Growth Hormone Responses to Thyroid Hormone in the Neonatal Rat: *RESISTANCE AND ANAMNESTIC RESPONSE*

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GH synthesis and GH mRNA activity studied in pituitaries of 28-d-old rats were expressed as percent total protein synthesis and percent mRNA activity, respectively. GH synthesis and mRNA activity were 3.0 and 2.6% in hypothyroid rats, 3.3 and 2.9% in hypothyroid rats given a single T3 injection 14 d earlier (T3-withdrawn rats), and 26.8 and 27.1% in normal rats. Administration of T3 to hypothyroid rats induced an increase in GH synthesis and GH […]

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RESISTANCE AND ANAMNESTIC RESPONSE

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ABSTRACT Differences in the growth hormone (GH) responses to primary and to secondary stimulation with triiodothyronine (T3) were studied in rats deprived of thyroid hormone from birth. Neonatal hypothyroidism was induced in pups by feeding pregnant rats an iodine-deficient, propylthiouracil-containing diet. T3 stimulation was carried out in pups by subcutaneous injection of a single dose of 50 μg T3/100 g body wt. Pituitary GH content, rate of GH synthesis in vitro, and GH messenger (m)RNA activity in a cell-free translation system were measured.

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Four conclusions can be drawn from these experiments. First, during neonatal life, GH accumulation in rat pituitaries is independent of thyroid hormone and is insensitive to T3. Second, GH dependence on and sensitivity to thyroid hormone is acquired between the 6th and 10th d of neonatal life. Third, secondary T3 stimulation produces an anamnestic response manifested by an increased rate of GH synthesis and mRNA activity. Fourth, primary T3 stimulation is not associated with a lag in the endogenous translation of the newly accumulated GH mRNA.

INTRODUCTION

We have previously shown differences in the rate and time of prolactin (PRL)[1] response to estrogen when given to male rats previously unexposed to the hormone (primary stimulation) and when it was given for a second time after a period of withdrawal (secondary stimulation) (1). Differences were of dual nature. First, the rate of cytoplasmic PRL messenger (m)RNA accumulation was much slower after primary stimulation, and second, a lag period between the accumulation of PRL mRNA and stimulation of endogenous synthesis of PRL was observed. A similar increase in the rate of accumulation of egg protein mRNA in the cytoplasm of oviduct and liver of birds and of amphibians previously exposed to estrogen has been observed by a number of investigators (2–7). After both primary and secondary stimulation, however, synthesis of the

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[1] *Abbreviations used in this paper*: GH, growth hormone; m, messenger; PRL, prolactin; T3, triiodothyronine; T4, thyroxine.

specific protein proceeded in parallel with the level of its mRNA. A lag before the newly accumulated vitellogenin mRNA became endogenously translated has been noted during primary stimulation of the male *Xenopus* with estrogen (8).

In a preliminary experiment on the correlation between thyroid hormone-induced growth hormone (GH) synthesis and mRNA activity, we failed to observe such a lag (9). Because of the possibility that even prolonged thyroid hormone deprivation may not abolish the anamnestic response to thyroid hormone, a neonatal rat model was developed to determine whether synthesis lag is a common phenomenon after primary exposure to hormones acting at the gene level. Results from experiments reported here show that the neonatal rat is unresponsive to thyroid hormone. Furthermore, the slower induction of GH mRNA after a primary exposure to thyroid hormone is not accompanied by a lag in the endogenous translation of the accumulated specific mRNA.

METHODS

Mature male and female rats of the C/D strain, weighing 200–250 g, were obtained from Charles River Breeding Laboratories, Inc., Wilmington, Mass. They were kept in a temperature-controlled (19–20°C) and artificially illuminated (light from 0700 until 1900 h) room. In all experiments, rats were killed between 1230 and 1400 h. Triiodothyronine (T3) treatment, described below, was planned accordingly.

Preparation of adult hypothyroid rats. Male rats were surgically thyroidectomized and placed on low iodide diet (Teklad Co., Madison, Wis.) and 0.9% calcium chloride in the drinking water. 1 mo later, each rat was injected intraperitoneally with 80 μCi of $Na^{125}I$ (Industrial Nuclear Co., St. Louis, Mo.). Normal controls were fed regular Purina laboratory chow (Ralston Purina Co., St. Louis, Mo.) and given tap water. Access to food and water was unrestricted and animals were housed 5–6/cage. Experiments were carried out 2 mo later as described below.

Preparation of neonatal hypothyroid rats. After a 1-wk period of acclimatization to the animal quarters, female rats, housed 5/cage, were exposed for 2 d to three male rats. 15 d later, a group of impregnated rats was started on low iodine diet containing 0.15% propylthiouracil (Teklad Co.), while another was continued on a regular diet, both provided ad lib. These diets were maintained after delivery and throughout the entire period of the experiments. 2 d before the expected day of delivery, each female was placed in an individual cage and visited every 6 h. Pups were weighed upon delivery and at various intervals thereafter. Pups were removed randomly from different litters at 1, 3, 6, and 10 d for the study of pituitary GH content as described below. On the 5th d after delivery, the number of pups per mother was readjusted to eight. GH synthesis and GH mRNA activity in response to primary and secondary treatment with T3 was studied in 28-d-old rats as described below.

Protocol for the study of the evolution of the pituitary GH content in neonatal rats. Three groups of three to four pups each were studied at ages 1, 3, 6, 10, and 28 d: (*a*) normal pups; (*b*) hypothyroid pups born from propylthiouracil-treated mothers; and (*c*) hypothyroid pups 24 h after the subcutaneous injection of 50 μg T3/100 g body wt. Pups were killed under light ether anesthesia by exsanguination into the chest cavity after heparinization and closed-chest needle injury of the heart. Blood accumulated in the diaphragmatic recess was collected with a Pasteur pipette, and the plasma separated by centrifugation. This technique allowed collection of from 0.2 to 0.4 ml of plasma in 1-d-old pups and from 0.3 to 0.6 ml of plasma in 10-d-old pups. 28-d-old rats were bled by direct cardiac puncture. Plasma was stored at −20°C until assayed for thyroxine (T4) and T3 concentrations.

After exsanguination, the pituitary was immediately exposed through an anterior craniotomy approach, and the posterior lobe was removed and discarded. The anterior pituitary was resected in toto, weighed, homogenized in 0.5 ml of phosphate-buffered saline (20 mM sodium phosphate, 140 mM sodium chloride, pH 7.4), and stored at −20°C until assayed for GH content.

Protocol for the study of T3-induced GH synthesis and GH mRNA activity in adult hypothyroid rats. 24 hypothyroid male rats, prepared as described above, were divided into four groups of 6 rats each. One group was untreated and the other three received a single intraperitoneal injection of 50 μg T3/100 g body wt, 2, 6, and 12 h before the termination of the experiment. In addition, 12 normal age-matched male rats were divided into two groups of 6. One group was not treated and the other received T3 as above, 12 h before termination of the experiment. Treatment was staggered so that all rats were killed together by exsanguination through cardiac puncture under light ether anesthesia. Pituitaries were immediately dissected as described above, and GH synthesis and mRNA activity determined as outlined below. Serum was stored at −20°C for measurement of T4 and T3 concentrations. On the day of the experiment, mean±SD weights of hypothyroid and normal rats were 190±17 and 419±36 g, respectively.

Protocol for the study of GH synthesis and GH mRNA activity in response to primary and secondary T3 stimulation. Neonatal hypothyroid rats were prepared as described above. All rats were 28-d-old at termination of the experiment. Seven groups of six rats each were studied: (*a*) untreated hypothyroid; (*b*, *c*) 4 and 12 h after primary T3 stimulation given at 28 days of age; (*d*, *e*) 4 and 12 h after secondary T3 stimulation given at age 28 d, after a primary T3 stimulation at 14 d; (*f*) withdrawn animals 28 d old, given a single T3 injection at age 14 d; and (*g*) normal controls. In all instances, T3 was given as a single subcutaneous dose of 50 μg/100 g body wt. Rats were killed, and the pituitaries were dissected out as described above.

Determination of pituitary GH synthesis. GH synthesis rate was assessed from measurement of newly synthesized hormone by pulse labeling in vitro as described in greater detail elsewhere (9). Briefly, pituitaries from adult rats were divided into eight fragments and those from pups into four fragments. Incubation was carried out in duplicate flasks containing three fragments from individual rats from the same group. After 1 h preincubation, protein synthesis was carried out at 37°C in the presence of 50 μCi [^{3}H]leucine/ml of glucose-supplemented Krebs-Ringer's lactate ([^{3}H]leucine from Amersham Corp., Arlington Heights, Ill.). The viability of the tissue and uninterrupted synthetic function under the in vitro conditions were previously demonstrated (9). Total protein synthesis was measured by trichloroacetic acid precipitation and GH synthesis by specific immunoprecipitation (9) of [^{3}H]leucine. The recovery of GH in the immunoprecipitates was determined in each sample by coprecipitation of an added ^{125}I-labeled GH tracer. Analysis of samples from each experiment was carried out in the same assay. Results are reported in terms of mean GH synthesized in duplicate determinations containing pituitary fragments from

the six rats in each group and expressed as percent total protein synthesis.

Determination of pituitary GH mRNA activity. Pituitary tissue from each group of six animals not used for the determination of GH synthesis (3/4 of each gland from neonatal and 7/8 of each gland from adult rats) was pooled and immediately frozen in liquid nitrogen. RNA was extracted with phenol-chloroform as previously described (9). Total mRNA and GH mRNA activities were measured using a micrococcal nuclease-treated, reticulocyte lysate cell-free translation system (10) under the conditions previously described in detail (9). All reactions were carried out simultaneously. Care was taken to use RNA concentrations in the range where the response of the cell-free translation system was linear with respect to RNA input (80 μg/ml). Results are reported in terms of mean GH mRNA activity in duplicate determinations and expressed as percent total mRNA activity.

Radioimmunologic techniques. Plasma T4 concentration was measured by a modification of a competitive protein-binding assay (11) with a sensitivity of 0.10 μg thyroxine/dl and a coefficient of variation of 5.9%. Plasma T3 concentration was measured by a double-antibody radioimmunoassay (12) with a sensitivity of 10 ng/dl and a coefficient of variation of 7.0%. GH content in pituitaries was measured by a double-antibody radioimmunoassay (13) with a sensitivity of 10 ng/ml and a coefficient of variation of 5.0%. Before analysis, the frozen pituitary homogenate, prepared as described above, was sonicated and centrifuged at 3,000 g for 10 min. Several dilutions of the supernate were prepared in order to make use of the most sensitive and most accurate portion of the standard curve.

RESULTS

Table I shows the evolution of body weight, serum T4 and T3 concentrations, and pituitary GH content in hypothyroid and in normal rats from ages 1–28 d. There was no significant difference in the birth weight between hypothyroid and normal rats. Weights ranged from 4.7 to 7.2 g and from 4.9 to 6.7 g, respectively. An inverse correlation was noted between the birth weight and the number of pups per litter. At the extremes, the mean±SD weight in litters of 5 pups was 6.9±0.2 g/pup, and in litters of 11–14 pups, 5.2±0.1 g/pup, irrespective of treatment. Significant arrest in growth of hypothyroid pups was first noted at 10 d. The mean body weight of normal pups was 49.7% higher than that of hypothyroid pups, and further increased to the value of 164% in 28-d-old pups. In contrast, serum T4 concentration was significantly lower in 1-d-old hypothyroid pups, 0.13±0.06 compared with 0.43±0.06 μg/dl in normal pups. Serum T4 concentration remained low in the hypothyroid pups throughout the entire period of observation, whereas it rose gradually in normal pups, reaching a value of 4.3±0.3 μg/dl at 28 d. Serum T3 concentration was <10 ng/dl in both normal and hypothyroid pups at 1 and 3 d. At 6 d, mean serum T3 level in the normal

TABLE I

Evolution of Body Weight, Serum T_4 and T_3, and Pituitary GH in the Neonatal Hypothyroid Rat and Effect of T_3

		Body weight			Serum T4		Serum T3*		Pituitary GH content		
Age		N	H	N + T3	N	H	N	H	N	N	H + T3
d		*g*			*μg/dl*		*ng/dl*		*μg*		
1	Mean	5.7	5.5	—	0.43	0.13	<10	<10	4.3	5.4	—
	SD	0.2	0.4	—	0.06	0.06			0.08	1.9	—
	P	NS			<0.005				NS		
3	Mean	6.1	6.3	5.3	0.83	0.20	<10	<10	6.7	8.3	9.2
	SD	0.7	1.0	0.6	0.12	0.10			0.8	1.6	1.2
	P	NS		NS	<0.001				NS		NS
6	Mean	10.4	9.1	9.9	1.33	0.17	15.0	<10	8.0	9.2	9.0
	SD	0.6	0.7	0.1	0.15	0.06	4.4		0.8	3.2	2.2
	P	NS		NS	<0.001		<0.05		NS		NS
10	Mean	18.1	12.1	11.1	2.03	0.17	48.3	<10	19.0	8.6	13.7
	SD	2.8	1.3	0.7	0.49	0.06	11.6		2.5	3.1	2.3
	P	<0.05		NS	<0.001		<0.005		<0.001		<0.05
28	Mean	76.3	28.9	29.7	4.34	0.40	72.2	<10–15.1	20.8	1.4	5.6
	SD	4.3	2.8	1.3	0.30	0.10	7.8		3.5	0.9	0.6
	P	<0.001		NS	<0.001		<0.001		<0.001		<0.001

Results are given in terms of mean±SD for each group containing three to four pups. *P* values derived from *t* tests compare the N and H, and the H and H + T3 groups at each age. N, normal controls. H, hypothyroid pups prepared by feeding low iodide and propylthiouracil-containing diet to pregnant mothers and throughout the period of the study. H + T3, hypothyroid pups given 50 μg T3/100 g body weight s.c. 24 h before the termination of each study period.

* Serum T3 concentration was >1,000 ng/dl in all H + T3 rats. Concentrations of T3 < 10 ng/dl were assigned a value of 9 ng/dl in the calculation of significance. Serum T3 values in 28-d hypothyroid pups are given as the range for the group.

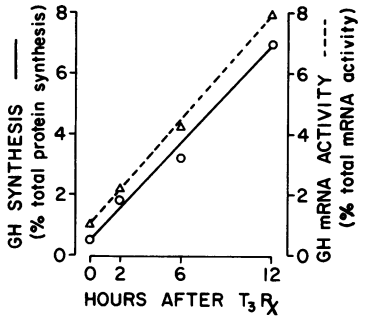

FIGURE 1 Response of GH synthesis and GH mRNA activity to T3 in pituitaries of adult hypothyroid rats. Hypothyroidism was induced 3 mo before the execution of the experiment. A single intraperitoneal dose of T3 (50 μg/100 g body wt) was given before the times indicated on the abscissa. Each point represents the mean values of duplicate determinations using pituitary tissue from six rats. The difference between duplicate determinations was within 20% of the mean value.

pups was 15.0±4.4 ng/dl, reaching a level of 72.2±7.8 μg/dl at 28 d. Serum T3 in hypothyroid pups remained either below 10 μg/dl or reached a maximal level of 15.1 ng/dl. 24 h after the administration of T3, serum levels were >1,000 ng/dl.

The results of pituitary GH content were least expected (Table I). No significant differences were found between hypothyroid and normal pups 1, 3, and 6 d. Furthermore, during the same period of time, no changes in the pituitary GH content were observed 24 h after administration of 50 μg of T3/100 g body wt to the hypothyroid pups. A significant increase in pituitary GH content in response to T3 occurred in 10-d-old pups, when differences between hypothyroid and normal animals also became significant. At 28 d, pituitary GH content in the hypothyroid rats decreased to levels approximately 3-fold lower than at birth, whereas that in normal rats increased by 48-fold, resulting in an average 150-fold difference between the two groups, as previously shown in adult rats (14–16).

The induction of GH synthesis and GH mRNA activity in response to T3 given to adult hypothyroid rats is shown in Fig. 1. The basal GH mRNA activity of 1.1% of the total mRNA doubled at 2 h after the administration of T3 and continued rising in a linear fashion over the period of 12 h, reaching 8.0%. GH synthesis rate increased in parallel. Of note is the good agreement between both parameters, GH synthesis expressed as percent total endogenous protein synthesis and GH mRNA as percent total translational activity in the cell-free system. GH synthesis and mRNA activity in age-matched normal controls were 35.9 and 36.8%, respectively, and did not significantly change (33.1 and 34.4%) 12 h after the administration of T3. Furthermore, total protein synthesis (incorporation of [^{3}H]leucine into TCA-precipitable material per milligram pituitary protein) and total mRNA activity (incorporation of [^{3}H]leucine into TCA-precipitable material per microgram RNA added in the cell-free translation system) were not different in hypothyroid compared with normal rats.

Similar studies were carried out in the neonatal hypothyroid rat. GH synthesis and GH mRNA activity were measured at 4 and 12 h after the primary and secondary stimulation with T3, and in untreated hypothyroid, T3-withdrawn, and normal rats. Data are shown in Fig. 2. GH synthesis and mRNA activity in rats treated with T3 14 d earlier (T3-withdrawn rats) were not different from those observed in the untreated hypothyroid rats. A significant increase in both parameters was observed at 4 h after the administration of T3. The response rate after secondary stimulation was 2.5-fold more rapid than after primary stimulation, despite the fact that, before the administration of the second T3 dose, values in the T3-withdrawn rats had returned to the pretreatment level. The rate of increase in GH mRNA activity in the adult hypothyroid rat mimicked that observed after secondary T3 stimulation in the pups: an approximately threefold increase at 4 h and eightfold increase 12 h after T3 injection. No lag was observed in GH synthesis in relation to the cytoplasmic accumulation of GH mRNA during the primary stimulation of neonatal hypothyroid rats. As in normal adult rats, GH synthesis and mRNA activity in the normal 28-d-old pups were higher than the values achieved 12 h after T3 treatment, and represented 26.8% of the total protein synthesis and 27.1% of the total mRNA activity, respectively. Serum T3 concentrations at 4 and 12 h after T3 injection were both >1,000 ng/dl.

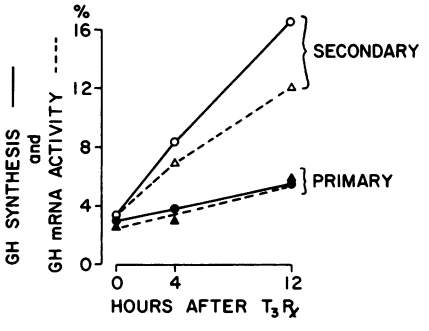

FIGURE 2 Responses of GH synthesis and GH mRNA activity to the primary and secondary stimulations with T3 in pituitaries of neonatal hypothyroid rats. All rats were 28-d-old at the termination of the experiment. A single subcutaneous dose of T3 (50 μg/100 g body wt) was given before the times indicated on the abscissa. Secondary stimulation involved the administration of the same dose of T3 14 d earlier. As in Fig. 1, pituitary GH synthesis and mRNA activity are expressed in terms of percent total protein synthesis and total mRNA activity, respectively.

DISCUSSION

The most salient and novel findings of the present study are (*a*) thyroid hormone-independent accumulation of GH in rat pituitary during early neonatal life; (*b*) insensitivity of GH to T3 during the same period of neonatal life; (*c*) acquisition of GH dependence on and sensitivity to T3 between the 6th and 10th d of neonatal life; (*d*) anamnestic response of the rate of GH mRNA accumulation with T3; and (*e*) absence of lag in the endogenous translation of the newly accumulated cytoplasmic GH mRNA in response to primary T3 stimulation.

The exquisite sensitivity and dependence of GH on thyroid hormone have been previously demonstrated in the adult rat and in experiments using GH-producing rat pituitary tumors in culture (9, 14–19). Indeed, in the hypothyroid rat, pituitary GH content is depressed by 90–99.9% (14–16). A 97–98% decrease in the synthesis of GH has also been demonstrated in pituitaries of hypothyroid rats (9, 15), as well as a 76–80% diminution in GH accumulation in the medium of GH-producing rat pituitary cell lines deprived of thyroid hormone (17, 18). Previous work has also shown that GH dependence on thyroid hormone can be fully explained by the requirement of the latter to induce the transcription of the GH gene (17–19). It is thus surprising that we failed to demonstrate an effect of T3 on GH during the first 6 d of neonatal life in the rat. Not only was there no difference in the pituitary GH content of hypothyroid and normal pups, but T3 administration to the thyroid hormone-deprived pups also failed to produce a change. The latter observation indicates that the hypothyroid pup behaved as the euthyroid adult rat, in which GH does not respond to supraphysiologic doses of thyroid hormone (16, 20). Thyroid hormone appears not to be necessary for intrauterine growth and early postnatal life. Indeed, pituitary GH is unaffected by hypothyroidism, and during the same period, no significant differences in growth rate were observed between the hypothyroid and normal pups. GH dependence on thyroid hormone became manifest at 10 d. Thyroid hormone deficiency was associated with a diminution in pituitary GH content, significant slowing in the growth rate, and appearance of GH response to T3.

The period between days 6 and 10 of the neonatal rat life, during which regulation of GH synthesis becomes dependent upon thyroid hormone, corresponds to the opening of the pups' eyes. In this species of mammals, normally born at a relatively immature stage of development, the time of appearance of GH dependence on T3 should correspond to the late fetal life in man. It could then be speculated that intrauterine supplementation of thyroid hormone to the athyrotic human fetus may not be necessary for normal development. Support in favor or against such a contention should become available in the future from results of early initiation of thyroid hormone replacement in the hypothyroid newborn detected by the neonatal screening programs (21, 22).

The rate of GH mRNA accumulation in T3-stimulated hypothyroid animals after a period of T3 withdrawal (secondary stimulation) was approximately 2.5-fold more rapid than after T3 administration to rats previously unexposed to thyroid hormone (primary stimulation). Thus, an anamnestic response to thyroid hormone, similar to that described for estrogen (1–7), has been demonstrated. The exact duration of the anamnestic response has not been defined, and it remains unknown whether it would persist throughout the entire life span of the animal. However, adult rats rendered hypothyroid responded to T3 with a rate of GH mRNA accumulation similar to that after secondary stimulation of neonatal hypothyroid rats T3 withdrawn for 14 d. From this experiment, it appears clear that the anamnestic response persists for at least 2.5 mo, which constituted the duration between establishment of hypothyroidism and T3 stimulation of the adult rats. On the other hand, we failed to show a lag in the endogenous translation of the newly accumulated pituitary GH mRNA during primary T3 stimulation. Synthesis of GH appeared to proceed in a congruous fashion with the accumulation of cytoplasmic GH mRNA. In this respect, it has been shown that the presence or absence of synthesis lag varied not only with the hormone used but also according to the responding substance, the animals species, and, unfortunately, the laboratory. A lag in the endogenous translation of newly accumulated rat pituitary PRL mRNA (1) and frog liver vitellogenin mRNA (8) has been demonstrated during the primary stimulation with estrogen. The same hormone failed to show a lag in the endogenous translation of newly accumulated ovalbumin mRNA in the oviduct (2) and vitellogenin in the liver of chickens (7) and *Xenopus* (6). Speculations concerning possible mechanisms responsible for the manifestations of the anamnestic response appear elsewhere (1–8, 23). These include irreversible differentiation and/or proliferation of specialized cells capable of specific response to hormonal stimulation, permanent alterations of the chromatin in hormone-primed cells, and rapid stabilization of the message during secondary stimulation.

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