Post-transcriptional Regulation of 3-Hydroxy-3-methylglutaryl-CoA Reductase mRNA in Rat Liver

GLUCOCORTICOIDS BLOCK THE STABILIZATION CAUSED BY THYROID HORMONES*

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Administration of dexamethasone to hypophysectomized rats treated with thyroid hormones blocked the increase in hepatic 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase mRNA and enzyme activity which occurred in response to thyroid hormone treatment. The rate of transcription of the rat liver HMG-CoA reductase gene measured by “run-on” assays in isolated nuclei was not diminished by dexamethasone. The half-life of HMG-CoA reductase mRNA was decreased from 12-15 to 2-3 h by dexamethasone treatment of hypophysectomized rats fed thyroid powder. Adrenalectomy caused the half-life of HMG-CoA reductase mRNA to increase from 3 to 10 h, suggesting that endogenous glucocorticoids also regulate reductase mRNA stability. Reductase mRNA levels were increased only 5-fold in thyroidectomized rats fed thyroid powder compared to a 20- to 40-fold increase in similarly treated hypophysectomized rats. In thyroidectomized rats, reductase mRNA had a half-life of only 1.5 h. Thyroid hormone treatment increased this to 4.5 h, significantly less than that of similarly treated hypophysectomized rats. Hydrocortisone, like dexamethasone, lowered reductase mRNA levels, but the biologically inactive analogue epihydrocortisone did not affect reductase mRNA or activity. These results suggest that glucocorticoids decrease the abundance of HMG-CoA reductase mRNA by stimulating its degradation.

The increase in reductase mRNA caused by treatment of hypophysectomized rats with thyroid hormones is attributable in part to an increase in the rate of transcription of the hepatic HMG-CoA reductase gene and in part to a stabilization of reductase mRNA (7).

In this communication, we report the effects of glucocorticoids on the levels of HMG-CoA reductase mRNA and activity in hypophysectomized rats treated with thyroid hormones. Our results suggest that glucocorticoids block the stabilizing effects of thyroid hormones on reductase mRNA, without affecting reductase gene transcription.

EXPERIMENTAL PROCEDURES

Animals—Hypophysectomized, adrenalectomized, and thyroparathyroidectomized male Sprague-Dawley rats were obtained from Harlan Industries, Madison, WI. They were housed in a reverse cycle, light-controlled room with a 14-h light period followed by a 10-h dark period. The animals were fed Purina Rodent Laboratory Chow 5001 ad libitum. Where indicated, rats were fed ground chow containing either 2% colestipol and 0.04% mevinolin, and/or 0.5% desiccated porcine thyroid powder as indicated. The inclusion of thyroid powder in the diet provided a continuous supply of hormone in order to achieve a steady state of induction. Hypophysectomized rats were used 14-24 days after surgery when they weighed 125-150 g. Adrenalectomized and thyroidectomized rats were used 10-14 days after surgery when they weighed about 200 g. Adrenalectomized rats were given 0.9% NaCl in their drinking water. Thyroparathyroidectomized rats received 1% calcium gluconate in their drinking water. Where indicated, rats were given daily subcutaneous injections of dexamethasone (50 μg/100 g body weight), hydrocortisone (0.4 mg/100 g body weight) or epihydrocortisone (0.4 mg/100 g body weight).

Determination of HMG-CoA Reductase Activity—Lysosome-free microsomes were prepared and assayed for HMG-CoA reductase activity as described (8).

Isolation of Rat Liver Poly(A)+ RNA and Quantitation of HMG-CoA Reductase mRNA Levels—Total RNA was prepared from liver by the guanidinium thiocyanate procedure (9). Poly(A)+ RNA was isolated by oligo(dT)-cellulose chromatography (10). Poly(A)+ RNA was denatured at 65 °C for 15 min in three volumes of 6.15 M sodium citrate, pH 7.0. Poly(A)+ RNA (0.1-2.0 μg) was applied to nitrocellulose using a Dot-blot apparatus. Hybridizations with 32P-labeled pRED227 cDNA and washes were as described (8). pRED227 is a full-length cDNA to hamster HMG-CoA reductase mRNA (11). The cDNA was obtained from American Type Culture Collection. After washing, the filters were subjected to autoradiography, and the amount of HMG-CoA reductase mRNA was determined by assaying radioactivity in the individual dots in a scintillation spectrometer. Northern blot analysis was performed as described (7).

Transcription Rate Analysis by Hybridization to Immobilized cDNA—The isolation of rat liver nuclei and the measurement of HMG-CoA reductase and β-actin transcription rates were performed as described previously (7).

RESULTS

Accumulation of HMG-CoA Reductase in Hypophysectomized Rats Treated with Thyroid Hormones Is Blocked by...
Glucocorticoids Destabilize HMG-CoA Reductase mRNA

Dexamethasone at a Post-transcriptional Step—In hypophysectomized rats, dexamethasone blocks the increase in reductase mRNA (Fig. 1) and activity caused by administration of thyroid hormones. Thyroid hormone treatment increased reductase mRNA levels by 20- to 40-fold. The extent to which this thyroid-induced increase was blocked was dependent on the duration of dexamethasone treatment. In rats given four daily injections of dexamethasone at 36 and 12 h prior to thyroid hormone treatment, reductase mRNA levels were markedly decreased in dexamethasone-treated animals. In hypophysectomized rats treated with thyroid hormones, β-actin mRNA levels were not significantly altered by giving dexamethasone. Metabolism of mevinolin was not affected by dexamethasone treatment as determined by bioassays for mevinolin.

When dexamethasone was administered to hypophysectomized rats treated with thyroid hormones before removing the mevinolin and colestipol diet, the rate of decay for HMG-CoA reductase mRNA was much faster than in animals not given dexamethasone (Fig. 3). The half-life of the mRNA was decreased from 12–15 to 2–3 h (Table I), suggesting that dexamethasone acts to stimulate degradation of HMG-CoA reductase mRNA.

**Endogenous Glucocorticoids Destabilize HMG-CoA Reductase mRNA**—If glucocorticoids stimulate turnover of HMG-CoA reductase mRNA, then adrenalectomized animals should exhibit a more stable mRNA. In adrenalectomized rats changed from a mevinolin and colestipol diet to a normal diet at zero time, HMG-CoA reductase mRNA decreases with a half-life of 8–10 h (Fig. 4 and Table II), about 3-fold longer than the half-life of reductase mRNA in normal animals (8). Thus, endogenous glucocorticoids may stimulate reductase mRNA degradation. When adrenalectomized rats were treated with dexamethasone, the half-life of reductase mRNA decreased to about 2 h (Fig. 4).

**Thyroid Hormone Treatment of Thyroidectomized Rats Increases Reductase mRNA Levels and Reductase mRNA Stabilization**

### Table I

**Effects of dexamethasone on the relative transcription rates of the hepatic HMG-CoA reductase gene and β-actin gene in hypophysectomized rats treated with thyroid hormones**

<table>
<thead>
<tr>
<th>Animal condition</th>
<th>Counts/min hybridized to pRED227 cDNA per 10^6 cpm total RNA</th>
<th>Counts/min hybridized to β-actin cDNA per 10^6 cpm total RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypophysectomized</td>
<td>246 ± 79 (n = 4)</td>
<td>1190 ± 62 (n = 4)</td>
</tr>
<tr>
<td>Hypophysectomized + thyroid</td>
<td>1180 ± 120 (n = 6)</td>
<td>1670 ± 326 (n = 4)</td>
</tr>
<tr>
<td>Hypophysectomized + thyroid + dexamethasone</td>
<td>1317 ± 185 (n = 7)</td>
<td>1800 ± 324 (n = 7)</td>
</tr>
</tbody>
</table>
Glucocorticoids Destabilize HMG-CoA Reductase mRNA

**Fig. 3.** Effect of dexamethasone on the thyroid hormone-promoted stabilization of HMG-CoA reductase mRNA in hypophysectomized rats. Hypophysectomized rats were fed a ground chow diet containing 2% mevinolin, 0.04% mevinolin, and 0.5% thyroid powder for 5 days. Where indicated (C—C), animals were injected with dexamethasone (0.5 μg/g body weight) at 36 h and again at 12 h before beginning the time course. At zero time, 48 h of the dark period, the mevinolin and colestipol were removed from the diet. The rats were killed at the indicated times after the change in diet and the levels of HMG-CoA reductase mRNA were determined in dexamethasone-treated animals (C—C) and nontreated animals (O—O). The inset shows the semi-log plot used to determine the half-life of HMG-CoA reductase mRNA. [mRNA]₀ is the concentration of reductase mRNA at the indicated time, t; [mRNA]ₜ, is the new steady-state concentration of reductase mRNA at 12 (C) and 72 (O) h.

**Table II**

<table>
<thead>
<tr>
<th>Animal condition</th>
<th>HMG-CoA reductase mRNA half-life</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypophysectomized + thyroid</td>
<td>13.5</td>
</tr>
<tr>
<td>Hypophysectomized + dexamethasone</td>
<td>2.5</td>
</tr>
<tr>
<td>Adrenalectomized</td>
<td>9</td>
</tr>
<tr>
<td>Adrenalectomized + dexamethasone</td>
<td>2</td>
</tr>
<tr>
<td>Thyroidectomized</td>
<td>1.5</td>
</tr>
<tr>
<td>Thyroidectomized + thyroid</td>
<td>4.5</td>
</tr>
</tbody>
</table>

**Fig. 4.** Effects of dexamethasone on the half-life of HMG-CoA reductase mRNA in adrenalectomized rats. The time course of decay in reductase mRNA levels in untreated (O—O) and dexamethasone-treated (C—C) adrenalectomized rats is shown. Adrenalectomized rats were fed a diet containing 2% mevinolin and 0.04% mevinolin for 5 days. Where indicated, rats were injected with dexamethasone (0.5 μg/g body weight) at 36 and 12 h before beginning the time course. At zero time, the mevinolin-and colestipol-supplemented diet was switched to normal rat chow and the decrease in abundance of reductase mRNA observed. The inset shows the semi-log plot used to determine the half-life of reductase mRNA, [mRNA]₀ concentration at the indicated time, t; [mRNA]ₜ, concentration at 72 (C) and 12 (O) h.

**Fig. 5.** Effects of thyroid hormones on HMG-CoA reductase mRNA levels in thyroidectomized rats. This Northern blot compares reductase mRNA levels in three thyroidectomized rats (lanes 1-3) and three thyroidectomized rats fed 0.5% desiccated thyroid powder for 5 days (lanes 4-6). Poly(A)^+ RNA (5 μg/lane) was isolated from liver as described under “Experimental Procedures” and separated by size on a 1.2% agarose gel containing 2.2 M formaldehyde. The separated RNAs were transferred to nitrocellulose and hybridized to 35P-labeled pRED227 cDNA.

**DISCUSSION**

Glucocorticoids exert both stimulatory and inhibitory effects on HMG-CoA reductase activity depending on dose and the presence of other hormones. These conflicting results make it difficult to determine the physiological role of glucocorticoids in the regulation of HMG-CoA reductase activity. Our results show that the inhibition by dexamethasone of the thyroid-induced stimulation of HMG-CoA reductase activity is accompanied by a decreased level of reductase mRNA. Dexamethasone appears to block the thyroid-induced increase
in reductase mRNA half-life without affecting reductase gene transcription.

In a previous study, we reported (6) that dexamethasone treatment of triiodothyronine-treated, hypophysectomized rats caused only a 50% decrease in HMG-CoA reductase mRNA levels as compared to the 90% decrease reported in Fig. 1. This difference may reflect different ratios of dexamethasone to thyroid hormones achieved in the two experiments. Animals treated for a longer period of time with dexamethasone do exhibit a greater suppression of reductase mRNA. In addition, differences due to the mode of administration of thyroid hormones (large single dose of triiodothyronine versus dietary thyroid powder) may be responsible.

The decreased effectiveness of thyroid hormones in stimulating expression of the HMG-CoA reductase gene in thyroidectomized rats compared to hypophysectomized rats suggests that glucocorticoids may be physiologically relevant regulators of thyroid-stimulated reductase gene expression. In animals which produce normal levels of glucocorticoids, the -fold increase in reductase mRNA and activity in response to thyroid hormone treatment was at least 80% lower than in animals (hypophysectomized rats) which do secrete adrenal hormones.

Although glucocorticoids mainly exert their effects on specific mRNAs through alterations in gene transcription (16-18), there have been several reports of effects on message stability (19, 20). In rat fibroblasts, for example, dexamethasone stimulated degradation of procollagen mRNAs without affecting transcription of the pro-coll(1) gene (21-23). Post-transcriptional mechanisms also have been proposed for dexamethasone-induced inhibitions of accumulation of the mRNAs for granulocyte-macrophage colony-stimulating factor in murine macrophages (24) and interferon in murine C127 cells (25).

Several hepatic genes expressed during the inflammatory response are suppressed by glucocorticoid hormones (26). A common feature of the mRNAs encoded by these genes is a consensus sequence, UUAAUUUAU, in the 3'-untranslated region. A similar sequence, UUAUUUAU, is repeated twice in the 3'-untranslated region of the hamster reductase mRNA. The core of this sequence, UUAUUU, is common to the 3'-untranslated region of a number of short-lived mRNAs (27). This core sequence is repeated 5 times in the 3'-untranslated region of the reductase mRNA (11). The inverse compliment of this sequence is also repeated five times. Thus, glucocorticoids may induce the expression of an enzyme that degrades mRNAs which contain such a sequence. Alternatively, glucocorticoids may counteract a thyroid-induced protein which stabilizes such mRNAs. The thyroid-induced stabilization of reductase mRNA requires on-going protein synthesis (7). Among other possibilities, glucocorticoids may promote the synthesis of a less stable mRNA due to utilizing alternative transcription initiation or polyadenylation sites. Such heterogeneity with respect to transcriptional initiation and polyadenylation occurs in the transcription of the hamster HMG-CoA reductase gene (28, 29).

REFERENCES

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