Hormonal Regulation of Lipogenic Enzymes in Chick Embryo Hepatocytes in Culture

EXPRESSION OF THE FATTY ACID SYNTHASE GENE IS REGULATED AT BOTH TRANSLATIONAL AND PRETRANSLATIONAL STEPS*

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Mechanisms involved in the multihormonal regulation of fatty acid synthase have been investigated by comparing levels of its mRNA with rates of enzyme synthesis in chick embryo hepatocytes in culture. Triiodothyronine or insulin caused about a 2.5-fold increase in the relative rate of synthesis of fatty acid synthase. Together, these hormones were synergistic, stimulating enzyme synthesis by nearly 40-fold (Fischer, P. W. F., and Goodridge, A. G. (1978) Arch. Biochem. Biophys. 190, 332-344). Addition of triiodothyronine stimulated increases in mRNA levels comparable to increases in enzyme synthesis whether insulin was present or not. Thus, triiodothyronine regulates fatty acid synthase primarily by controlling the amount of its mRNA. Addition of insulin, in the presence of triiodothyronine, stimulated enzyme synthesis by 14-fold and mRNA levels by only 2-fold. In the absence of triiodothyronine, insulin had no effect on mRNA levels. Thus, insulin has a major effect on the translation of fatty acid synthase mRNA.

After the addition of triiodothyronine, fatty acid synthase mRNA accumulated with sigmoidal kinetics, approaching a new steady state about 48 h after the addition of hormone. Puromycin, an inhibitor of protein synthesis, blocked the effect of triiodothyronine. We suggest that the abundances of both fatty acid synthase and malic enzyme mRNAs are regulated by a common triiodothyronine-induced peptide intermediate which has a relatively long half-life.

Glucagon caused an 80% decrease in the synthesis of fatty acid synthase (Fischer, P. W. F., and Goodridge, A. G. (1978) Arch. Biochem. Biophys. 190, 332-344) and a 60% decrease in the level of fatty acid synthase mRNA. Thus, glucagon regulates fatty acid synthase by controlling the concentration of its mRNA. The synthesis of malic enzyme also was inhibited by glucagon at a pretranslational step, but the inhibition was almost complete. Thus, despite coordinated regulation of the concentrations of these enzymes during starvation and refeeding, individual hormones sometimes regulate synthesis of the two enzymes at the same step and to about the same degree and sometimes at different steps or to very different degrees.

The synthesis of fatty acid synthase is regulated by nutritional status and by insulin, thyroid hormone, and glucagon (Zehner et al., 1977; Fischer and Goodridge, 1978). In vivo, starvation inhibits and refeeding stimulates the synthesis of fatty acid synthase, primarily by regulating the rate of transcription of the fatty acid synthase gene (Morris et al., 1982, 1984; Goodridge et al., 1984; Back et al., 1986b). Malic enzyme (EC 1.1.1.40) is another of the "lipogenic" enzymes which are stimulated by feeding and inhibited by starvation. In vivo, the concentration of malic enzyme is regulated at both transcriptional and post-transcriptional steps (Tepperman and Tepperman, 1964; Silpananta and Goodridge, 1971; Winberry et al., 1983; Morris et al., 1984; Goodridge et al., 1984; Goldman et al., 1985).

In hepatocytes in tissue culture, thyroid hormone alone stimulates synthesis of malic enzyme by 30- to 50-fold, whereas insulin alone has little effect and, in the presence of thyroid hormone, augments the synthesis rate by 2- or 3-fold (Goodridge and Adelman, 1976). By contrast, stimulation of the synthesis of fatty acid synthase required both hormones; thyroid hormone alone had little effect (Fischer and Goodridge, 1978). Furthermore, glucagon inhibited the synthesis of malic enzyme much more than that of fatty acid synthase (Goodridge and Adelman, 1976; Fischer and Goodridge, 1978). Regulation of the synthesis of malic enzyme by these hormones is exerted primarily at a pretranslational step and, in the case of thyroid hormone, appears to involve a protein intermediate (Winberry et al., 1983; Back et al., 1986a). The objectives of this study were: 1) to determine if hormonal regulation of synthesis of fatty acid synthase occurred at translational or pretranslational steps and 2) to assess the involvement of a protein intermediate in the stimulation of fatty acid synthase concentration by triiodothyronine.

EXPERIMENTAL PROCEDURES

Preparation and maintenance of isolated cells, isolation of RNA, quantitation of mRNA, source of materials, and statistical analyses of the results were carried out as described (Back et al., 1986a) except that hybridizations were carried out at 37 °C and the washes in 50% formamide at 38 °C. M13GFA3B, the template for preparing single-stranded 32P-labeled cDNA, is a subclone of a previously characterized plasmid clone, pGFAS3 (Morris et al., 1982; Back et al., 1986b). Fatty acid synthase activity was measured in soluble extracts of hepatocytes as previously described (Goodridge, 1972).

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RESULTS AND DISCUSSION

The Actions of Insulin and Triiodothyronine—Triiodothyronine caused a 5-fold increase in the concentration of fatty acid synthase mRNA and a 3-fold increase in enzyme synthesis. In the presence of insulin, triiodothyronine was more potent, causing mRNA levels and enzyme synthesis to increase by 9- and 14-fold, respectively, with respect to cells incubated with insulin alone (Table I). Thus, triiodothyronine stimulated enzyme synthesis primarily by increasing mRNA abundance and did so more effectively in the presence of insulin than in its absence.

Insulin alone had no effect on the level of fatty acid synthase mRNA, but stimulated enzyme synthesis by 3-fold. If triiodothyronine was in the medium, however, insulin caused a 2-fold increase in mRNA concentration and a 14-fold increase in enzyme synthesis, with respect to cells incubated with triiodothyronine alone. Insulin, therefore, has a major effect on translation of pre-existing fatty acid synthase mRNA, an action which explains its synergistic effect with triiodothyronine on the synthesis of this enzyme. Insulin did not regulate the translation of pre-existing malic enzyme mRNA (Back et al., 1986a). Thus, triiodothyronine regulates the synthesis of both enzymes by regulating accumulation of their mRNAs, while insulin regulates the translational efficiency of the mRNA for fatty acid synthase, but not that for malic enzyme.

The measurements of enzyme synthesis were performed several years prior to the mRNA measurements, raising the possibility that the difference in the responses of enzyme synthesis and mRNA level to insulin was due not to differences in mechanism, but to differences in the animals or in the methods used to prepare and incubate the cells. However, the strain of chickens was the same, and the procedures for preparing and incubating the cells were identical. It is improbable, therefore, that the 2-fold versus a 14-fold difference \( (p < 0.02; \text{Mann-Whitney Test}) \) was due to differences in the animals or experimental procedures. Nevertheless, to be certain that hepatocytes assayed for mRNA levels responded in the same way as those prepared several years ago, we measured fatty acid synthase enzyme activity. Both sets of cells responded identically (Table I; Fischer and Goodridge, 1978).

Since enzyme activity and enzyme synthesis were regulated in parallel (Fischer and Goodridge, 1978), we conclude that the older enzyme synthesis data represent valid responses of these cells to insulin and triiodothyronine.

We next examined the time course for the accumulation of fatty acid synthase mRNA after addition of triiodothyronine. Since accumulation of fatty acid synthase mRNA was greater when insulin was present (Table 1), all of the subsequent experiments were performed with cells incubated for 2 days in medium containing insulin; insulin also was present throughout the experimental treatments. After triiodothyronine was added, the level of fatty acid synthase mRNA began to increase with no detectable lag period and, at 9 h, was 3 times that before adding hormone (Fig. 1, left panel). Fatty acid synthase mRNA continued to accumulate at about this rate for 24 h. After 24 h, the rate of accumulation of mRNA increased, approaching an apparent new steady state at 48 h, at which time the level of fatty acid synthase mRNA had increased 9- and 14-fold, respectively, with respect to cells incubated for 3 h with triiodothyronine \( \pm \text{S.E. of 3, 5, 3, and 2 h} \). An approximate value for time 0 was determined by averaging the values for control samples at 1.5, 3, 6, and 9 h, respectively. The results of all experiments were averaged together. Total RNA was isolated and the level of fatty acid synthase mRNA was determined as described under "Experimental Procedures." In the left panel, the results are expressed as a percentage of the value for cells incubated for 3 h with triiodothyronine \( \pm \text{S.E. of 3, 5, 3, and 2 h} \). An approximate value for time 0 was determined by averaging the values for control samples at 1.5, 3, and 6 h. In the right panel, the results are expressed as a percentage of the value for cells incubated for 24 h with triiodothyronine \( \pm \text{S.E. of 5 independent experiments except for the 36- and 48-h points which were 4 experiments each} \).
increased 47-fold with respect to time 0 (Fig. 1, right panel). Over the same time period, the abundance of fatty acid synthase mRNA increased almost 5-fold in cells incubated with insulin alone, so that, at 48 h, the increase due to triiodothyronine was 10-fold. The accumulation of mRNA in the control cells may be related to the increase in synthesis of fatty acid synthase which occurred in hepatocytes incubated in culture whether hormones were added or not (Fischer and Goodridge, 1978). Triiodothyronine caused the level of β-tubulin mRNA to decrease about 50% in a 48-h period (Back et al., 1986a). Thus, the triiodothyronine-stimulated accumulation of fatty acid synthase mRNA was evident whether expressed relative to total RNA or relative to β-tubulin mRNA, an mRNA which was little affected by the hormone.

The half-life of fatty acid synthase mRNA was estimated by measuring mRNA abundance as a function of time after adding inhibitors of transcription. The abundance of fatty acid synthase mRNA began to decrease immediately after adding actinomycin D, but not until about 3 h after adding α-amanitin (Fig. 2). The 3-h delay in α-amanitin-treated cells probably was due to slow uptake of the drug. Incorporation of [3H]Juridine into total RNA was inhibited maximally by actinomycin at 1.5 h (>90%), whereas α-amanitin required 3 h to achieve maximum inhibition (about 50%). Under the same conditions, inhibition of the synthesis of malic enzyme by α-amanitin had a similar lag period, followed by decay with first order kinetics from 3 h to 24 h after adding the drug (Goodridge and Adelman, 1976). Fatty acid synthase mRNA decayed with a half-life of 2.5 to 4 h in hepatocytes in culture (Fig. 2), similar to that estimated for this mRNA in the livers of intact ducklings (3 to 6 h; Back et al., 1986b).

The time required for the level of fatty acid synthase mRNA to reach a new steady state was long relative to this mRNA’s half-life. In addition, the kinetics of accumulation for fatty acid synthase mRNA were sigmoidal. These results suggested that an intermediate might be involved in the accumulation of fatty acid synthase mRNA caused by triiodothyronine. The involvement of a protein intermediate was tested by inhibiting total protein synthesis with puromycin. Incorporation of [3H]leucine into total protein was inhibited by 99% under the conditions used in these experiments (data not shown). Puromycin, added 30 min prior to time 0, caused a significant decrease in the level of fatty acid synthase mRNA in cells incubated with (Fig. 3, right panel) or without (Fig. 3, left panel) triiodothyronine. The rapid increase in fatty acid synthase mRNA caused by triiodothyronine was prevented completely by puromycin, but the drug had no effect on the level of β-tubulin mRNA (Back et al., 1986a). Since β-tubulin mRNA has a short half-life (Ben-Ze'ev et al., 1979; Stimac et al., 1984), a general effect of puromycin on the synthesis or degradation of all mRNA should have decreased the concen-
trations of both $\beta$-tubulin mRNA and fatty acid synthase mRNA to similar extents. The involvement of a peptide intermediate in the triiodothyronine-induced accumulation of fatty acid synthase mRNA also was tested in cells incubated for 24 h with triiodothyronine (Fig. 4). Puromycin resulted in cessation of the accumulation of fatty acid synthase mRNA with a lag time of less than 1.5 h, followed by a slow decrease in mRNA concentration. Addition of puromycin at 24 h after adding triiodothyronine did not cause a decrease in the level of $\beta$-tubulin mRNA (Back et al., 1986a). Ongoing protein synthesis, therefore, was required to allow triiodothyronine to exert its stimulatory effect on fatty acid synthase mRNA levels both early and late in the accumulation phase.

The response of fatty acid synthase mRNA to triiodothyronine is very similar to that of malic enzyme mRNA. For each of these mRNAs, the time course for accumulation of mRNA was sigmoidal. For both malic enzyme mRNA and fatty acid synthase mRNA, the triiodothyronine-induced accumulation was blocked by an inhibitor of protein synthesis (Back et al., 1986a). These results suggest that concentrations of both mRNAs may be regulated by proteins which are increased in amount by triiodothyronine. We suggest that the same triiodothyronine-induced protein may regulate the abundance of both mRNAs.

The Action of Glucagon—When glucagon was added to cells which were incubated for 3 days with insulin plus triiodothyronine, the rate of synthesis of fatty acid synthase and the levels of fatty acid synthase mRNA were inhibited by 80% and 60%, respectively (Table I). Thus, glucagon controls synthesis of this protein by regulating the abundance of its mRNA. When glucagon was added to cells incubated with triiodothyronine for 24 h, accumulation of fatty acid synthase mRNA came to steady state after a lag period of about 1.5 h (Fig. 4). Glucagon had either no effect or caused a rapid decrease in the concentrations of the mRNAs for $\beta$-tubulin and malic enzyme, respectively (Back et al., 1986a). The apparent steady-state concentration of fatty acid synthase mRNA in glucagon-treated cells was about 120% of the level at 24 h (Fig. 4) or about 33% of that attained at 48 h after adding triiodothyronine (cf. Fig. 1). Thus, the degree of inhibition is similar to that observed in freshly prepared cells incubated with glucagon for 3 days. Fatty acid synthase mRNA came to its new steady-state concentration shortly after the addition of glucagon, suggesting that the hormone rapidly alters activity of the process which controls the abundance of this mRNA.

Glucagon inhibits the accumulation of malic enzyme mRNA (93–98%; Back et al., 1986a) to a much greater degree than it inhibits accumulation of fatty acid synthase mRNA (60–70%). In the paper by Back et al., 1986a, we suggested that glucagon may control the activity of a putative intermediate protein involved in regulation of the concentration of malic enzyme mRNA by triiodothyronine, a protein which may regulate the level of fatty acid synthase mRNA. The discrepancy in the relative effectiveness of glucagon in regulating the levels of the two mRNAs has several potential interpretations, including the possibility that glucagon acts independently of the triiodothyronine-induced protein. Further experiments will be required to resolve this issue.

Inhibition of the accumulation of fatty acid synthase mRNA by glucagon in cells in culture (60 to 70%) is smaller than the inhibition caused by starvation in intact animals (about 90%; Morris et al., 1984; Back et al., 1986b). This means that increasing the glucagon concentration from 0 to 1 ng/ml in culture medium had a smaller effect on the concentration of fatty acid synthase mRNA than increasing the plasma concentration of glucagon from 100 to 40 (ducklings starved 48 h and refed 6 h, n = 3) to 220 ± 80 pg/ml (ducklings starved 48 h, n = 3). Glucagon, therefore, may not play an important role in the nutritional regulation of the level of fatty acid synthase mRNA.

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