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Effect of thyroidectomy and subsequent treatment with triiodothyronine on kidney mitochondrial oxidative phosphorylation in the rat

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Abstract. The effect of thyroidectomy (Tx) and subsequent treatment with triiodothyronine (T_3) on rat kidney mitochondrial oxidative phosphorylation was examined. Thyroidectomy resulted in lowering of state 3 respiration rates and cytochrome contents. Thyroidectomized animals administered with T_3 (20 μg/100 g body wt) resulted in the nonsynchronous stimulation of state 3 respiration rates in kidney mitochondria with glutamate, β-hydroxybutyrate, succinate and ascorbate+TMPD as substrates. Cytochrome contents were also elevated differentially. Increase in the state 4 respiration rates was transient and reversible. However, primary dehydrogenases were not generally altered in the Tx and T_3- treated Tx animals. The results thus indicate that the T_3-treatment to Tx animals brings about differential and nonsynchronous increase in the respiratory parameters and respiratory chain components of kidney mitochondria.

Keywords. Thyroidectomy; triiodothyronine; kidney mitochondria; respiratory parameters.

1. Introduction

Thyroid hormones influence multiple physiological functions such as cell growth and differentiation, protein synthesis and basal metabolic rate. Their effects, especially on the mitochondrial metabolic activities are well documented (Tata *et al* 1963; Tata 1964, 1966; Satav *et al* 1973; Rajwade *et al* 1975; Katyare *et al* 1977; Nunez 1988). Thus, hypothyroidism in general, results in decreased metabolic activities and the treatment of hypothyroid animals with physiologic doses of thyroid hormones restores these activities to an almost normal level (Tata *et al* 1963; Katyare *et al* 1970, 1977).

Earlier studies from our laboratory had shown that the thyroid hormone effects on mitochondrial metabolism were tissue-specific and brought out an interesting point that the kidney mitochondria were most sensitive to the hormonal action, whether it was thyroid deficiency (Rajwade *et al* 1975; Katyare *et al* 1977) or T_3-induced thyrotoxicosis (Satav and Katyare 1982). Additionally, it was also found that thyroid hormone deficiency caused a non-synchronous turnover of protein components in the kidney mitochondria (Rajwade *et al* 1975) i.e. the turnover of insoluble proteins and cytochrome c decreased without any changes being seen for the turnover of the 'other cytochromes' fraction (Rajwade *et al* 1975).

Since most of the earlier studies on thyroid hormone action on mitochondria reported in the literature deal with liver and muscle mitochondria (Tata 1964, 1966; Katyare *et al* 1970), it was of interest to examine the metabolic responses of kidney

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mitochondria to thyroid hormone treatment of thyroidectomized rats. Such studies assume importance in elucidating the tissue-specific action of thyroid hormone *e.g.*, the kidney nuclei possess practically the same number of binding sites for T_3 as the liver nuclei (Oppenheimer 1979), nevertheless, the response with respect to stimulation of phosphoenolpyruvate kinase is differential (Muller *et al* 1982; Sibrowski *et al* 1982; Muller and Seitz 1984).

Therefore, we have examined the time course of effects of a single injection of 3,3′, 5-triiodothyronine (T_3) at physiologic dose (Satav and Katyare 1981) to thyroidectomized rats on various parameters of energy metabolism of kidney mitochondria.

2. Materials and methods

2.1 *Animals and T_3-treatment*

Weanling male albino rats of Wistar strain were surgically thyroidectomized (Katyare *et al* 1970; Satav *et al* 1973; Satav and Katyare 1981) and used after 8–10 weeks for further studies. Thyroidectomized (Tx) animals received a single injection of T_3 at a dose of 20 μg/100g body weight as described earlier (Satav *et al* 1973). Control animals received only saline vehicle. The animals were killed at the end of 12, 24, 48 and 72h after hormone/saline administration and kidney cortex mitochondria were isolated and washed once (Satav and Katyare 1982). State 3 and state 4 respiration rates were measured using Clark-type oxygen electrode as described by Katyare and Satav (1989). Substrates used were: glutamate, β-hydroxybutyrate, succinate and ascorbate + TMPD; with latter two substrates, 1 μM rotenone was also employed (Satav and Katyare 1982).

2.2 *Enzyme assays*

Succinate dehydrogenase and glutamate dehydrogenase activities were measured as described earlier (Rajwade *et al* 1975; Katyare and Satav 1989). β-hydroxybutyrate dehydrogenase activity was measured in sonicated mitochondria as described by Katyare and Satav (1989). Mitochondrial cytochrome contents were determined following the methods described earlier (Satav and Katyare 1982). Protein was estimated by the method of Lowry *et al* (1951).

Fine chemicals were purchased from sources described in an earlier communication (Katyare and Satav 1989).

3. Results

The results of the effects of thyroidectomy (Tx) and T_3-treatment of Tx animals on state of 3 respiration rates with various substrates are given in figure 1. Figure 1A gives the respiration rates as per cent of control while figure 1B shows these values as per cent of Tx. It is clear that the state 3 respiration rates in isolated kidney mitochondria from Tx animals decreased from 36 to 51 % with four substrates used (figure 1A). After T_3-treatment to Tx animals, the respiration rates with glutamate and ascorbate + TMPD reached a value comparable to control on day 1 and then

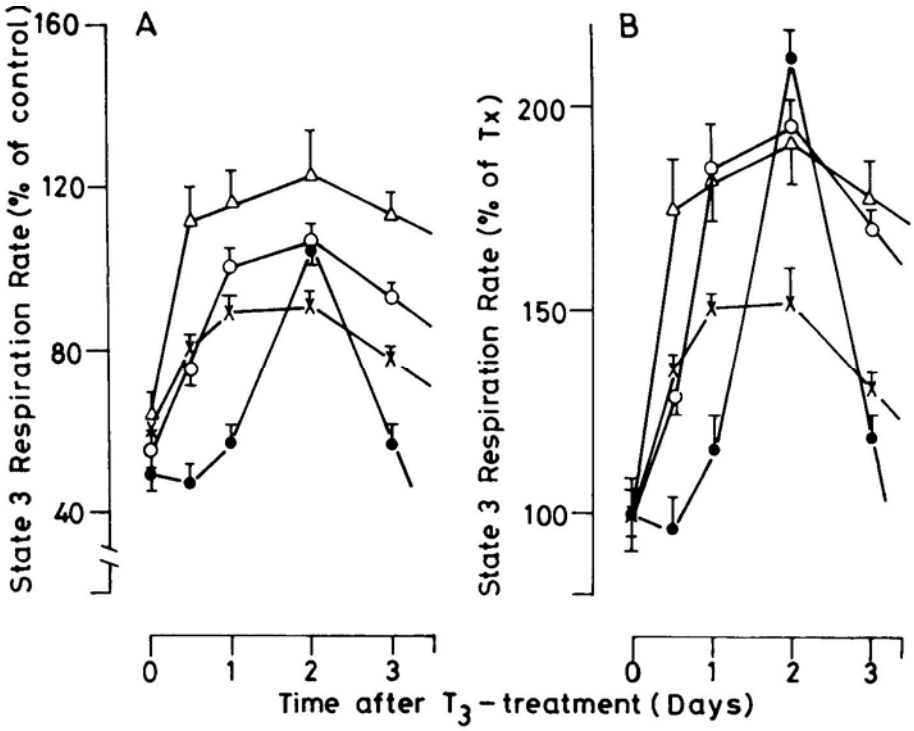

Figure 1. Effect of T_3-treatment to thyroidectomized (Tx) animals on state 3 respiration rates in kidney mitochondria.

Tx animals were injected with 20 μg T_3/100g body wt (s.c.) and were killed at various time intervals as indicated. Respiration rates are expressed as (**A**) per cent of control and (**B**) per cent of Tx. '0' time values represents the respiration rates in Tx. Substrates used: glutamate (O); β-hydroxybutyrate (●); succinate (×) and ascorbate + TMPD (Δ). Results and mean ± SEM of 10-18 observations. State 3 respiration rates in control for glutamate, β-hydroxybutyrate, succinate and ascorbate + TM PD were 40·2 ± 1·24; 30·81 ± 1·28; 119·43 ± 3·91 and 141·81 ± 17·78, nmol O_2/min/mg protein, respectively.

increased only slightly on day 2 and declined on day 3. For succinate, the control value was already reached within 24 h of T_3-treatment and at 72 h the value was still high as compared with control. For β-hydroxybutyrate the respiration was not affected up to 24 h, but reached the peak value comparable to control on day 2 and came back to Tx level on day 3. Thus, the patterns were quite different and specific for the substrate employed. These differences were further accentuated when the values were expressed as per cent of Tx. The profile for β-hydroxybutyrate was different as compared to the other substrates; a sharp peak on day 2 was seen with this substrate (figure 1B).

The pattern for state 4 respiration rates is shown in figure 2. When the respiration rates for various substrates were expressed as per cent of control (figure 2A), it was clear that the maximum stimulatory effect could be noted on day 1 after T_3 treatment; at 12 h post T_3-treatment respiration was stimulated only with glutamate and β-hydroxybutyrate. On day 2, the rates for these two substrates were still high (20 to 25 % higher) compared to the controls but declined to about 80% of the

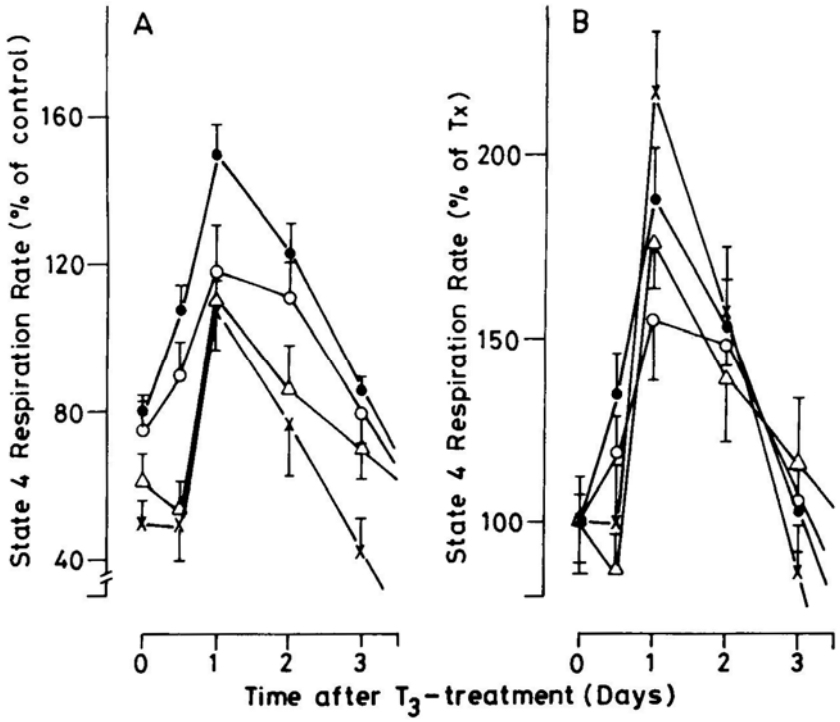

Figure 2. Effect of T_3-treatment to thyroidectomized (Tx) animals on state 4 respiration rates in kidney mitochondria.

Respiration rates are expressed as (**A**) per cent of control and (**B**) per cent of Tx. '0' time values represent the respiration rates in Tx animals. Results are given mean ± SEM of 10–20 observations. Other details are same as given in figure 1. Substrate used: glutamate (O); *β*-hydroxybutyrate (●); succinate (×) and ascorbate + TMPD (Δ). State 4 respiration rates in control mitochondria for glutamate, *β*-hydroxybutyrate, succinate and ascorbate+ TMPD were 6·93 ± 0·74; 2·56 ± 0·59; 31·73 ± 2·80 and 52·71 ± 10·90, nmol O_2/min/mg protein, respectively.

control value by day 3. For succinate and ascorbate + TMPD, the rates had already decreased further to the basal Tx value by day 3 of the T_3 treatment. The effects were comparable even when expressed as per cent of Tx (figure 2B).

The results on mitochondrial cytochrome contents are given in table 1. Thyroidectomy brought about 52% and 66% decrease respectively in cytochrome aa_3, b and c + c_1 content which is consistent with our earlier findings (Katyare *et al* 1977). After T_3-treatment of the Tx animal, contents of cytochrome aa_3 and b increased as early as 12 h reaching the peak value on day 1 (figure 3 A). These values then declined on day 2 and became comparable to those found at 12 h. The content of Cytochrome b then decreased on day 3 considerably while the values of aa_3 remained more or less at the same level without further decrease. Cytochrome c + c_1 content increased marginally up to 24 h and reached a peak value on day 2 of T_3-treatment to subsequently decline on day 3. It was thus clear that the patterns of accretion and the turnover profiles were also specific for the given cytochrome species. In this context it is interesting to note that in T_3-treated thyroidectomized rats, the profile for cytochromes accretion in liver and skeletal muscle mitochondria was found to be synchronous (Tata *et al* 1963; Tata 1964, 1966). It is also evident

Table 1. Effect of thyroid hormone deficiency on cytochrome content of kidney mitochondria.

	pmol of cytochrome/mg protein		
Cytochrome	Normal (7)	Tx (5)	Decrease (%)
aa_3	215 ± 11	103 ± 5*	52
b	273 ± 9	90 ± 30*	67
$c+c_1$	666 ± 26	229 ± 9*	66

Cytochromes were determined in Triton X-100 solubilized mitochondria from difference spectra of dithionite reduced minus ferricynide oxidized cytochromes.

Results are given as mean ± SEM of number of observations given in parentheses.

*$P < 0{\cdot}001$ compared with normal.

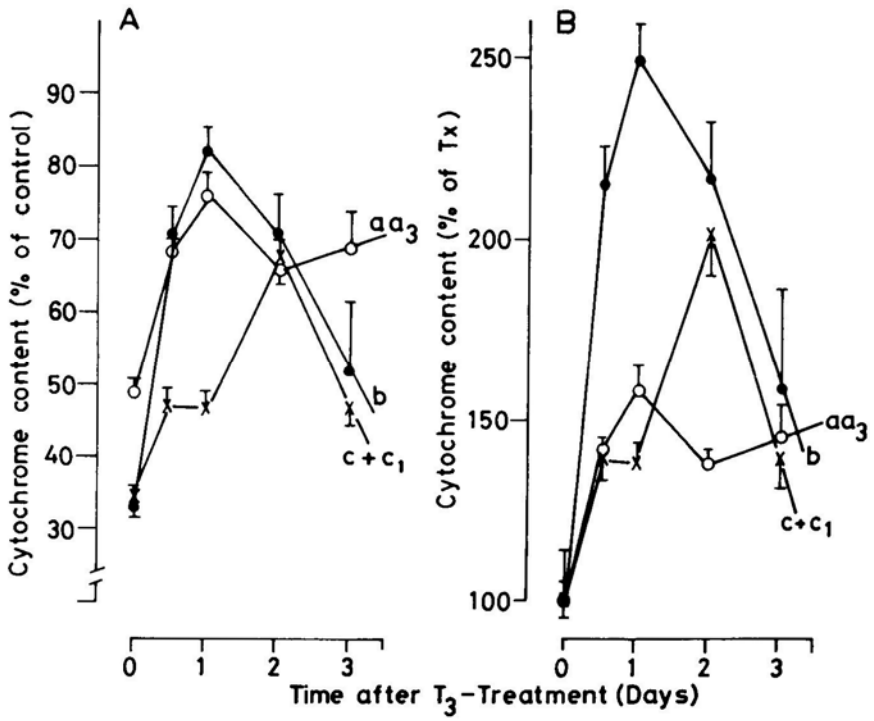

Figure 3. Changes in the cytochrome content of kidney mitochondria after T_3-treatment.

Cytochromes were determined as described in the text. Results are given as (**A**) per cent of control and (**B**) per cent of Tx. '0' time values represents the cytochrome content in kidney mitochondria from Tx animals. Cytochrome aa_3 (O), b (●) and $c + c_1$ (×). Results are mean ± SEM of 6–8 independent observations.

from figure 3 that although the contents of all the cytochromes were elevated, the values were still 25–30% lower than the euthyroid controls. When the patterns were represented as per cent of Tx, it was apparent that the maximum stimulation was seen in cytochrome b synthesis followed by cytochrome $c + c_1$; synthesis of aa_3 was least stimulated amongst these cytochrome classes (figure 3B).

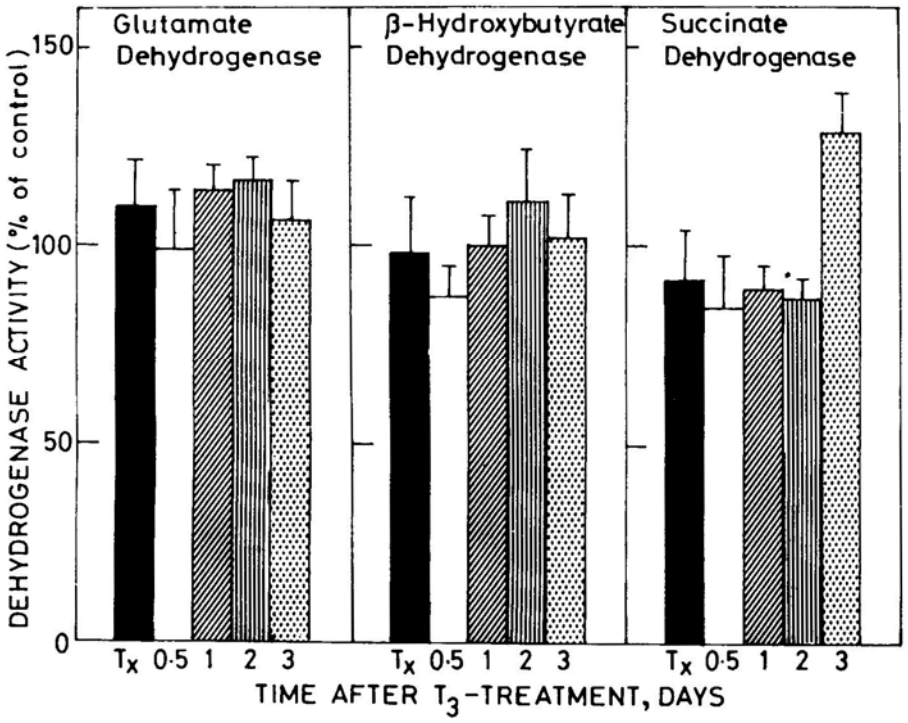

Figure 4. Changes in kidney mitochondrial dehydrogenases after T_3-treatment.

Enzyme activities were measured as described in text. Results are expressed as per cent of controls and are mean of 8 independent observations. 'Tx' represents the enzyme activities obtained in kidney mitochondria from thyroidectomized animals. Enzyme activities in control animals were: GDH: 52·47 ± 12·58 nmol NADH formed/min/mg protein; BDH: 219·74 ± 11·88 nmol NADH formed/min/mg protein; SDH:55·29 ± 7·60 nmol DCIP reduced/min/mg protein.

*$P < 0{\cdot}001$ compared with Tx or control.

We then examined the effects of thyroidectomy and T3-treatment on the primary dehydrogenase activities. It is apparent that the dehydrogenase activities did not change significantly except in the case of succinate dehydrogenase which registered a small but reproducible increase at 72 h post T_3-treatment (figure 4).

4. Discussion

Since the early studies of Barker (1956) and Pittman *et al* (1961), the kidney has been recognized as a thyroid hormone responsive tissue with respect to oxygen consumption. This agrees well with the good correlation between the thyromimetic action of the hormone metabolites and nuclear receptor binding sites in the tissues reported earlier (Oppenheimer 1979, 1983). It has thus been reported that the number of T_3-binding sites which the kidney nuclei possesses is almost comparable to those of liver (Oppenheimer 1979, 1983; Muller and Seitz 1984). However, much of the work on thyroid hormone effects on the mitochondrial metabolism has been carried out either with liver or with skeletal muscle mitochondria rather than with kidney (Tata *et al* 1963; Tata 1964, 1966). Few scattered studies on the kidney are available which relate to thyroid hormone deficiency (Katyare *et al* 1977; Rajwade

et al 1975), or where excessively high doses of thyroid hormones have been used (Hoch and Lipmann 1954; Maley and Lardy 1955; Satav and Katyare 1982) or thyroid powder was fed to animals (Kadenbach 1966). Besides, some of these studies deal mainly with kidney slices where a co-relationship between respiration and Na^+ + K^+-ATPase activity is sought (Somjen *et al* 1981). Occasionally, studies on thyroid-status dependent changes in Na^+ + K^+-ATPase activity are reported (Silva *et al* 1976).

Our interest in the thyroid hormone effects on kidney mitochondrial metabolism arose from our earlier observations that: (i) the kidney was the most sensitive tissue to the thyroid status (Rajwade *et al* 1975; Satav and Katyare 1982) and (ii) thyroid hormone deficiency caused a nonsynchronous turnover of kidney mitochondrial proteins (Rajwade *et al* 1975). Therefore it seemed most appropriate to find out in systematic studies if, as in the case of liver and skeletal muscle mitochondria (Gustafsson *et al* 1965; Gear 1970; Gross 1971; Rajwade *et al* 1975), thyroid hormones also constitute a factor for maintaining the synchrony of kidney mitochondrial functions.

The present results show that thyroid hormone deficiency resulted in impaired respiratory functions, but the effects were not uniform; rather they were substrate-specific (*e.g.* figures 1 and 2). Also, the contents of cytochromes decreased in a differential manner (table 1 and figure 3). Subsequently when the Tx animals were given T_3, the mitochondrial functions were restored more or less to normalcy, but once again the effects were differential and interestingly, non-synchronous. It would thus seem that the T3-treatment triggered a series of non-synchronous events leading to a stimulation of respiration in kidney mitochondria. The results thus point out that the thyroid hormone effects on mitochondrial functions in the kidney are different from those described for liver and skeletal muscle mitochondria (Gustafsson *et al* 1965; Gear 1970; Gross 1971; Rajwade *et al* 1975). This observation is consistent with our earlier findings on nonsynchronous turnover of kidney mitochondrial protein components (Rajwade *et al* 1975). Such an observation is not altogether unexpected since thyroid hormone action is known to be tissue-specific (Katyare *et al* 1977; Nikodem *et al* 1981; Satav and Katyare 1981, 1982).

The tissue-specific action of thyroid hormones has been explained partly on the basis of the number of nuclear binding sites present in the responsive tissues (Oppenheimer 1979, 1983). Although, the mechanism of thyroid hormone action is not yet clearly understood, there exists a good correlationship between the number of nuclear T3-receptors and thyromimetic action of hormone metabolites (Oppenheimer 1979, 1983; Muller *et al* 1982). It is believed that the early action of T_3 may be the regulation of synthesis of rapidly turning over mRNA (Seeling *et al* 1982) whose translational product secondarily exerts effects on DNA-dependent RNA polymerase, thus, regulating the synthesis of specific mRNA species (Muller and Seitz 1984; Mutvei and Nelson 1989). Recently, in a model system using rat liver nuclei, it has been shown that in thyroidectomized rats, 102 out of 500 proteins disappeared, but 13 reappeared at 6 h after thyroid hormone administration (Nikodem *et al* 1981; Muller and Seitz 1984); 67 additional proteins could be detected 24 h later (Nikodem *et al* 1981). Similar studies on mitochondrial proteins especially in the kidney should be interesting.

While the presence of nuclear receptors is well documented (Oppenheimer 1979, 1983), the thyroid hormone action at the target cell level seems to be pleiotypic

(Muller and Seitz 1984). Thus, the presence of thyroid hormone receptors, or at least binding sites in mitochondria, plasma membranes and cytosol has been reported (Oppenheimer 1983; Muller and Seitz 1984; Nunez 1988). Although the mitochondrial receptor is a controversial matter, it still deserves a comment. The presence of a specific binding site for thyroid hormones in mitochondria from various organs *i.e.* liver, kidney, intestine, heart, lung, skeletal muscle, etc. has been well described (Oppenheimer 1979; Hashizume and Ichikawa 1982; Muller and Seitz 1984; Nunez 1988). Interestingly, in kidney mitochondria, it has been reported that there are four and two binding sites, respectively, for the outer and inner mitochondrial membranes (Hashizume and Ichikawa 1982). Other workers, however, could demonstrate only non-specific binding sites on the outer membrane (Wahl *et al* 1977), while others failed to demonstrate a specific mitochondrial binding protein for T_3 (Greif and Sloane 1978). Interestingly, there is an isolated report by Shivakumar and Jayaraman (1986) claiming that the fish gill mitochondria have a T_4-specific receptor. The functional significance of the putative mitchondrial receptor is obscure, although it has been implicated in the early effects of thyroid hormones on mitochondrial respiration (Mutvei and Nelson 1989; Sterling 1986). Recently, Sterling (1986) has shown that T_3 at nM concentrations can stimulate respiration in mitoplasts from rat liver under in *vitro* conditions.

The earliest time point in the present studies was 12 h post T_3-treatment. Besides, physiological and not nM concentrations of T_3 were used in these studies. The effects we observed here are therefore not the early effects reported by others (Sterling 1986) but relate mostly to specific gene activation by thyroid hormones. In this connection the reported presence of totally six different binding sites for T_3 on kidney mitochondrial membranes seems to be interesting and deserves further elucidation (Hashizume and Ichikawa 1982).

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