Regulation of cytosolic 3-hydroxy-3-methylglutaryl-CoA synthase mRNA levels by L-tri-iodothyronine

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In hypophysectomized and thyroidectomized rats, cytosolic 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) synthase activity, immunoreactive protein and mRNA levels were all considerably decreased. Administration of L-tri-iodothyronine (T$_3$) resulted in a large increase in all three in hypophysectomized rats and in only a 2-fold increase (reaching the values of control rats) in thyroidectomized rats.

INTRODUCTION

The synthesis of cholesterol from acetyl-CoA is subject to feedback suppression by the sterol end-products of the pathway. It has been demonstrated by various authors that sterols or enzymically formed oxygenated cholesterol derivatives [1–3] suppress the cholesterol biosynthetic pathway by means of a decrease in the activities of several rate-limiting enzymes, two of the most important of which are 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase [4] and HMG-CoA synthase [5–7].

Two distinct forms of HMG-CoA synthase are found in liver: a mitochondrial form and a cytoplasmic form. The HMG-CoA produced by the mitochondrial enzyme is largely converted by HMG-CoA lyase into acetoacetate and then into other ketone bodies. The HMG-CoA produced by the cytoplasmic enzyme acts as a substrate for HMG-CoA reductase [8–10].

Many studies have reported about the regulation of the liver HMG-CoA reductase activity by dietary and hormonal signals. The best studied regulatory process is the low-density-lipoprotein-mediated feedback system [11]. It has been shown that the mechanism responsible for the decrease in reductase activity caused by this system is double: suppressed transcription of the gene [12] and accelerated degradation of the reductase protein [13]. Several studies have been carried out on the influence of thyroid hormones on transcription of the gene and stability of the protein in hypophysectomized and thyroidectomized rats [14,15].

The gene for the cytosolic HMG-CoA synthase is also under transcriptional control by oxysterols [16]. Mehrabian et al. [17] have described the influence of cholesterol and two different drugs on transcription and translation of this gene, but there is no report on the influence of hormones on the fate of this enzyme.

We have studied the effect of L-tri-iodothyronine (T$_3$) on liver cytosolic HMG-CoA synthase in hypophysectomized or thyroidectomized rats, determining the changes induced in mRNA levels, protein mass and activity of this enzyme by hormonal treatment.

MATERIALS AND METHODS

Animals

Hypophysectomized and thyroidectomized male Wistar rats weighing 150–175 g at the time of surgery were obtained from Panlab, Barcelona, Spain. Hypophysectomized, thyroidectomized and normal rats were housed in a reverse-cycle light-controlled room with a 12 h light period followed by a 12 h dark period for 15 days before the initiation of the experiment, and were fed ad libitum. Animals ate normally in accordance with their condition and were alert and active. L-Tri-iodothyronine was given as a single intraperitoneal injection of 100 μg/100 g body wt. This dose is sufficient to saturate nearly 90% of the nuclear T$_3$ receptors. At times indicated in the Figures, the animals were killed by decapitation at the same hour of the cycle (7 h of the dark period). The treatments were performed in triplicate, as were controls.

Materials

[1-^{14}C]Acetyl-CoA (59.3 mCi/mmol) was from New England Nuclear, and ^{125}I-labelled Protein A was from Amersham. Acetoacetyl-CoA, acetyl-CoA and T$_3$ were from Sigma. DEAE-cellulose DE-32 was obtained from Whatman; BA 83 nitro-cellulose paper was from Schleicher and Schuell; blotting membranes were from Bio-Rad.

Cytosolic HMG-CoA synthase assay

A modification of the radiochemical method of Clinkenbeard et al. [10] was used to measure the synthesis of [^{14}C]HMG-CoA. The standard reaction mixture contained the following component, in a final volume of 0.2 ml: 0.1 M Tris/HCl; 20 μM acetoacetyl-CoA; 0.1 mM EDTA; 20 mM MgCl$_2$; 20 μg of cytosolic protein, partially purified by DEAE-cellulose fractionation; and 0.2 mM [1-^{14}C]acetyl-CoA (4000 c.p.m./nmol), pH 8.0. All subsequent steps were performed as mentioned in reference [18].

Immunoblotting analysis of cytosolic HMG-CoA synthase protein

This was carried out as described by Beisiegel et al. [19], with minor modifications. The antibodies used were obtained against synthetic peptides corresponding to amino acid sequences of hamster cytosolic HMG-CoA synthase [20,21]. These antibodies
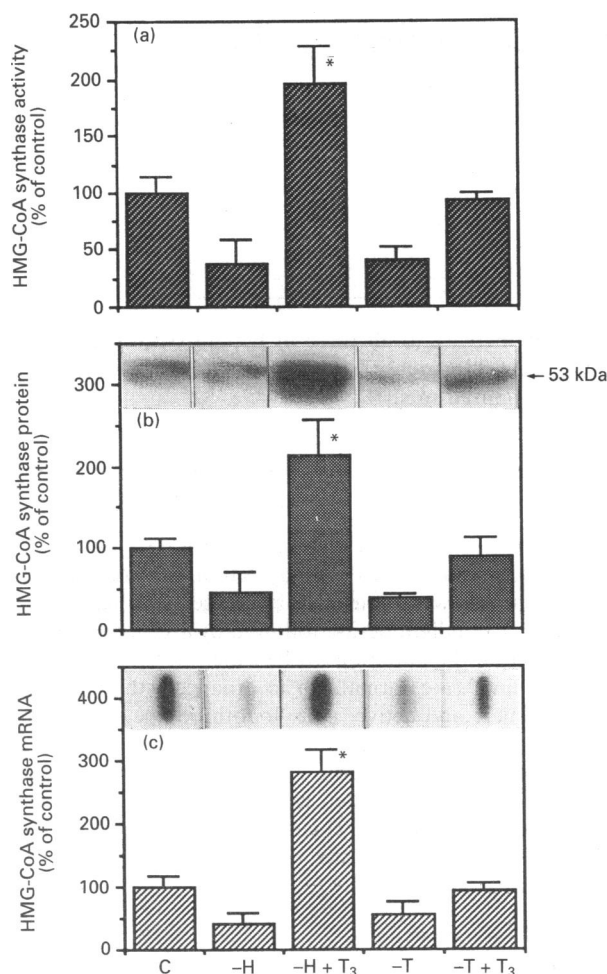

Figure 1 Quantitative comparison of cytosolic HMG-CoA synthase specific activity and levels of cytosolic HMG-CoA synthase protein and mRNA in hypophysectomized or thyroidectomized rats treated or not with T₃

Hypophysectomized (−H) or thyroidectomized (−T) rats were injected intraperitoneally with a single dose of T_3 and killed 72 h later, and liver cytosol and total RNA were prepared. Cytosolic HMG-CoA synthase activity was determined as described in the Materials and methods section. Levels of cytosolic HMG-CoA synthase protein and mRNA were quantified by densitometric scanning of Western and slot-blot analysis respectively; values corresponding to normal rats (C) were taken as 100% for the purpose of comparison (0.8 ± 0.11 m-unit for HMG-CoA synthase activity, and arbitrary units for protein and mRNA levels). Data are means ± S.D. from at least three different experiments: *$P < 0.05$ for difference between control and treated animals. The inset in (b) shows a representative Western-blot experiment in which the same amount of protein (100 μg) was applied to each lane. Arrow indicates the molecular mass (kDa) of cytosolic HMG-CoA synthase. The inset in (c) shows a representative slot-blot analysis in which 0.6 μg of total RNA was loaded in each slot.

specifically recognized a single band of protein with a molecular mass of 53 kDa [18]. The 53 kDa band corresponds exclusively to the cytosolic synthase, because our antibodies did not cross-react with mitochondrial HMG-CoA synthase [18].

RNA analysis

Total RNA from rat liver was isolated as described [22], with minor modifications. Slot-blotting was performed by the manufacturer's recommendations (Zeta-Probe Blotting Membranes; Bio-Rad). To measure the cytosolic HMG-CoA synthase mRNA by slot-blotting analysis, 0.6 μg of RNA was loaded in each slot and then transferred to a nylon membrane and probed with the ^{32}P-labelled 1.1 kb fragment (ApaI–SacI) of

the rat liver cytosolic HMG-CoA synthase cDNA [23]. The probe used was specific for rat liver cytosolic synthase [18]. To quantify a large number of samples, we decided to use the slot-blot analysis because of its simplicity and high sensitivity with a minimum quantity of RNA. To confirm that the RNA obtained was not degraded, 1–2 μg samples were fractionated on 1% agarose gel with ethidium bromide and made visible by u.v. radiation.

RESULTS

First we examined the effect of hypophysectomy and thyroidectomy on rat liver cytosolic HMG-CoA synthase by measuring mRNA levels, protein and enzymic activity. As shown in Figure 1, the two treatments decreased these parameters to approx. 50% of their value in control rats. The strong correlation between mRNA and protein levels and enzymic activity in hypophysectomized and thyroidectomized rats suggests that the absence of hormones of these organs has no influence on translational or post-translational processes.

The effect of T_3 treatment on levels of immunoreactive cytosolic HMG-CoA synthase, mRNA levels and enzymic activity was then examined in different groups of hypophysectomized and thyroidectomized rats at 72 h of treatment (Figure 1). This period was chosen since it is the optimum time in which T_3 influences HMG-CoA reductase mRNA and protein levels in hypophysectomized and thyroidectomized rats. Hepatic HMG-CoA synthase levels increased only by 2-fold in thyroidectomized rats at 3 days after T_3 injection (reaching the values of control rats), whereas they increased 7-fold in hypophysectomized rats. HMG-CoA synthase activity and protein correlated with the mRNA levels under this treatment.

In the second part of the study, we examined the effect of T_3 treatment on the cytosolic HMG-CoA synthase protein, enzyme activity and mRNA levels in livers of hypophysectomized rats after different periods of time after the hormone administration. As shown in Figure 2, the action of T_3 on the three parameters is produced almost without lag. At 24 h, values of mRNA were near those of control rats. Protein and enzymic activity showed less recovery with respect to non-treated hypophysectomized rats. These three parameters increased progressively up to 60 h after treatment and then began to decline. The maximum value reached was 13-fold for mRNA levels, 14-fold for protein and 14.6-fold for activity, suggesting that no control was exerted at the post-translational levels. These increases were not parallel at 48 h, when the mRNA increased 8-fold but protein and enzymic activity rose by only 3-fold.

DISCUSSION

The effect of thyroidectomy and hypophysectomy on HMG-CoA synthase resembles the same effect on HMG-CoA reductase, although in general, effects on the reductase are much greater than on the cytosolic synthase. The decrease in reductase mRNA in hypophysectomy is more than 22-fold [14], whereas that in synthase mRNA is only 2-fold. Analogous values are seen in thyroidectomy, from which it is concluded that in liver the absence of these hormones has less influence on synthase than on reductase mRNA levels.

Analogously, the influence of T_3 is not so marked on the cytosolic synthase as on the reductase. Under optimal conditions, mRNA levels are increased 14-fold for synthase and more than 40-fold for reductase, by the effect of T_3 in hypophysectomized rats. Similar increases were observed in immunodetectable HMG-
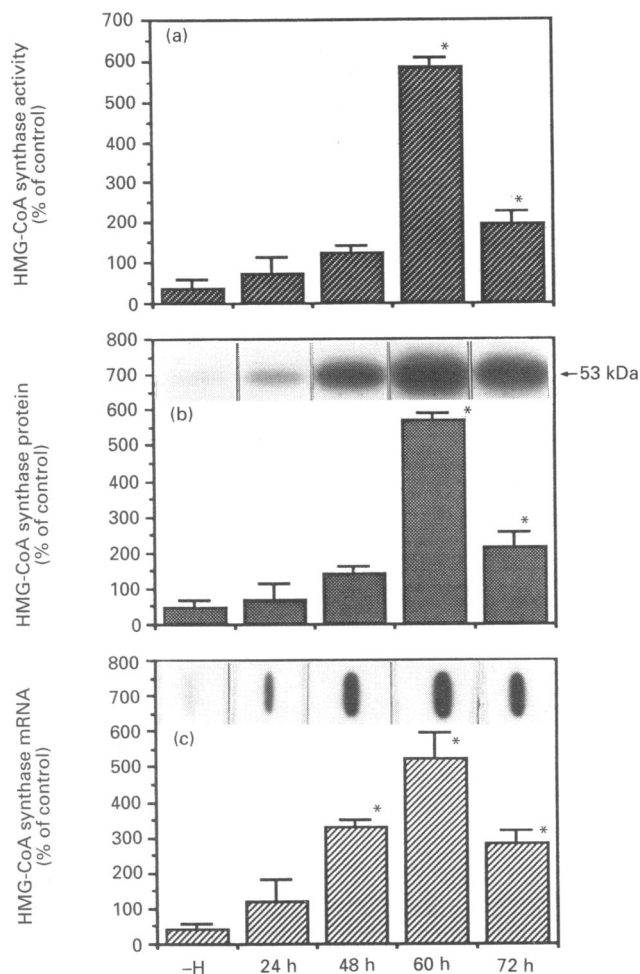
Figure 2   Time-dependent effect of T_3 administration on cytosolic HMG-CoA synthase specific activity, protein and mRNA levels in hypophysectomized rats

Liver cytosol and total RNA was prepared from hypophysectomized (— H) rats at the indicated periods of time after the administration of a single dose of T_3. HMG-CoA synthase activity, protein and mRNA levels were determined as described in Figure 1. Data are means ± S.D. for at least three different experiments: $*P < 0.05$ for difference between control and treated animals. Insets in the Figure show representative Western and slot-blot experiments. The same amount of protein (100 μg, except for the lane corresponding to the 60 h period, in which 50 μg were used) and total RNA (0.6 μg) were used for each experiment.

CoA synthase protein and enzymic activity compared with those of HMG-CoA reductase.

It is noteworthy, however, that the time of response to T_3 is shorter for synthase than for reductase. We have shown that in 24 h there is a 2-fold increase in mRNA levels for synthase, whereas as shown by Simonet and Ness [14], there is no change for HMG-CoA reductase. The time in which maximal re-activation was achieved is also different: 60 h for synthase and 72 h for reductase [14].

The decreased effectiveness of thyroid hormones in stimulating expression of the cytosolic HMG-CoA synthase gene in thyroidectomized rats compared with hypophysectomized rats suggests that glucocorticoids may be physiologically relevant regulators of thyroid-stimulated synthase gene expression. In rats which produce normal levels of glucocorticoids, the increase in synthase mRNA and activity in response to thyroid hormone treatment was at least 71 % lower than in animals (hypo-

physectomized rats) not secreting adrenal hormones. This observation agrees with results obtained with the same effectors on HMG-CoA reductase [15].

These results seem to confirm the role of HMG-CoA as a major effector of mevalonate synthesis. We have previously shown that cytosolic HMG-CoA synthase has a circadian rhythm similar to that of HMG-CoA reductase, but with the peak delayed by 4 h [18]. When protein levels and enzymic HMG-CoA synthase activity were low, HMG-CoA reductase was at its maximal activity, and when these parameters for synthase were maximal, those of HMG-CoA reductase were declining. Gibbons et al. [8] reached the same conclusion with rat hepatocytes treated with insulin and glucagon, showing that HMG-CoA present in the cytoplasmic compartment is the main factor responsible for the increase in HMG-CoA reductase activity, and that this was an inverse correlation. These circumstances also resulted in changes in cholesterol biosynthesis.

We have shown here that hypophysectomy does not abolish the expression or activity of liver cytosolic HMG-CoA synthase, from which it is concluded that HMG-CoA is synthesized at a decreased (50 %) but measurable level. At this time HMG-CoA reductase is minimal. Under the influence of T_3, there is an increase in synthase and reductase expression, although the latter is delayed with respect to the former by 36 h. When the influence of T_3 on expression of reductase is maximal, mRNA and protein levels, and enzymic activity for synthase have already begun to decline. It seems that the HMG-CoA levels produced by HMG-CoA synthase activity modulate the reductase expression and activity.

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