Regulation of Ornithine Aminotransferase mRNA Levels in Rat Kidney by Estrogen and Thyroid Hormone*

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The regulation of the mitochondrial matrix enzyme, ornithine aminotransferase, by estrogen and triiodothyronine (T3) in rat kidney was examined using a cloned cDNA probe and in vitro translation of poly(A) RNA. After a single, acute dose of either 17β-estradiol or T3, the rate of enzyme synthesis and the levels of translatable and hybridizable ornithine aminotransferase mRNA all increase in parallel. Levels of hybridizable mRNA were estimated by hybridization of randomly 32P-labeled RNA to filter-bound plasmid DNA. Maximal levels of induction by estrogen and T3 were about 15- and 3-fold, respectively. Lag times of at least 5 h and less than 3 h were observed for induction by T3 and estrogen. T3 and estrogen exert a synergistic effect in increasing ornithine aminotransferase mRNA levels, 16 h after T3 administration and 24 h after estrogen administration, a 1.6- and 13-fold increase in mRNA levels were observed. Both of these treatments in combination for the indicated time periods resulted in a 21-fold increase in ornithine aminotransferase mRNA. From the mRNA accumulation curves, half-lives of 10 to 14 h and 12 to 16 h were estimated for the mRNA after estrogen and T3 induction, respectively. These similar half-lives suggest that an increase in the rate of mRNA production is primarily responsible for the induction observed after estrogen administration.

Ornithine aminotransferase (l-ornithine:2-oxo-acid aminotransferase, EC 2.6.1.13) is a mitochondrial matrix enzyme (1, 2) present in many tissues, including liver and kidney (1-5). The liver polyprotein is synthesized on cytoplasmic polysomes as a precursor molecule of 49,000 daltons and enters the mitochondrion post-translationally (6). The liver and kidney enzymes exhibit some distinct physical properties, including different heat labilities and cysteine contents (4). In addition, the synthesis of the liver and kidney isozymes is subject to regulation by a different set of factors (7, 8). The kidney isozyme is induced by the administration of estrogen and T3 (8). The half-life of kidney ornithine aminotransferase is unchanged by estrogen administration (10).

We recently reported the cloning of DNA complementary to ornithine aminotransferase mRNA (11). We have used this cloned DNA as a probe to quantitate ornithine aminotransferase mRNA levels in kidney following estrogen and T3 induction. We report herein that hybridizable ornithine aminotransferase mRNA levels increase in parallel with the rate of enzyme synthesis and the level of translatable mRNA after induction by these two hormones. Furthermore, estrogen and

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1 The abbreviations used are: T3, 3,5,3'-triiodothyronine; SDS, sodium dodecyl sulfate.

T3 administered in combination exert a synergistic effect on the level of ornithine aminotransferase mRNA.

MATERIALS AND METHODS

Measurement of Ornithine Aminotransferase Activity and Rate of Synthesis

Estrogen and T3 were obtained from Sigma Chemical Company. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

2 The "Materials and Methods" are presented in miniprint as prepared by the authors. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 82M-1714, cite the authors, and include a check or money order for $1.20 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.
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RESULTS

Previous work from this laboratory demonstrated that multiple injections of triiodothyronine and 17β-estradiol to male rats increase the levels of kidney ornithine aminotransferase activity, immunoprecipitable protein, and rate of synthesis by 2-3-fold and 10-11-fold, respectively (8). We sought to obtain more detailed information on the induction of ornithine aminotransferase by these hormones, and to elucidate the level at which these hormones act to induce the enzyme.

Fig. 1A shows the increase in the rate of synthesis up to 38 h after a single acute administration of 17β-estradiol. The 38-h point represents the approximate plateau value at which there is a 15-fold increase in the rate of ornithine aminotransferase synthesis over the value in control rats. In order to determine whether the increase in the rate of synthesis observed is the result of an increase in the level of functional ornithine aminotransferase mRNA, total kidney poly(A') RNA was translated in a rabbit reticulocyte lysate system in the presence of [35S]methionine and the products subjected to immunoprecipitation with anti-ornithine aminotransferase IgG. The immunoprecipitates were electrophoresed on a 10% polyacrylamide-SDS gel. A fluorogram of the dried gel is shown. The position of pre-ornithine aminotransferase (pOAT) is indicated.

RNA was translated in a rabbit reticulocyte lysate system in the presence of [35S]methionine and the products subjected to immunoprecipitation with monospecific anti-ornithine aminotransferase IgG. Fig. 2 shows the progressive increase in functional ornithine aminotransferase mRNA activity 8, 12, and 24 h after estrogen administration. This increase could be due to an increase in the level of mRNA molecules, or an increase in the relative in vitro translational efficiency of pre-existing mRNA. To distinguish between these two possibilities, it was necessary to quantitate absolute mRNA sequence levels using a cDNA probe. We have recently reported the cloning of DNA complementary to liver ornithine aminotransferase mRNA (11). The recombinant plasmid pOAT-2 contains about 1100 bases of the liver ornithine aminotransferase mRNA sequence (11). A hybrid select translation experiment (not shown) using this cloned DNA and total kidney poly(A') RNA indicated that there is significant homology between liver and kidney ornithine aminotransferase mRNAs. Thus, pre-ornithine aminotransferase can be used as a probe to quantitative the level of the kidney message.

A quantitative estimate of changes in ornithine aminotransferase mRNA levels after estrogen administration was obtained by hybridizing total kidney poly(A') RNA that had been labeled (after partial alkaline hydrolysis) using T4 polynucleotide kinase and [γ-32P]ATP to pOAT-2 immobilized on nitrocellulose filters (15). If the efficiency of hybridization and the proportion of the total mRNA sequence contained within the probe are known, the relative percentage of a specific mRNA sequence within a heterogenous population can be determined. Fig. 1B demonstrates that the time course of accumulation of ornithine aminotransferase mRNA after acute administration of 17β-estradiol closely parallels the increase in the rate of enzyme synthesis (Fig. 1A). For comparison, the increase in translatable levels of mRNA up to 24 h after estrogen treatment are also given. At 24 h post-estrogen, the rate of synthesis and the relative levels of translatable and hybridizable mRNA are all increased about 13-fold over the control values. Thus, the increase observed in the rate of enzyme synthesis after estrogen administration can be quantitatively accounted for by an increase in ornithine aminotransferase mRNA. The half-life of the mRNA under the conditions of estrogen induction can be estimated directly
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Fig. 3. Increase in ornithine aminotransferase rate of synthesis and mRNA levels in rat kidney following an acute dose of T3. Kidney high speed supernatant fractions were prepared from rats injected intraperitoneally with 50 µg/100 g of body weight of T3 at various times before killing. Rats were injected with 1 mCi [35S]methionine 90 min before killing. Ornithine aminotransferase was immunoprecipitated and electrophoresed on 10% polyacrylamide-SDS gels as described under "Materials and Methods." The bands corresponding to ornithine aminotransferase were excised from the gels and radioactivity quantitated as described under "Materials and Methods." Rate of synthesis is expressed as the fold increase in the percent of radioactive incorporation into ornithine aminotransferase relative to that incorporated into total trichloroacetic-acid-precipitable protein. Values represent the average of 2 independent experiments. Total kidney poly(A)+ RNA was isolated from rats at various times after the injection of 60 µg/100 g of body weight of T3. Ornithine aminotransferase mRNA levels were determined as described in the legend to Fig. 1. Values represent mean ± S.E. of 4-8 determinations, using RNA pooled from 8-16 kidneys.

Fig. 4. Synergistic effect of combination estrogen-T3 administration on hybridizable levels of ornithine aminotransferase mRNA. Total kidney poly(A)+ RNA was isolated from rats after administration of T3, estrogen, or both. For the combination treatment, estrogen was given for 24 h and T3 for 16 h. Levels of ornithine aminotransferase mRNA were determined as described in the legend to Fig. 1. Values represent mean ± S.E. of 4 determinations using RNA pooled from 8 kidneys.

from the accumulation curve, assuming a zero order rate of mRNA synthesis and a first order rate of decay (18). The half-life may be defined as the time required for one-half the maximal increase in mRNA observed at the new steady state level after induction (18). Assuming no lag time, by this analysis the half-life of ornithine aminotransferase mRNA in the presence of estrogen is 10 to 14 h.

In Fig. 3 the increases in the rate of ornithine aminotransferase synthesis and mRNA level up to 40 h after a single acute dose of T3 are presented. Fig. 3 demonstrates that the increase in the rate of enzyme synthesis is closely paralleled by an increase in the relative level of ornithine aminotransferase mRNA. In contrast to estrogen induction, there appears to be at least a 5-h lag before the increase in enzyme synthesis or mRNA levels commence. A 2.6-fold increase in the rate of enzyme synthesis and a 3.9-fold increase in mRNA were observed 40 h post-T3 administration. The half-life of the mRNA in the presence of T3 estimated from Fig. 3 as described above is 12-16 h, assuming a lag time of 5 h. Since there is a 5-fold difference in the maximal levels of induction by estrogen and T3, the similar half-lives observed in the presence of the two hormones suggests that a change in the rate of degradation of the mRNA plays little, if any, role in the induction by estrogen.

It was reported previously (8) that estrogen and T3 have a synergistic effect on the increase in the rate of kidney ornithine aminotransferase synthesis. After a combined 24 h of estrogen and 16 h of T3, there is a multiplicative effect on the level of hybridizable ornithine aminotransferase mRNA. Fig. 4 shows that there is a 1.6-fold increase in mRNA 16 h post-T3, a 13-fold increase 24 h post-estrogen, and a 21-fold increase after both of these treatments in combination for the above time periods.

DISCUSSION

Estrogen and T3 induce ornithine aminotransferase synthesis in kidney by increasing levels of its mRNA. At least in the case of estrogen induction, this increase in mRNA level is probably mediated primarily by an increased rate of mRNA production. At present, we cannot differentiate between primary and secondary effects of these hormones on ornithine aminotransferase synthesis, although the rapidity of the response to estrogen suggests that this hormone acts directly on the kidney to induce the enzyme. Although we have not analyzed the early response in detail, the data indicate that the lag period before the initial effect of estrogen is observed is less than 3 h. It is possible that T3 acts indirectly by increasing serum levels of another hormone, e.g. growth hormone, which in turn acts directly on the kidney. The full effect of experimental hyperthyroidism on ornithine aminotransferase synthesis in kidney is dependent on the presence of adrenal glands. Adrenalectomy of rats reduces, but does not abolish, the induction and has no effect on basal levels of ornithine aminotransferase synthesis (8). Thyroidectomy decreases the basal level of enzyme synthesis by a factor of 2 (8). Thus, ornithine aminotransferase induction by T3 is augmented by, but not dependent on, an adrenal factor(s). Work is currently in progress to elucidate the nature of these and other potential inter-dependent hormonal effects on T3 induction of ornithine aminotransferase mRNA.

The availability of a cloned probe for ornithine aminotransferase mRNA provides the opportunity to study the differential hormonal responsiveness of an mRNA coding for a low abundance, "housekeeping" type enzyme in different target tissues. The liver and kidney ornithine aminotransferase isozymes exhibit distinct physical properties (4) and are expressed at different stages of the HeLa cell cycle (19). The liver isozyme is induced by the feeding of a high protein diet to rats, the administration of glucagon, or the addition of dibutyryl cAMP to primary rat hepatocyte cultures (20). The kidney isozyme is refractory to these treatments, but is induced by estrogen or T3, neither of which affects the liver isozyme (7, 8). We have recently demonstrated that glucagon and high dietary protein induce liver ornithine aminotransferase at the level of translation. Thus, the differential hor-
monal responsiveness of the liver and kidney isozymes is also reflected in unique induction mechanisms in the two tissues. Elucidation of the cause of this differential hormonal responsiveness will be facilitated by the determination of the structure and sequence of the ornithine aminotransferase mRNA(s) and gene(s). This work is currently in progress.

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