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Role of Thyroxine in the Postnatal Development of Rat Hepatic Tryptophan Oxygenase and Ornithine Aminotransferase (42067)

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Abstract. The activities of tryptophan oxygenase and ornithine aminotransferase are known to increase markedly in rat liver during the postnatal period. The aim of this study was to determine whether thyroxine regulates the development of these two enzymes. It was found that hyperthyroidism had no effect on the activity of tryptophan oxygenase, but caused a modest increase of ornithine aminotransferase activity at 10 days of age. The latter effect persisted in adrenalectomized animals, indicating that it was not secondary to elevation of plasma corticosterone. When thyroxine was administered together with cortisone acetate, elevation of ornithine aminotransferase activity was substantially greater than that observed with cortisone acetate alone. It is concluded that the postnatal development of hepatic ornithine aminotransferase is primarily controlled by glucocorticoids, but that the effect of these hormones may be potentiated by thyroxine.

The postnatal development of the rat is characterized by a high degree of temporal coordination of enzymic changes (1). In many tissues there are clusters of enzymes which display large increases or decreases of activity during the third postnatal week (1, 2). The hormones thyroxine and corticosterone have been implicated as regulators of many of these enzymic changes (1, 2). Physiological roles for these hormones are indicated by the fact that their serum concentrations are markedly increased during the second postnatal week, i.e., just preceding the changes of enzyme activity (3–5).

For hepatic enzymes, the ontogenic roles of thyroxine and corticosterone have not been fully elucidated. A number of liver enzymes display increasing activity during the third postnatal week, e.g., glucokinase (6), tryptophan oxygenase (7), ornithine aminotransferase (8), histidase (9, 10), malate: NADP oxidoreductase (11), and hexosaminidase (12). Of these, thyroxine has been shown to have an important regulatory role in the ontogenic increases of glucokinase (6) and malate:NADP oxidoreductase (11). Glucocorticoid control has been implicated in the postnatal development of tryptophan oxygenase and ornithine aminotransferase (2, 7, 8, 13–17). The possibility of a role for thyroxine in the ontogenic increases of tryptophan oxygenase and ornithine aminotransferase activity has not been previously investigated. Exogenous thyroid hormone and glucocorticoids have opposing effects on the natural rise of hepatic histidase development (9, 10) and hepatic hexosaminidase (12). Thus, four possibilities exist for the role of thyroxine in the development of hepatic tryptophan oxygenase and ornithine aminotransferase in the late suckling period: (i) a permissive role mediated by glucocorticoids; (ii) a role synergistic with corticosterone; (iii) a role independent of glucocorticoids (negative or additive); or (iv) no effect. The aim of the current study was to evaluate these possibilities using hyperthyroid, hypothyroid, and adrenalectomized rats.

Materials and Methods. *Chemicals.* Pyridoxal-5-phosphate, L-ornithine, α-ketoglutarate, *o*-aminobenzaldehyde, trichloroacetic acid (TCA: 10% w/v solution), L-tryptophan, L-ascorbate, L-kynurenine, disodium EDTA, methemoglobin, L-thyroxine (T_4), D-aldosterone, 6-n-propylthiouracil (PTU), Dextran (500,000 mol wt), and corticosterone were obtained from Sigma Chemical Company (St. Louis, Mo.). Cortisone acetate (CA) was obtained from Merck, Sharp and Dohme (West Point, Pa.). [1,2,6,7-^{3}H]Corticosterone was obtained from New England Nuclear Corporation (Boston, Mass.). The scintillation fluid (Liquiscint) was obtained from National Diagnostics (Sommerville, N.J.). Charcoal

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(neutral Norit) was obtained from Fisher Scientific (King of Prussia, Pa.). All other chemicals were reagent grade.

Animals. Timed-pregnant Sprague-Dawley rats (Crl:CD(SD)BR) were obtained from Charles River Breeding Laboratories (Wilmington, Mass.) on Day 15 of gestation. They were housed individually in opaque polystyrene cages and provided with food (rodent laboratory chow 5001; Ralston Purina, St. Louis, Mo.) and water *ad libitum.* Animal quarters were air-conditioned (21 ± 1°C) and maintained on a 12-hr light/dark cycle. On the due date, cages were checked every 2 hr for births and the birth date was regarded as day 0. Approximately 24 hr following birth, litters were culled to eight or nine pups. Pups remained with their mother throughout the experiment.

Experimental conditions. Hyperthyroidism was induced by daily subcutaneous injections of thyroxine (0.5 μg/g body wt) for 3 days preceding sacrifice. This dosage regimen was chosen because it was previously found to maximally stimulate hepatic production of corticosteroid-binding globulin in the neonatal rat liver (18). Controls received the appropriate volume of vehicle (0.3 m*M* NaOH).

Hypothyroidism was induced by giving the dams 0.01% propylthiouracil (PTU) in their drinking water starting the day after the birth. Previous studies have shown that this dosage of propylthiouracil is sufficient to abolish the developmental rise of circulating thyroxine in the pups (3) when given from midgestation onwards. We have recently discovered that the same is true when the propylthiouracil regimen begins on postnatal Day 1 (19).

Cortisone acetate was administered by subcutaneous injection (4 μg/g body wt) approximately 24 hr before sacrifice. This dosage regimen was chosen because Herzfeld and Greengard (8) have shown it to increase ornithine aminotransferase activity in 8-day-old rats. Controls received an appropriate volume of vehicle (0.3 m*M* NaOH).

Bilateral adrenalectomy was performed via a dorsal incision under ether anesthesia 3 days before sacrifice. Sham-operated animals served as controls. All adrenalectomized pups received daily subcutaneous injections of D-aldosterone (0.05 μg/g body wt) after the operation to reduce mortality rates (14). The sham-operated pups received the appropriate volume of vehicle (0.3 m*M* NaOH). Serum corticosterone was monitored in all adrenalectomized pups to check the efficacy of the surgery. Adrenalectomized animals with serum corticosterone levels higher than 0.1 μg/dl were considered to be incompletely adrenalectomized and their liver enzyme values were rejected as invalid. This criterion led to the rejection of two values.

Assay of serum corticosterone. Animals were killed by decapitation. Trunk blood was collected and allowed to clot at room temperature for 15 min. The blood was centrifuged at 3000*g* for 10 min at 4°C. Serum was removed and stored in small polypropylene tubes at −10°C. Corticosterone concentrations were determined by a competitive protein-binding assay as previously described (20) with four modifications. The amount of [^{3}H]corticosterone added to the corticosteroid-binding globulin solution was doubled, the incubation time was extended to 1 hr, the pH of the sodium phosphate buffer was changed from 7.2 to 7.9, and the concentration of the Dextran solution used to make Dextran-coated charcoal was increased from 3.75 mg/ml to 10 mg/ml. These changes greatly improved the stability of binding.

Enzyme assays. Liver ornithine aminotransferase and tryptophan oxygenase were assayed simultaneously on fresh tissue. Rat litters were isolated overnight (with their dams) before sacrifice to avoid stress-induced enzyme elevation. Livers were quickly removed after sacrifice by decapitation and chilled in normal saline on ice. Each liver was blotted dry, chopped finely with a razor, mixed thoroughly, and then divided into two tared portions. One liver portion was used to determine ornithine aminotransferase activity by the method of Herzfeld and Knox (21) with two modifications. The homogenizing buffer used was 0.14 *M* KCl in 0.02 *M* sodium phosphate buffer (pH 7.4) and a 0.2-ml aliquot of homogenate was used for the incubation because preliminary studies indicated that enzyme activity declined if larger volumes were used. The second liver portion was used to assay the cytosolic activity of tryptophan oxygenase by the method of Franz

and Knox (7) with one modification: for preparation of cytosol, a 20% homogenate was used instead of a 25% homogenate. For both enzymes, activities were expressed as micromoles of product per gram tissue per hour.

Statistics. All data are expressed as means ± SE. Statistical significance of differences was evaluated using Student's two-tailed *t* test for unpaired values.

Results. *Effects of hyperthyroidism.* The effect of hyperthyroidism on hepatic tryptophan oxygenase activity was examined at 10 and 16 days of age. Preliminary studies confirmed earlier findings (7) that these ages are before and during the developmental increase of tryptophan oxygenase activity, respectively. Figure 1A shows that there were no significant differences between the tryptophan oxygenase activities of vehicle-injected and thyroxine-injected pups at either age ($P > 0.7$). In the 16-day-old rat pups, the activity of tryptophan oxygenase was significantly higher in the thyroxine-treated pups than in untreated pups ($P < 0.02$). There was a parallel but statistically insignificant ($P > 0.1$) difference between the untreated and vehicle-injected groups, indicating a stress effect associated with injection.

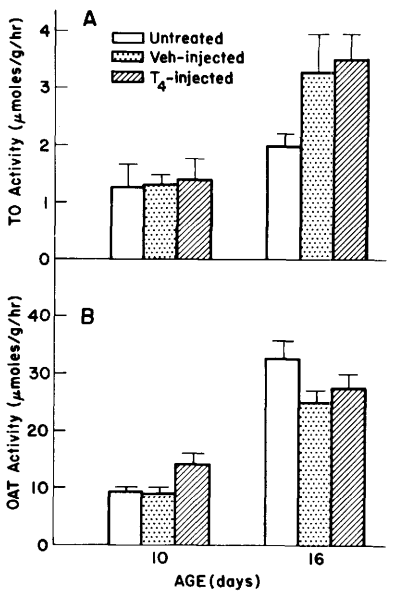

FIG. 1. Effect of hyperthyroidism on hepatic tryptophan oxygenase (TO) and ornithine aminotransferase (OAT) activities. Two litters of nine pups were used for enzyme determination at ages 10 and 16 days (total of eight litters). In each litter, three pups (□) were not treated, three pups (▨) received vehicle (veh) injections, and three pups (▨) received thyroxine (T_4) for the 3 days preceding sacrifice. Values are given as means ± SE ($n = 6$).

The effect of hyperthyroidism on hepatic ornithine aminotransferase activity was also examined at 10 and 16 days of age. Liver ornithine aminotransferase activity has been shown to rise from birth, peaking by Day 35 (21). Figure 1B shows that at 10 days of age, ornithine aminotransferase activity was significantly increased in the thyroxine-treated pups as compared with both the untreated and vehicle-treated controls ($P < 0.02$). On Day 16, both the hyperthyroid pups and the vehicle-injected pups displayed a small but nonsignificant decrease of ornithine aminotransferase activities as compared with untreated controls ($P > 0.2$ and $P > 0.05$, respectively).

Effect of thyroxine on ornithine aminotransferase in adrenalectomized rats. A previous study has shown that serum corticosterone levels in the infant rat are increased by thyroxine doses greater than 0.02 μg/g body wt (22). In addition, glucocorticoid administration is known to raise liver ornithine aminotransferase activity in developing rats (8, 13). Since Fig. 1B showed that thyroxine at a dosage of 0.5 μg/g body wt significantly increased the activity of hepatic ornithine aminotransferase in 10-day-old rats, we investigated the effect of hyperthyroidism in adrenalectomized pups in order to determine whether the thyroxine effect in Fig. 1B was mediated through elevated corticosterone. Sham-operated littermates receiving glucocorticoid treatment were included for purposes of comparison. The results are shown in Fig. 2.

Comparison of the adrenalectomized (adX) + vehicle (veh) group and the sham + veh group in the two panels shows that adrenalectomy significantly decreased serum corticosterone concentrations from sham-operated levels ($P < 0.001$) without affecting liver ornithine aminotransferase activity ($P > 0.80$). This is not surprising in view of the fact that at this age (10 days), the developmental increase of serum corticosterone concentrations has not yet begun (1). The lowness of

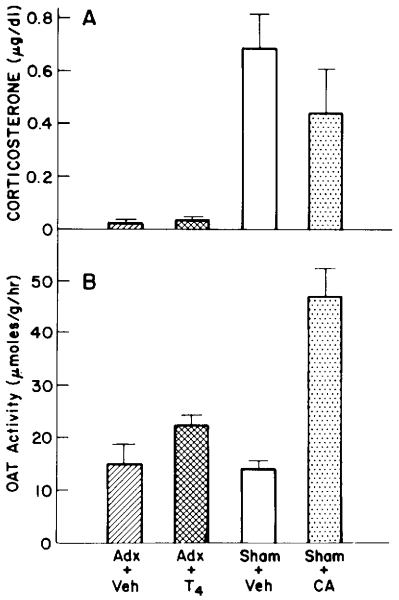

FIG. 2. Effect of hyperthyroidism on serum corticosterone and hepatic ornithine aminotransferase (OAT) activity in adrenalectomized (adX) pups at 10 days. Three litters of eight or nine pups were used. In each litter, two or three pups (▨) were adX and subsequently injected with vehicle (veh) for 3 days; two or three pups (▩) were adX and then treated with thyroxine (T_4) for 3 days; two pups (□) were sham-operated and then given a single injection of vehicle 24 hr before sacrifice; and two pups (□) were sham-operated and then given a single injection of cortisone acetate (CA) suspension 24 hr before sacrifice. Serum corticosterone concentrations for each group were determined. All values are given as means ± SE (n = 6).

these corticosterone values, even in sham-operated animals (Fig. 2A), is illustrated by the fact that during normal development, serum corticosterone reaches >5 μg/dl by 17 days of age and >11 μg/dl by 24 days of age (4).

Figure 2 shows that thyroxine treatment of adrenalectomized pups did not significantly elevate serum corticosterone levels as compared with vehicle-treated adrenalectomized pups ($P > 0.60$) but caused a modest increase of liver ornithine aminotransferase activity ($P < 0.025$). Cortisone treatment of sham-operated animals caused a marked increase of liver ornithine aminotransferase activity as compared with sham-operated animals receiving vehicle injections ($P < 0.001$). The effect of cortisone was significantly greater than that of thyroxine ($P < 0.001$).

Effect of thyroxine and cortisone acetate on liver ornithine aminotransferase activity of hypothyroid rat pups. Since thyroxine significantly increased liver ornithine aminotransferase levels in adrenalectomized pups (Fig. 2B), we further investigated the relationship between thyroxine and glucocorticoids in the control of ornithine aminotransferase development. The aim of this experiment was to determine whether the effect of thyroxine was additive, permissive, or synergistic with that of glucocorticoids.

Pups were made hypothyroid by administration of propylthiouracil and then were treated with either vehicle, thyroxine, cortisone acetate, or cortisone acetate plus thyroxine. The results (Fig. 3) show that thyrox-

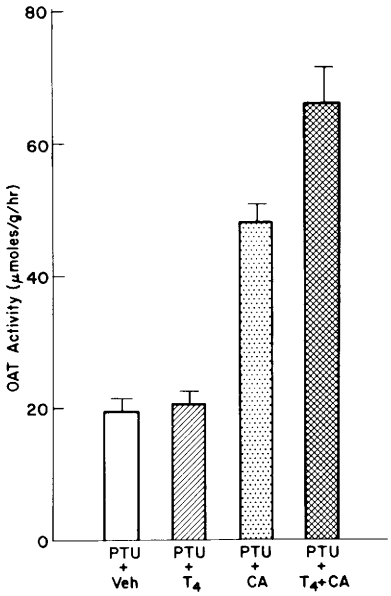

FIG. 3. Effect of hyperthyroidism and cortisone acetate (CA) on hepatic ornithine aminotransferase (OAT) activity in propylthiouracil (PTU)-treated rats aged 10 days. Six litters of eight or nine pups each were used for this experiment. In each litter, two rats (□) received vehicle (veh) injections, two or three rats (▨) were given thyroxine (T_4), two or three (□) rats were given cortisone acetate (CA), and two or three rats (▩) were given thyroxine plus cortisone acetate (T_4 + CA). Thyroxine treatment was for 3 days preceding sacrifice while cortisone acetate treatment was given once 24 hr before sacrifice. Values are given as means ± SE (n = 12–14).

ine alone did not significantly increase the liver ornithine aminotransferase activities of these propylthiouracil-treated pups over their vehicle-injected littermates ($P > 0.6$). In contrast, both cortisone acetate and thyroxine plus cortisone acetate treatment caused marked increases of ornithine aminotransferase activities over those of the vehicle-injected littermates ($P < 0.001$ on both cases). The combined treatment with the two hormones gave values significantly higher ($P < 0.005$) than those with cortisone acetate alone. However, the effect of cortisone acetate alone was no different ($P > 0.70$) in these hypothyroid animals (Fig. 3) than in euthyroid animals (Fig. 2B).

Discussion. The experiments in this paper were designed to determine the role of thyroxine in controlling the developmental rise in the activity of two liver enzymes that have previously been shown to be responsive to glucocorticoid induction. Hyperthyroidism had no effect on tryptophan oxygenase activity during its developmental rise. However, ornithine aminotransferase activity was increased by hyperthyroidism at 10 days after birth. The adrenalectomy experiments showed that thyroxine increased ornithine aminotransferase activity independent of adrenal glucocorticoids. Nevertheless, the effect of thyroxine on ornithine aminotransferase at 10 days of age is modest compared with the normal developmental increase of ornithine aminotransferase activity (Fig. 1) and with the effect of exogenous glucocorticoid (Fig. 2). Although a dose–response study was not performed, it is unlikely that higher doses of thyroxine would have had a greater effect, because the dose used was that previously shown to be saturating with respect to induction of another liver protein during the same developmental period (18, 22). The lack of effect of hyperthyroidism on ornithine aminotransferase activity at 16 days of age is not surprising in view of the fact that the developmental increase of endogenous thyroxine is complete by this age (3, 5), i.e., stimulating effects of thyroxine would have already been expressed.

Although hepatic ornithine aminotransferase activity was increased 60% by thyroxine treatment, this increase is small in comparison to the threefold increase of renal ornithine aminotransferase activity that occurs in adult rats following administration of triiodothyronine (23). The hepatic ornithine aminotransferase effect appears similar to the statistically significant but quantitatively minor effect of thyroid status on DNA synthesis in postnatal rat lung (24). The induction of ornithine aminotransferase by triiodothyronine in the adult kidney is due to increased ornithine aminotransferase mRNA levels (25). This may not be the mechanism of action in the developing liver because liver and kidney ornithine aminotransferase enzymes are nonidentical (26, 27) and are induced by different stimuli (28). Moreover, the ornithine aminotransferase activity of infant and adult livers responds differently to glucocorticoids, the former being reliably increased (8, 13) and the latter being either unaffected (8, 13, 28) or reduced (29).

The data from the propylthiouracil experiment show that although cortisone can act in hypothyroid animals, the effect of cortisone is greatly enhanced by the concurrent administration of thyroxine. The lack of effect of thyroxine alone in the hypothyroid studies (Fig. 3) is difficult to explain in view of the consistency of the thyroxine effect in Figs. 1 and 2. Since propylthiouracil is known to partially inhibit the conversion of thyroxine to its biologically active form (triiodothyronine) (30, 31), one could argue that there was insufficient triiodothyronine generated following injection of thyroxine. It seems unlikely, however, in view of the fact that the combination of propylthiouracil and a five-fold lower dose of thyroxine has previously been reported to cause pronounced stimulation of corticosteroid-binding globulin production in the infant rat (22). A more plausible explanation is that in the current study, propylthiouracil had a direct stimulatory action on hepatic ornithine aminotransferase activity, and the elevated values in the control animals (Fig. 3, cf. Fig. 1) obscured the detection of a thyroxine effect. Such action of propylthiouracil does not jeopardize the comparison of animals treated with cortisone acetate plus thyroxine and with cortisone acetate alone (Fig. 3) because the background effect of propylthiouracil would have been present in both of these groups of animals. Thus, we take the latter data as

indicating that thyroxine, although having only a modest effect alone, can synergize with exogenous glucocorticoid to cause marked enhancement of hepatic ornithine aminotransferase activity in the 10-day-old rat. This synergism may be analogous to that between triiodothyronine and epinephrine found by Mueckler and Pitot in the induction of ornithine aminotransferase in adult rat kidney (23).

In summary, the current study together with previous ones indicates that thyroxine seems to act in a variety of ways on the liver during the suckling period of rats. It can be the primary modulator, as for glucokinase (6) and corticosteroid-binding globulin (18, 22); be synergistic with glucocorticoids, as for ornithine aminotransferase (current study); have an independent effect opposing glucocorticoids, as for histidase (9, 10) and hexosaminidase (12); or it can have no effect at all despite glucocorticoid involvement, as for tryptophan oxygenase (current study).

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