Hypothalamic-Pituitary Somatotropic Function in Prepubertal Hypothyroid Rats: Effect of Growth Hormone Replacement Therapy (43212)

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Abstract. **The effect of thyroid hormone deficiency and growth hormone (GH) treatment on hypothalamic GH-releasing hormone (GHRH)/somatostatin (SS) concentrations, GHRH/SS mRNA levels, and plasma GH and somatomedin-C (IGF-I) concentrations were studied in 28- and 35-day-old rats made hypothyroid by giving dams propylthiouracil in the drinking water since the day of parturition. Hypothyroid rats, at both 28 and 35 days of life, had decreased hypothalamic GHRH content and increased GHRH mRNA levels, unaltered SS content and SS mRNA levels, and reduced plasma GH and IGF-I concentrations. Treatment of hypothyroid rats with GH for 14 days completely restored hypothalamic GHRH content and reversed the increase in GHRH mRNA, but did not alter plasma IGF-I concentrations. These data indicate that, in hypothyroid rats, the changes in hypothalamic GHRH content and gene expression are due to the GH deficiency ensuing from the hypothyroid state. Failure of the GH treatment to increase plasma IGF-I indicates that the feedback regulation on GHRH neurons is operated by circulating GH and/or perhaps tissue but not plasma IGF-I concentrations. Presence of low plasma IGF-I concentrations would be directly related to thyroid hormone deficiency.**

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Thyroid hormone deficiency is associated both in rodents and humans with reduced basal and stimulated growth hormone (GH) release (1–5) and diminished plasma somatomedin-C (IGF-I) levels (6–9). In hypothyroid rats, diminished pituitary GH (1–4, 10) and GH mRNA content (11), and decreased number of pituitary somatotrophs (12, 13) have been documented. Hypothyroidism also affects central nervous system GH regulatory mechanisms. In fact, in adult rats, it induces a decrease of the content of hypothalamic growth hormone-releasing hormone (GHRH) and increase of GHRH mRNA content, respectively (14–16). Regarding somatostatin (SS), the other GH regulatory neuropeptide, unaltered (4) or diminished (17) hypothalamic SS concentrations, depending on the length of the hypothyroid state, have been reported in thyroidectomized rats.

All of these studies refer to adult rats. In infant hypothyroid rats, as in the adult rats, impairment in GH secretion has also been established (13, 18, 19), though pertinent data are more scanty. In 21-day-old rats made hypothyroid since birth, we have recently evidenced a marked increase in hypothalamic GHRH gene expression associated with a decrease in hypothalamic GHRH content (20). There were changes neither in hypothalamic SS gene expression nor in SS-LI content (20). Since in weanling hypothyroid rats, as in adult rats (14–16), changes in hypothalamic GHRH content and mRNA levels occur later than changes in GH secretion (14–16, 20), we hypothesized that the former are caused by the GH deficiency associated with the hypothyroid state rather than by a direct effect of thyroid hormone deficiency on the hypothalamus. To test this hypothesis, we have now evaluated the effect of GH treatment on hypothalamic GHRH and SS

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content and GHRH and SS mRNA levels in 28- and 35-day-old rats made hypothyroid since birth. Additionally, with the aim to assess the possible contribution of somatomedin-C (IGF-I) to the feedback effect of GH on GHRH neurons, plasma IGF-I levels were also assayed before and after GH treatment.

Materials and Methods

Animals. Pregnant Sprague-Dawley rats were obtained from a commercial supplier (Charles River, Calco, Italy) on the 13th day of gestation. Animals were housed in individual cages under standard environmental conditions (temperature 22 ± 2°C, 65% humidity, lights on 0600–2000 hr) and provided food and water *ad libitum*. Dams were assigned randomly to the treatment or the control group, with the dams of the treatment group receiving 0.05% propylthiouracil (PTU) in the drinking water from the day of parturition (Day 0). Offspring were weaned on Day 21 and only weanling male rats were selected for the study. Starting from the day of weaning, hypothyroid rats were either given biosynthetic human GH (0.4 μg/g, sc, twice daily, Genotropin, KabiVitrum, Stockholm) dissolved in 0.9% saline solution (PTU + GH group) or isovolumetric amounts of saline (PTU group). Rats were treated with GH for 7 or 14 days and were sacrificed by decapitation on Day 28 or 35, respectively, 12 hr following the last GH injection.

Tissue Extraction and Radioimmunoassay (RIA). Blood was collected, and anterior pituitary glands and brains were rapidly removed. Blood was collected into EDTA-containing tubes, and plasma was separated and stored at −20°C until RIA determination of GH, IGF-I, and thyroid-stimulating hormone (TSH). Pituitary glands were weighed and homogenized in 0.5 ml of 0.01 *M* $NaHCO_3$ and assayed for GH content by a specific RIA, as described previously (20).

Hypothalami were dissected (21) and assayed for GHRH/SS content or GHRH/SS mRNA levels. For RIA determinations, the tissue was put in 0.1 *M* HCl and boiled for 10 min. after boiling, it was homogenized and the supernatant was kept frozen at −70°C until RIA was performed. For evaluation of mRNA levels, four hypothalami from each experimental group were pooled (about 120 mg of tissue), immediately frozen on dry ice, and stored at −70°C until used.

Plasma and pituitary GH content and plasma TSH levels were assayed by specific RIA as described previously (20). For GH, results were expressed in nanograms/milliliter (plasma) or micrograms per pituitary in terms of the NIADDK GH-RP-2 reference standard. The sensitivity of the assay was 0.5 ng/ml; intraassay variability was 5%. In our rat GH assay system, cross-reactivity with human GH (hGH) corresponded to 4.3%. For TSH, results were expressed in nanograms/milliliter (plasma) in terms of the NIADDK TSH-RP-2 (AFP-5135B) reference standard. The sensitivity of the assay was 0.1 ng/ml; intraassay variability was less than 5%. Plasma IGF-I concentrations were evaluated by a homologous RIA in plasma extracted with 2 *N* HCl, 12.5%, plus 87.5% ethanol, using reagents provided by NIADDK. The sensitivity of the assay was 100 pg/ml; intra-assay variability was less than 10%.

GHRH content in the hypothalamus was evaluated by a homologous RIA, previously described and validated (22), and the results were expressed as nanograms of GHRH per hypothalamus. The assay sensitivity was 15 pg/tube, and the intraassay variation was 5%.

SS concentrations in the hypothalamus were evaluated by a homologous RIA, described previously (23). Results were expressed in picomoles per hypothalamus. The sensitivity of the assay was 3.75 fmol/tube; intraassay variability was 6%. To avoid possible interassay variations, all samples were assayed in the same RIA.

GHRH and SS Gene Expression. Total RNA was isolated from pooled hypothalami by the guanidinium thyocyanate/CsCl method (24, 25). Poly(A^+)RNA was then purified by chromatography on oligo(dT)-cellulose (Pharmacia type 7; Pharmacia, Uppsala, Sweden) (26). Poly(A^+)RNA samples (1 μg of each sample) were spotted onto nitrocellulose sheets (BA 85, 0.45 μm; Schleicher & Schuell, Dassel, Federal Republic of Germany) (prewetted with 5× standard saline citrate) using a slot blot apparatus (Minifold II; Schleicher & Schuell). For each age group examined, two filters were obtained, one of which was hybridized with the plasmid containing the rat GHRH cDNA sequence, prGRF2 (27), and the other with the plasmid containing the rat SS cDNA sequence, pSR-1 (28). Control of the amount of the RNA blotted was performed as described previously (20).

Both the prGRF2 and pSR-1 were labeled by the Multiprime DNA labeling system (Amersham) with [α-^{32}P]dCTP to a specific activity of 2×10^8 dpm/μg cDNA. Hybridization conditions have been already reported (29).

Quantitative measurements of slot blots were performed by densitometric scanning of the autoradiograms using an LKB Ultroscan XL Laser Densitometer (20). The individual densitometric values were averaged for each experimental group and expressed as arbitrary densitometric units.

Statistical Analysis. Differences between groups in individual experiments were determined by Dunnett's *t* test preceded by analysis of variance. A value of $P < 0.05$ was taken to be statistically significant.

Results

Hypothyroid rats, irrespective of whether they were treated with GH or not, had significantly higher basal plasma TSH levels than control rats (CO) (28 days: PTU, 5.1 ± 0.2; PTU + GH, 5.8 ± 0.3; CO, 0.4 ± 0.0

ng/ml, $P < 0.01$; 35 days: PTU, 5.0 ± 0.2; PTU + GH 4.5 ± 0.2; CO, 0.6 ± 0.1 ng/ml, $P < 0.01$). Concurrent with the almost 10-fold increase in plasma TSH was a significant decrease in baseline plasma GH levels in hypothyroid rats, irrespective of treatment (29.6% and 13.7% of control values at 28 and 35 days of age, respectively) (Fig. 1). Pituitary GH concentrations were also strikingly lower than in control rats, and GH treatment did not increase the barely detectable pituitary GH concentrations (1% of control value at both ages studied) (Fig. 2).

In both 28- and 35-day-old hypothyroid rats, plasma IGF-I levels were markedly reduced (22% and 13% of age-matched control values, respectively). GH administration did not enhance plasma IGF-I levels (Fig. 3).

Hypothalamic GHRH content was significantly decreased in hypothyroid rats at both ages (Fig. 4). Treatment with GH did not alter hypothalamic GHRH concentrations in 28-day-old rats; in contrast, in 35-day-old rats, GH treatment completely restored hypothalamic GHRH content (Fig. 4).

A 2.6-fold and 2.8-fold increase in GHRH mRNA was present in 28- and 35-day-old hypothyroid rats, respectively (Fig. 5). Administration of GH for 7 days decreased only moderately GHRH mRNA (85% of PTU value), whereas prolongation of GH treatment up to 14 days lowered GHRH mRNA (34% of PTU value) to levels statistically indistinguishable from those found in age-matched controls (Fig. 5).

Hypothyroid rats, treated or not with GH, had no significant differences in hypothalamic SS concentrations vs CO (28 days: PTU, 3.6 ± 0.1; PTU + GH 3.3 ± 0.1; CO, 3.7 ± 0.1 pmol/hypothalamus, P value not significant; 35 days; PTU, 3.6 ± 0.1; PTU + GH, 3.7 ± 0.1; CO, 4.2 ± 0.1 pmol/hypothalamus, P value not significant) or SS mRNA levels (28 days: PTU, 0.75 ± 0.046; PTU + GH 0.77 ± 0.013; CO, 0.81 ± 0.023; arbitrary densitometric unit, P value not significant).

Discussion

We have recently studied the hypothalamo-pituitary somatotropic function in growing rats, after induction of thyroid hormone deficiency at birth (20). In 14- and 21-day-old hypothyroid rats, a striking decline in plasma GH and pituitary GH levels was evident, whereas the functional activity of GHRH neurons was modified only at 21 days of age. In fact, at this age, decreased hypothalamic GHRH content and a striking rise in GHRH mRNA levels were observed (20). The marked decrease in GH synthesis and release, preceding changes in the functional activity of GHRH neurons, indicated the crucial role of GH deficiency in modifying the hypothalamic function. To test directly the hypothesis that changes in GHRH neural activity were in fact caused by GH deficiency, in the present study male rats made hypothyroid at birth were given GH for 7 and 14 days, respectively, from the day of weaning. Our data indicate that changes in hypothalamic GHRH content and gene expression associated with a long-lasting hypothyroid state are due to GH deficiency ensuing the hypothyroid state rather than to a direct effect of thyroid hormone deficiency.

A 14-day GH treatment restored to normal both hypothalamic GHRH content and gene expression, despite persistence of hypothyroidism. Failure of a shorter lasting GH treatment to affect significantly either index of GHRH neuronal activity would denote some refractoriness of the hypothalamus of prepubertal hypothyroid rats to GH autofeedback.

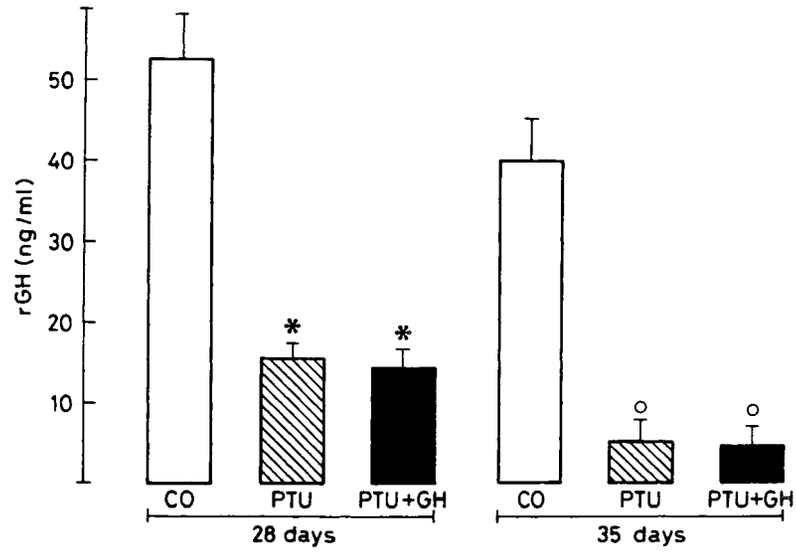

Figure 1. Plasma GH levels (ng/ml) in 28- and 35-day-old control and hypothyroid rats (PTU) treated or not with hGH. Each point is the mean and SE of 10–14 determinations. *$P < 0.01$ vs 28-day-old controls; °$P < 0.01$ vs 35-day-old controls.

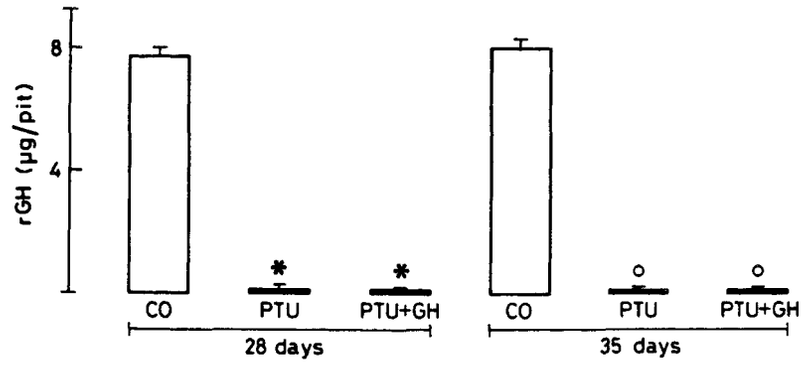

Figure 2. Pituitary GH concentrations (μg/pit) in 28- and 35-day-old control and hypothyroid rats, treated or not with hGH. Each point is the mean and SE of 10–14 determinations. *$P < 0.01$ vs 28-day-old controls; °$P < 0.01$ vs 35-day-old controls.

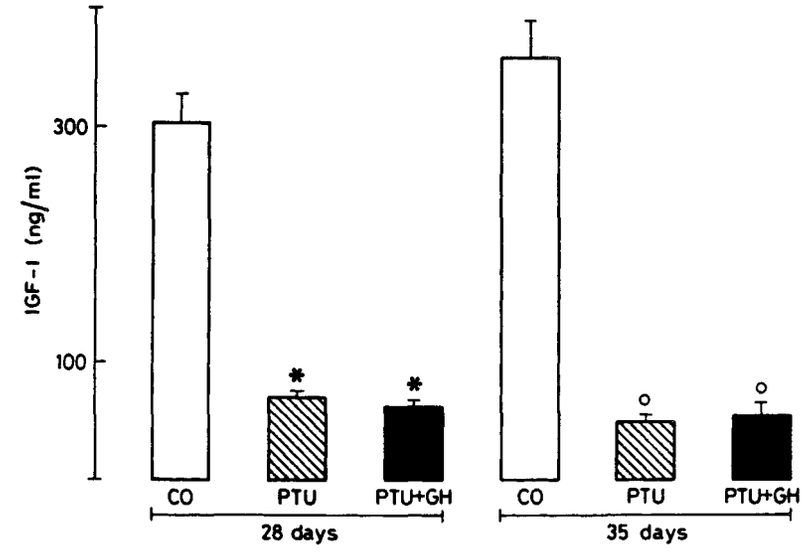

Figure 3. Plasma IGF-I levels (ng/ml) in 28- and 35-day-old control and hypothyroid rats, treated or not with hGH. Each point is the mean and SE of 10 determinations. *$P < 0.01$ vs 28-day-old controls; °$P < 0.01$ vs 35-day-old controls.

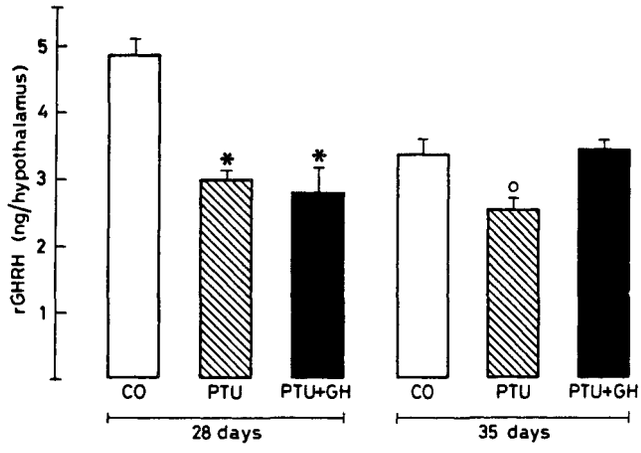

Figure 4. Hypothalamic GHRH concentrations (ng/hypothalamus) in 28- and 35-day-old control and hypothyroid rats, treated or not with hGH. Each point is the mean and SE of 10 determinations. *$P < 0.01$ vs 28-day-old controls; °$P < 0.01$ vs 35-day-old controls.

Recently, replacement therapy with GH at doses superimposable on those used in our study, i.e., 75 μg/rat, twice daily and for only 5 days, has been shown capable of decreasing the elevated GHRH mRNA levels of adult thyroidectomized rats (16).

In contrast to the changes of GHRH neuronal activity in the hypothalamus of untreated or GH-treated hypothyroid rats stands the uniform pattern of hypothalamic SS activity. No significant change occurred in either the hypothalamic SS concentrations or mRNA levels, strengthening the view that the SS neuronal system is rather refractory to perturbations of the pituitary-thyroid axis. Supporting our findings, unaltered hypothalamic SS concentrations and basal and K^+-stimulated SS release *in vitro* have been reported, respectively, in 5-week and 8-week thyroidectomized adult rats (4, 30). Reduction of SS content and release from the hypothalamus were reported only at 6 and 12 weeks postsurgery (17, 30).

Based on our and these findings, it is tempting to

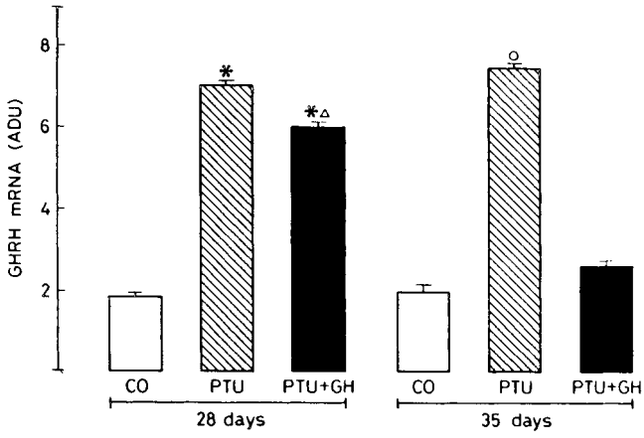

Figure 5. Hypothalamic GHRH mRNA levels, expressed as arbitrary densitometric units (ADU), in 28- and 35-day-old control and hypothyroid rats, treated or not with hGH. Each point is the mean and SE of five determinations. *$P < 0.01$ vs 28-day-old controls; ▲, $P < 0.05$ vs 28-day-old untreated hypothyroid rats; °$P < 0.01$ vs 35-day-old controls and hypothyroid GH-treated rats.

speculate that in hypothyroid rats the involvement of hypothalamic SS function is delayed since the concomitant rise of GHRH neuronal activity stimulates SS secretion (31) and gene expression (32). Alternatively, the high plasma TSH levels may preserve the functional activity of SS neurons in long-lasting hypothyroidism. It is known, in fact, that TSH stimulates SS secretion by cultured hypothalamic cells (33).

GH treatment failed to increase the low plasma IGF-I concentrations in both 28- and 35-day-old rats, a finding favoring the view that GH itself (29, 34), and not plasma IGF-I levels (34), is responsible for the feedback regulation of GHRH gene expression. However, the possibility cannot be ruled out that exogenous GH may increase hypothalamic IGF-I concentrations, thus influencing GHRH neurons through paracrine mechanisms (35, 36).

Diminished plasma IGF-I levels are a common finding in hypothyroid rats and humans (6, 9) and it is still unclear whether this is due to a curtailed GH secretion or to the effect of thyroid hormone deficiency on somatomedin synthesis and release (37, 38). In this respect, contrasting data have been reported. Burnstein *et al.* (6) did not find any rise in plasma IGF-I levels after 7 days of GH treatment in adult hypothyroid rats, whereas in other studies GH proved capable to restore plasma IGF-I concentrations in both hypothyroid humans (9) and rats (7).

Our results showing the failure of exogenous GH to raise plasma IGF-I levels agree with those of Burnstein *et al.* (6) and suggest that the low plasma IGF-I titers are due at least partially to thyroid hormone deficiency. In keeping with this view is the finding that T_3, in addition to GH and cortisol, is needed to restore to normal plasma IGF levels in hypophysectomized rats (39).

In all, these data demonstrate that in prepubertal hypothyroid rats GH restores to normal the altered pattern of hypothalamic GH neuroregulatory systems, and reinforce the notion that in both adult (16) and neonatal (20) rats a primary defect of somatotroph function is the underlying mechanism. Of the two GH neuroregulatory systems, the GHRH system is the one susceptible to GH deficiency and replacement, whereas the somatostatinergic system appears to be refractory to either condition. Since GH failed to alter plasma IGF-I levels, the restoration of the hypothalamic GHRH function seems to be carried out by GH itself. However, a paracrine mechanism mediated by increased hypothalamic IGF-I concentrations cannot be ruled out.

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