], and urea synthesis [4], as well as adenine nucleotide translocation from mitochondria to cytosol [11]. When the T_3 concentration was gradually decreased from 1 μM to 10 pM, a concomitant decrease in gluconeogenesis was detectable [40], indicating the metabolic significance of our finding.

If the rapid T_2 effect is present not only in the liver, but also in other organs (e.g. heart, muscle), T_2 might play a significant role in thermogenesis. Energy intake, for example, influences T_2 levels in humans: after eating a high-energy diet for several days, serum T_2 concentrations were 36% higher than after a low-energy diet, and the T_2 production rate was even doubled [41]. Thus T_2 might take part in the high-energy-induced thermogenesis observed in humans [42]. This phenomenon might also contribute to obesity. Deiodinase activity and serum free T_3 concentration were decreased in Zucker rats [43]; consequently, T_2 levels and T_2 -influenced mitochondrial activity should be lowered, thereby probably limiting energy consumption.

Taken together, a new scheme of regulation of mitochondrial activity can be concluded: the novel metabolically active thyroid hormone, T_2 , acts directly via the mitochondrial pathway in a short-term fashion, whereas T_3 exhibits its long-term effects via the nuclear pathway by the induction of certain mitochondrial proteins. It should be investigated whether this scheme of regulation is limited to the liver, or is also apparent in other organs.

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