Triliodothyronine Stimulates Transcription of the Fatty Acid Synthase Gene in Chick Embryo Hepatocytes in Culture

INSULIN AND INSULIN-LIKE GROWTH FACTOR AMPLIFY THAT EFFECT*

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The principal enzymes of fatty acid synthesis are acetyl-CoA carboxylase, a biotin-containing multifunctional enzyme that catalyzes the synthesis of the major precursor, malonyl-CoA, and fatty acid synthase, a multifunctional protein that synthesizes fatty acids using acetyl-CoA as a primer and malonyl-CoA for the elongating units. Animal fatty acid synthases obtained from bovine, human, rodent, and avian tissues are homodimers, each with an approximate subunit molecular weight of 260,000 (1, 2). Each subunit contains all of the catalytic sites required for palmitate synthesis, but only the dimer is active (1).

The levels and synthesis rates of avian hepatic fatty acid synthase are correlated during various nutritional and hormonal manipulations (3, 4). 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 (5). The effects of starvation and refeeding on hepatic fatty acid synthase are mimicked in chick embryo hepatocytes in culture in defined medium. The addition of insulin alone or triiodothyronine alone causes increases of 2- to 6-fold, but in fatty acid synthase activity over the course of a 3-day incubation (6-8). When insulin plus triiodothyronine are added to the medium, fatty acid synthase increases up to 20-fold over that in cells without hormone. When glucagon or cyclic AMP (6-8) are added in the presence of insulin plus triiodothyronine, the activity of fatty acid synthase is decreased 70-80%. The abundance of fatty acid synthase mRNA is regulated in parallel with the rate of enzyme synthesis, indicating that regulation is primarily pretranslational (8).

We show here that the increase in abundance of fatty acid synthase mRNA caused by triiodothyronine in chick embryo hepatocytes in culture is correlated with a quantitatively comparable increase in transcription of the fatty acid synthase gene; regulation is thus transcriptional. Insulin alone had no effect but amplified the stimulation of transcription caused by triiodothyronine; insulin also has a transcriptional action.

IGF-1 (Insulin-like growth factor 1) had the same effect as insulin, while glucagon or dibutyryl cyclic AMP had little or no effect on transcription of the gene for fatty acid synthase.

EXPERIMENTAL PROCEDURES

Materials—Crystalline bovine insulin (26.6 units/mg) and glucagon (1.05 units/mg) were obtained from Eli Lilly Corp. Proteinase K and human recombinant IGF-1 were purchased from Boehringer Mannheim. SP6-grade ([3P]UTP (800 Ci/mmol) and [32P]dCTP (800 Ci/mmol) were purchased from Amersham Corp. and ICN Biochemicals, respectively. Nucleotides used in the transcription assays were from Pharmacia LKB Biotechnology Inc. Unless specifically stated in the

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The abbreviations used are: IGF-1, insulin-like growth factor 1; EGTA, [ethylenebis(oxyethylenenitrilo)]tetraacetic acid; HEPES, (2-hydroxyethyl)-1-piperazineethanesulfonic acid; SDS, sodium dodecyl sulfate.
text, all other enzymes or hormones were from Sigma.

Preparation and Maintenance of Isolated Cells—Unincubated embryonated eggs from white Leghorn chickens were obtained from Hy Vac Laboratory Eggs, Gowrie, IA, and incubated in an electric forced-draft incubator at 37.5 ± 0.5 °C and 60% relative humidity. Embryos (18 or 19 days of incubation) were killed by decapitation. Liver cells were isolated (9) and suspended in Waymouth’s MD705/1 medium (Gibco Laboratories, Life Technologies, Inc., 1 ml of packed cells/9 ml of medium) containing penicillin (60 pg/ml) and streptomycin (100 µg/ml) and incubated in untreated 90-mm Petri dishes at 40 °C in an atmosphere of 95% air, 5% CO2. One milliliter of the cell suspension (2–3 mg of total protein, about 1 × 1010 cells) was incubated with insulin (50 µg/ml) in Waymouth’s medium. After about 20 h, the medium was changed. Hormones were added as indicated in the figure legends. Male enzyme activity (10), fatty acid synthase activity (11), and protein (12) were assayed by the indicated methods.

DNA Probes—The cDNA probes for chick fatty acid synthase were generously provided by Dr. Gordon G. Hammes (University of California, Santa Barbara) (13). FAS 1 and FAS 2 correspond to bases of the DNA for avian malic enzyme was isolated in our laboratory.1

Isolation and Measurement of mRNA Levels—After removing the medium, RNA was isolated from the cells by the guanidinium thiocyanate phenol chloroform method (15). Total RNA was treated with RNase-free DNase I (BRL). After 10 min at room temperature, the RNA was isolated as described under “Experimental Procedures.” This blot is representative of five identical experiments using different preparations of hepatocytes. A, transcription run-on assay. B, autoradiography of nuclear run-on transcription. C, Northern blot analysis of fatty acid synthase mRNA.

RESULTS AND DISCUSSION

Triiodothyronine Stimulates Transcription of the Fatty Acid Synthase Gene—Transcription of the fatty acid synthase gene was measured in nuclei isolated from chick embryo hepatocytes incubated in chemically defined media containing no hormones, insulin alone, or insulin plus triiodothyronine. If added, insulin was in the medium from the time the cells were put in culture to the time they were harvested (96 h). Triiodothyronine was added for 48 h, beginning at 48 h of culture. Activity of fatty acid synthase (7, 8) and abundance of its mRNA (Fig. 1B) did not increase appreciably in the presence of insulin alone. Addition of triiodothyronine in the presence of insulin caused an 8-fold increase in fatty acid synthase activity and a 6- to 10-fold increase in mRNA level relative to cells incubated with no hormone. In the presence of insulin, triiodothyronine also caused a 6- to 10-fold increase in transcription of the fatty acid synthase gene (Fig. 1A). Insulin alone had no effect on fatty acid synthase transcription (Fig. 1A). None of these treatments affected transcription of the β-actin gene, indicating that the effects were selective for the fatty acid synthase gene. These results indicate that insulin by itself does not stimulate mRNA accumulation or transcription and that the stimulation of enzyme activity caused by triiodothyronine is primarily transcriptional.

In the presence of insulin, accumulation of fatty acid synthase mRNA began within as little as 1 h after triiodothyronine was added (8). Triiodothyronine-stimulated transcription of fatty acid synthase followed a similar time course. In the presence of insulin, triiodothyronine-stimulated transcription was indicated in the legend to Table I and incubated in a chemically defined medium for 48 h with no hormones or with insulin. Then, no hormones (lane 1), insulin (300 ng/ml) (lane 2), or insulin plus triiodothyronine (1 µg/ml) (lane 3) were added, insulin was in the medium from the time the cells were put in culture to the time they were harvested (96 h). Total RNA or nuclei were isolated as described under “Experimental Procedures.” This blot is representative of five identical experiments using different preparations of hepatocytes.

Slot-blot filters for the nuclear transcription assays were prepared as follows. DNA (2 µg) was denatured in 0.4 N NaOH for 10 min at room temperature, transferred to ice, neutralized, adjusted to a final concentration of 6 X SSC, and “slotted” onto GeneScreen membranes as recommended by the manufacturer. The filter bound DNA was pre-hybridized for 6 h at 65 °C in a solution containing 10 mM HEPES, pH 7.5, 1% SDS, 10 mM EDTA, 0.3 M NaCl, 1 X Denhardt’s solution (21) (0.02% polyvinylpyrrolidone (M, 40,000), 0.02% ficoll (M, 40,000), 0.02% bovine serum albumin (fraction V)), and 250 µg/ml yeast tRNA. The labeled transcripts (20–30 × 106 cpm) were added and hybridization continued at 65 °C for 72 h. The filters were washed sequentially in 2 X SSC (1 X SSC, 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0) (4 times for 5 min each) at room temperature, 2 X SSC + 10 µg/ml RNase A (once for 15 min) at 37 °C, 0.3 X SSC, 1% SDS (once for 15 min) at 65 °C, and in 0.3 X SSC (once for 15 min) at room temperature. Autoradiography and densitometric scanning were as described above.

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Footnote:

1 D. A. Fantozzi and A. G. Goodridge, unpublished results.

Fig. 1. Triiodothyronine stimulates increased accumulation of fatty acid synthase mRNA and increased transcription of the fatty acid synthase gene. Hepatocytes were prepared as indicated in the legend to Table I and incubated in a chemically defined medium for 48 h with no hormones or with insulin. Then, no hormones (lane 1), insulin (300 ng/ml) (lane 2), or insulin plus triiodothyronine (1 µg/ml) (lane 3) were added for an additional 48 h. Total RNA or nuclei were isolated as described under “Experimental Procedures.” This blot is representative of five identical experiments using different preparations of hepatocytes. A, transcription run-on assay. B, autoradiography of nuclear run-on transcription. C, Northern blot analysis of fatty acid synthase mRNA.
occurred about 24 h after adding thyroid hormone. The maximal increase in the rate of transcription of the fatty acid synthase gene was about 10-fold and occurred about 24 h after adding triiodothyronine (data not illustrated).

The increase in transcription of fatty acid synthase within 1 h after adding triiodothyronine may implicate a direct effect of triiodothyronine on transcription of the fatty acid synthase gene. On the other hand, the further increase in transcription rate that occurs between 1 and 24 h could be due to indirect effects. For example, triiodothyronine may stimulate accumulation of a factor essential for transcription of this gene. Insulin also may stimulate accumulation of an important transcription factor or an increase in its activity. In the absence of insulin, triiodothyronine had no effect on transcription of the fatty acid synthase gene, even after 12 h with the hormone. Fatty acid synthase mRNA level was similarly unaffected up to 12 h (Fig. 3). By 48 h after adding triiodothyronine fatty acid synthase transcription had increased by 4- to 6-fold; this relative increase corresponded to that for mRNA accumulation (Fig. 3). Insulin thus acts at the transcriptional level to amplify the action of triiodothyronine. The long time required to manifest the insulin effect suggests that it may stimulate accumulation of a rate-limiting factor in the transcription of the fatty acid synthase gene. These triiodothyronine-induced changes in abundance of fatty acid synthase mRNA at 48 h of hormone addition were determined with Northern blots and confirm earlier work from our laboratory (8) that used a dot-blot analysis.

IGF-1 Mimics the Effect of Insulin on Transcription of the Fatty Acid Synthase Gene—Insulin and IGF-1 are members of a family of related polypeptide hormones. The amino acid sequence of IGF-1 is about 45% identical with that of insulin (22). As might be expected from the structural similarity, each of the receptors has some affinity for the other hormone as well (23). This prompted us to determine the effect of recombinant IGF-1 on the activity, mRNA level, and transcription of fatty acid synthase in chick embryo hepatocytes in culture. IGF-1 stimulated fatty acid synthase activity less than 2-fold when added by itself and amplified the triiodothyronine effect by more than 2-fold when added with that hormone (Table I). IGF-1 plus triiodothyronine stimulated an 8-fold increase in enzyme activity over cells incubated without hormones. The effects of IGF-1 on transcription and mRNA accumulation for fatty acid synthase also were similar to those of insulin. IGF-1 alone had little or no effect (Fig. 4). In combination with triiodothyronine, however, transcription was stimulated 3-fold within as little as 1 h and maximally induced (10- to 12-fold) after 24 h (Fig. 4). The same response was noted for accumulation of fatty acid synthase mRNA in the presence of IGF-1 and triiodothyronine (Fig. 4).

The maximum effect of IGF-1 was similar to or greater than that of insulin (data not illustrated). The maximum

T<sub>3</sub> Stimulates Transcription of Fatty Acid Synthase

![Fig. 2](image_url)  
**FIG. 2.** Time course of the effect of triiodothyronine fatty acid synthase (FAS) transcription in the presence of insulin. Hepatocytes were prepared and incubated in a chemically defined medium containing insulin (300 ng/ml). After about 20 h of incubation, the medium was changed to one of the same composition. Twenty-four hours later, triiodothyronine (1 μg/ml) was added. Nuclei were isolated as described under "Experimental Procedures" and used in the transcription run-on assay. The numbers indicate hours after adding triiodothyronine. This experiment was replicated 10 times. All samples on each strip were from the same hybridization; strips of the prints of the autoradiograph were reorganized to keep the same order in all figures.

![Fig. 3](image_url)  
**FIG. 3.** Stimulation of transcription of the fatty acid synthase (FAS) gene by triiodothyronine in the absence of insulin. Experimental conditions were the same as described in the legend to Fig. 2 except that insulin was omitted. After the indicated time with triiodothyronine, total RNA or nuclei were isolated and treated as indicated in the legend to Fig. 1. These cells were the same as those used in the experiment illustrated in Fig. 2, except that the autoradiograph was exposed for a longer time. The transcription and mRNA measurements were replicated 10 and 4 times, respectively. All samples on each strip were from the same hybridization; strips of the prints of the autoradiograph were reorganized to keep the same order in all figures.

![Fig. 4](image_url)  
**FIG. 4.** IGF-1 acts like insulin to amplify the effects of triiodothyronine on accumulation of fatty acid synthase (FAS) mRNA and of its gene. Hepatocytes were prepared and incubated in a chemically defined medium containing no hormone (NO) or IGF-1 (10 ng/ml) as indicated in the table legend. Triiodothyronine was added about 48 h later and either total RNA or nuclei isolated at the indicated hours after hormone addition. Hepatocytes incubated in medium without IGF-1 were harvested at time "zero." Transcription and RNA assays were carried out as described in the legend to Fig. 1. The transcription and mRNA measurements were replicated 3 and 4 times, respectively.

<table>
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<th>Additions</th>
<th>No triiodothyronine</th>
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</thead>
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<tr>
<td></td>
<td>millimolars/mg protein</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>5.0 ± 0.4</td>
<td>40 ± 6</td>
</tr>
<tr>
<td>IGF-1</td>
<td>8.5 ± 1.2</td>
<td>90 ± 12</td>
</tr>
<tr>
<td>IGF-1 + insulin</td>
<td>6.7 ± 3.1</td>
<td>86 ± 3</td>
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</table>

TABLE I

Insulin and IGF-1 have the same effects on fatty acid synthase activity in chick embryo hepatocytes in culture

Hepatocytes were isolated from the livers of 18- or 19-day-old chick embryos and incubated in chemically defined medium as described under "Experimental Procedures." No hormones were added when the cells were plated. At about 20 h of incubation, the medium was changed to contain no additions, insulin (300 ng/ml (5 x 10<sup>-8</sup> M)), or IGF-1 (10 ng/ml (1 x 10<sup>-7</sup> M)). Approximately 24 h later, triiodothyronine (1 μg/ml (1.5 x 10<sup>-8</sup> M)) was added. The cells were harvested 48 h later, and fatty acid synthase activity was assayed as described under "Experimental Procedures." Values are means ± S.E. of 10 experiments, except for cells incubated with insulin plus IGF-1 and with triiodothyronine plus insulin plus IGF-1 which had two and four experiments (± S.E.D.), respectively.
effects of the two hormones were not additive (Table I), suggesting action through similar intracellular mechanisms. In addition, the maximum effect of IGF-1 was achieved at 3% of the dose of insulin that gives a maximal response (cf. Table I and Ref. 24). Lack of additivity of the maximum responses to insulin and IGF-1 may be due to convergence of the two intracellular pathways prior to their interactions with the factors that regulate transcription of the fatty acid synthase gene. IGF-1 alone also had no effect on the activity of malic enzyme or the abundance of malic enzyme mRNA, but in the presence of triiodothyronine, IGF-1 stimulated both enzyme activity and mRNA level (data not illustrated). These are the same as the responses of malic enzyme activity and mRNA abundance to insulin with and without triiodothyronine (25). Based on dose-response curves for enzyme activity and mRNA abundance, the IGF-1 doses required for 50% of maximal effects on fatty acid synthase or malic enzyme were 1–5 ng/ml (1–7 × 10^{-6} M), whereas that for insulin on malic enzyme activity was about 250 ng/ml (4 × 10^{-6} M) (24). In liver membranes from chick embryos of the same age, the ED_{50} values of the IGF-1 receptor for binding of IGF-1 and insulin were about 8 and 250 ng/ml, respectively. Conversely, the ED_{50} values of the insulin receptor for binding of IGF-1 and insulin were about 100 and 2 ng/ml (28). Similarly, IGF-1 receptors measured intact chick embryo hepatocytes bind insulin with about 2% of the affinity of IGF-1 (27). In summary, our results and the binding results suggest that substantial parts of the effects of insulin on fatty acid synthase and malic enzyme may be mediated through the IGF-1 receptor.

Our earlier results (7, 8) indicated that triiodothyronine alone had only a small stimulatory effect on enzyme activity in the absence of insulin, even though abundance of fatty acid synthase mRNA was stimulated by triiodothyronine in the absence of insulin. In the present experiments, triiodothyronine clearly had a marked stimulatory effect on fatty acid synthase enzyme activity; mRNA abundance increased in parallel with enzyme activity. One significant difference in protocol for the present measurements of fatty acid synthase activity was incubation of the cells without any hormone for about 1 day before adding insulin or IGF-1 (2 days before adding triiodothyronine). In the earlier experiments, insulin or insulin plus triiodothyronine were present from the time the cells were plated. Although the exact nature of the discrepancy is unknown, it is clear that insulin and IGF-1 do stimulate transcription of the fatty acid synthase gene in triiodothyronine-treated hepatocytes, especially at early times after adding triiodothyronine. Thus, at least part of the stimulatory effects of insulin and IGF-1 on expression of fatty acid synthase are transcriptional.

The physiological significance of the effects of IGF-1 on fatty acid synthase and malic enzyme activities is not known. Note, however, that starvation lowers plasma IGF-1 concentration in rats and humans (28–30) and possibly chickens (31, 32). Thus, IGF-1 may be important in signaling the liver about changes in the state of alimentation of the whole animal, roles previously assigned in large part to insulin and glucagon.

Neither Glucagon nor Dibutyryl Cyclic AMP Inhibit Transcription of the Gene for Fatty Acid Synthase—In vivo, starvation decreases abundance of fatty acid synthase mRNA (5). The increased blood concentrations of glucagon in the starved state may be responsible, in part at least, for the starvation-induced decrease in fatty acid synthase. In chick embryo hepatocytes in culture with insulin and triiodothyronine, dibutyryl cyclic AMP decreased fatty acid synthase activity approximately 70% and mRNA levels approximately 50% (7, 8). Expression of fatty acid synthase is also inhibited by cyclic AMP in other systems. In the presence of insulin, treatment of mature 3T3-Ll cells with dibutyryl cyclic AMP caused a 60% decrease in accumulation for fatty acid synthase mRNA (33). If starved mice were injected with dibutyryl cyclic AMP at the same time that refeeding was initiated, accumulation of fatty acid synthase mRNA was inhibited by 90%. This latter decrease correlated with an inhibition of transcription of the gene (34).

The elevated rate of transcription of the fatty acid synthase gene caused by triiodothyronine was unaffected by cyclic AMP in chick embryo hepatocytes in culture (Fig. 5). Glucagon (1 μg/ml (3 × 10^{-3} M)) was equally ineffective (data not illustrated). A cloned genomic DNA for malic enzyme (ME) served as a positive control for the effects of dibutyryl cyclic AMP. This experiment was replicated 5 times. All samples on each strip were from the same hybridization; strips of the prints of the autoradiograph were reorganized to keep the same order in all figures.

**Fig. 5.** Dibutyryl cyclic AMP has no effect on transcription of the fatty acid synthase (FAS) gene. Hepatocytes were prepared and incubated in a chemically defined medium containing insulin (300 ng/ml). The medium was changed at about 20 h of incubation to one of the same composition. After an additional 24 h of incubation, either no additions (lane 1), triiodothyronine (1 μg/ml) (lane 2), or triiodothyronine + dibutyryl cyclic AMP (50 μM) (lane 3) were added. Twenty-four hours later the cells were harvested and nuclei isolated as described under "Experimental Procedures." A cloned genomic DNA for malic enzyme (ME) served as a positive control for the effects of dibutyryl cyclic AMP. This experiment was replicated 5 times. All samples on each strip were from the same hybridization; strips of the prints of the autoradiograph were reorganized to keep the same order in all figures.

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plasma hormones may regulate expression of the gene in question. Eventual isolation and characterization of the regulatory regions of the fatty acid synthase gene will permit a better understanding of the involved mechanisms.

REFERENCES

Triiodothyronine stimulates transcription of the fatty acid synthase gene in chick embryo hepatocytes in culture. Insulin and insulin-like growth factor amplify that effect.

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