MECHANISM OF TRIIODOTHYRONINE STIMULATION ON MICROSOMAL FATTY ACID CHAIN ELONGATION SYNTHESIS IN RAT LIVER

G. V. GNONI, C. LANDRISCINA and E. QUAGLIARIELLO

Centro di Studio sui Mitocondri e Metabolismo Energetico presso l'Istituto di Chimica Biologica dell'Università di Bari, Via Amendola 165/A, 70126 Bari, Italy

Received 31 July 1978

1. Introduction

Thyroid hormones have been found to influence several enzymatic activities [1–3]. Concerning lipid metabolism the hyperthyroid state is associated with increased oxidation of cholesterol [4] and fatty acids [5]. At the same time in these conditions an enhanced activity in acetate incorporation into lipids has been observed [6,7]. This rise in the liver is essentially due to a strong increase in both acetyl-CoA carboxylase and fatty acid synthetase activities [8–10]. As for the latter system Roncari and Murthy [9], by using specific antibodies, have proposed that the effect of L-thyroxine is mediated through an increase in enzyme level.

Recently we have reported strong stimulation of rat liver microsomal chain elongation of fatty acids in addition to acetyl-CoA carboxylase and fatty acid synthetase in hyperthyroidism [11]. The purpose of the present investigation was to determine the details of the hormonal effect at the molecular level in the microsomal system. Liver microsomal fatty acid chain elongation synthesis activity was therefore measured both in triiodothyronine and in triiodothyronine plus cycloheximide or actinomycin D-treated rats. Results obtained provide evidence for the involvement of the hormone in the stimulation of synthesis of microsomal enzymes responsible for fatty acid chain elongation.

2. Materials and methods

2.1. *Animals*

Triiodothyronine (30 μg/100 g body wt/day), dissolved in 0.9% NaCl–propyleneglycol (v/v), was injected intraperitoneally in male Wistar rats weighing 150–160 g, maintained on standard diet. Cycloheximide or actinomycin D, dissolved in the same solvent, was injected i.p. alone or with triiodothyronine, at the dose of 20 μg and 10 μg/100 g body wt/day, respectively. Control rats received only 0.9% NaCl–propyleneglycol (v/v). The animals were killed by decapitation 24 h after final administration. All rats were fed ad libitum. The weight of treated animals did not significant differ from that of controls throughout.

2.2. *Preparation of subcellular fractions*

The livers were immediately removed, minced in 10 ml icecold 0.25 M sucrose/g tissue and homogenized by mean of a Potter-Elvehjem homogenizer with a teflon pestle. All the preparations were carried out at 0°C. Microsomes and cell sap were prepared as in [11]. Protein was determined by the biuret method [12].

2.3. *Incubation procedure*

The assay for chain elongation of fatty acids by rat liver microsomes was carried out at 37°C for 10 min under N_2 with constant shaking. Substrate and cofactor concentration was as in [11]. The reaction was started by adding the subcellular fraction after flushing the sealed incubation vessels with N_2 for 10 min. The incorporation of [1,3-^{14}C]malonyl-CoA into fatty acids by crude liver cell sap was accomplished as in [11]. $(NH_4)_2SO_4$ precipitated cell sap (40% saturation) was used for assaying acetyl-CoA carboxylase [13].

2.4. Radioactivity measurement

At the end of the incubation time, the reaction was stopped, fatty acids extracted and their total radioactivity determined as in [11].

3. Results and discussion

The effect of hyperthyroidism on rat liver microsomal fatty acid chain elongation synthesis has been studied [11]. Figure 1 shows that after 3 daily intraperitoneal triiodothyronine administrations to normal rats, specific activity almost doubles, rising from 0.32–0.65 nmol [1,3-^{14}C]malonyl-CoA incorporated into fatty acids/min/mg protein and increasing by 184% after 5 days of hormone administration. These results are significantly different from those recently

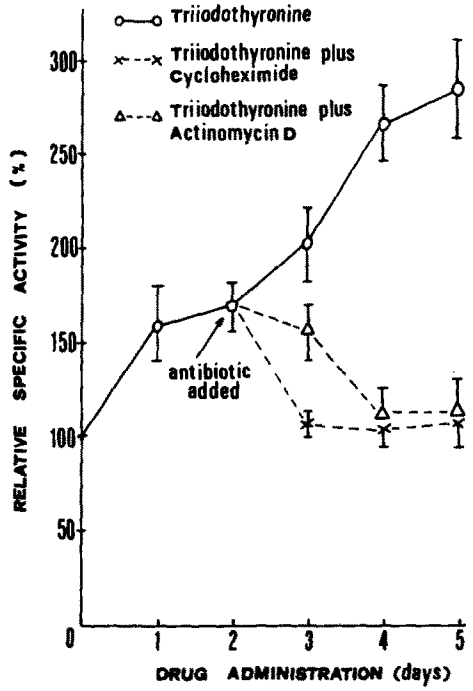

Fig.1. Liver microsomal fatty acid chain elongation synthesis following cycloheximide or actinomycin D in hyperthyroid rats. All animals received triiodothyronine for the entire period. When indicated, cycloheximide or actinomycin D was administered from the day 2 on. Rats were sacrificed at the times shown, i.e., 24 h after final administration. The verticle bars represent standard deviations. Microsomal protein, 0.45 mg.

found [14], where in vitro observed only very low stimulation of microsomal fatty acid chain elongation activity by the thyroid hormone. It is worthwhile to underline that incubation conditions used were as much as possible under anaerobiosis in order to minimize microsomal desaturation and thus study only fatty acid chain elongation in the hyperthyroid state.

Roncari and Murthy [9] have proposed that increased activity of soluble rat liver fatty acid synthetase in hyperthyroidism is essentially due to an increased level of enzyme protein. Tata [2] on the other hand has provided evidence that increased protein synthesis following triiodothyronine administration is preceeded by stimulation of RNA synthesis. Since actinomycin D and cycloheximide have been currently used as in vivo inhibitors of both RNA and protein synthesis [15–18], to clarify the mechanism of microsomal fatty acid chain elongation stimulation by thyroid hormones either of these antibiotics in addition to the hormone was injected for 3 days to rats to which only triiodothyronine had been previously administered for 2 days. The results of fig.1 show that already after 1 day of antibiotic administration, the increase of [1,3-^{14}C]malonyl-CoA incorporation into fatty acids due to the hormone was strongly reduced, especially in the case of cycloheximide. In any case, either with cycloheximide or actinomycin D, the triiodothyronine-mediated stimulation of microsomal fatty acid chain elongation activity is almost completely abolished after 2 days antibiotic administration. It must be specified that some experiments (data not shown), in which only cycloheximide or actinomycin D was given to rats at the same dose as in conjuction with triiodothyronine, indicated an activity of microsomal chain elongation after 1 day administration about 52% less than in untreated animals. In contrast, it is worth pointing out that when the antibiotic was administered together with triiodothyronine [1,3-^{14}C]malonyl-CoA incorporation still showed values slightly higher than that for control rats (cf. fig.1). The reduction of triiodothyronine-induced increase in microsomal fatty acid chain elongation activity consequent to antibiotic administration could thus be ascribed to its antagonizing effect on protein synthesis as a consequence of inhibition of RNA synthesis. These results are in line with [2,16] where triiodothyronine-stimulation

Table 1
Effect of cycloheximide or actinomycin D on liver acetyl-CoA carboxylase in triiodothyronine-treated rats

Rats	Spec. act. (nmol [1-^{14}C]malonyl-CoA formed/min/mg protein)	Relative spec. act. (%)
Control	1.35 ± 0.18	100
Triiodothyronine-treated	5.46 ± 0.46	405
Triiodothyronine-treated + cycloheximide	1.68 ± 0.15	125
Triiodothyronine-treated + actinomycin D	1.05 ± 0.09	78

Data: the mean value ± SD of 3 pairs of livers pooled each. Triiodothyronine was injected intraperitoneally for 5 days. Cycloheximide or actinomycin D was administered in conjunction with triiodothyronine for 3 days to the animals which had previously received only triiodothyronine for 2 days. $(NH_4)_2SO_4$ precipitated cell sap protein (40% saturation) was used as enzyme source. Protein, 0.23 mg

of RNA synthesis in thyroidectomized rats was reduced by actinomycin D, giving the antibiotic at the dose of 8 μg/100 g body wt, an amount similar to that we used in our experiments.

Table 1 shows the activity of rat liver acetyl-CoA carboxylase (EC 6.4.1.2) in hyperthyroid rats and following antibiotic administration. As is evident from this table a strong stimulation (305%) of the specific activity of acetyl-CoA carboxylase can be observed in rats to which triiodothyronine was administered. This stimulation almost disappears when cycloheximide or actinomycin D was injected in addition to the hormone. The same behaviour is shown by soluble fatty acid synthetase from rat liver. In fact from table 2 it appears that de novo fatty acid synthesis from [1,3-^{14}C]malonyl-CoA by cell

Table 2
Effect of cycloheximide or actinomycin D on liver fatty acid synthetase in triiodothyronine-treated rats

Rats	Spec. act. (nmol [1,3-^{14}C]malonyl-CoA incorporated into fatty acids/min/mg protein)	Relative spec. act. (%)
Control	0.45 ± 0.03	100
Triiodothyronine-treated	1.49 ± 0.18	331
Triiodothyronine-treated + cycloheximide	0.46 ± 0.05	102
Triiodothyronine-treated + actinomycin D	0.37 ± 0.04	82

Data the mean value ± SD of 3 pairs of livers pooled each. Triiodothyronine, cycloheximide and actinomycin D were administered as indicated in table 1. Crude cell sap protein, 0.35 mg

sap is greatly increased (231%) in the hyperthyroid state. The administration of cycloheximide or actinomycin D associated with triiodothyronine strongly reduces the stimulated incorporation of the labelled precursor due to the hormone.

The observed increase in both acetyl-CoA carboxylase and fatty acid synthetase activities displayed in the hyperthyroid state is in agreement with [8–10]. Reduced stimulation in the presence of cycloheximide or actinomycin D strongly supports the hypothesis that hyperthyroidism is responsible for a higher amount of these hepatic lipogenic enzymes [10] analogously to gluconeogenic ones [19,20]. Moreover similarities in the behaviour of acetyl-CoA carboxylase, fatty acid synthetase and microsomal fatty acid chain elongation system to hormone administration suggest that these may be linked as a unit by a common control mechanism.

The fact that the induction of the enzyme synthesis responsible for the microsomal fatty acid chain elongation system observed in triiodothyronine-treated animals is clearly antagonized by cycloheximide or actinomycin D, well-known inhibitors of protein synthesis, suggests that, analogously to acetyl-CoA carboxylase and fatty acid synthetase [8–10], increased microsomal fatty acid synthesis in this state is probably due to an increase in de novo enzymic protein synthesis and not to activation of pre-existing enzymes. Lastly we wish to note that the present data well agree with the proposals [21,22] where, in the hyperthyroid state, a higher glycerophosphate acylating enzyme level occurs both in liver and heart. Then in this condition a larger amount of saturated and unsaturated long chain fatty acids would be available in microsomes for the enhanced esterifying activity.

Acknowledgment

The authors are grateful to Vito Petragallo for his valuable assistance in this project.

References

[1] Babior, B. M., Creagan, S., S. H. and Kipnes, R. S. (1973) Proc. Natl. Acad. Sci. USA 70, 98–102.
[2] Tata, J. R. (1970) in: Biochemical Action of Hormones (Litwach, G. ed) pp. 89–133, Academic Press, New York.
[3] Evans, T. C. and Hoch, F. L. (1976) Biochem. Biophys. Res. Commun. 69, 635–640.
[4] Bressler, R. and Wittels, B. (1966) J. Clin. Invest. 45, 1326–1333.
[5] Danielsson, H. (1963) Adv. Lipid Res. 1, 335–385.
[6] Dayton, S., Dayton, J., Drimmer, F. and Kendall, F. E. (1960) Am. J. Physiol. 199, 71–76.
[7] Gompertz, D. and Greenbaum, A. L. (1966) Biochim. Biophys. Acta 116, 441–459.
[8] Diamant, S., Gorin, E. and Shafrir, E. (1972) Eur. J. Biochem. 26, 553–559.
[9] Roncari, D. A. K. and Murthy, V. K. (1975) J. Biol. Chem. 250, 4134–4138.
[10] Kumar, S., Das, D. K., Dorfman, A. E. and Asato, N. (1971) Arch. Biochem. Biophys. 178, 507–516.
[11] Landriscina, C., Gnoni, G. V. and Quagliariello, E. (1976) Eur. J. Biochem. 71, 135–143.
[12] Gornall, A. G., Bardawill, C. J. and David, M. M. (1949) J. Biol. Chem. 177, 751–766.
[13] Quagliariello, E., Landriscina, C. and Coratelli, P. (1968) Biochim. Biophys. Acta 164, 12–24.
[14] Faas, F. H., Carter, W. J. and Wynn, J. O. (1977) Arch. Biochem. Biophys. 182, 71–81.
[15] Young, C. W., Robinson, P. F. and Sacktor, B. (1963) Biochem. Pharmacol. 12, 855–865.
[16] Tata, J. R. and Widnell, C. C. (1966) Biochem. J. 98, 604–620.
[17] Becker, F. F. and Brenowitz, J. B. (1970) Biochem. Pharmacol. 19, 1457–1462.
[18] Viarengo, A., Zoncheddu, A., Accomando, R. and Molinari, M. P. (1975) Ital. J. Biochem. 24, 231–239.
[19] Bottger, I., Kriegel, H. and Wieland, O. (1970) Eur. J. Biochem. 13, 253–257.
[20] Singh, S. P. and Snyder, A. K. (1978) Endocrinology 102, 182–187.
[21] Young, D. L. and Lynen, F. (1969) J. Biol. Chem. 244, 377–383.
[22] Kako, K. J. and Liu, M. S. (1974) FEBS Lett. 39, 243–246.