Changes in the Hypothalamic-Pituitary Somatotropic Function of Infant Hypothyroid Rats (43028)

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Abstract. **The effects of the perturbation of the pituitary-thyroid axis induced during development on the functional activity of the growth hormone (GH) regulatory neuronal systems, GH-releasing hormone (GHRH), and somatostatin (SS) were studied in 14- and 21-day-old rats made hypothyroid by giving dams propylthiouracil in the drinking water since the day of parturition. Infant hypothyroid rats, both at 14 and 21 days of life, had elevated plasma thyroid-stimulating hormone levels and decreased pituitary and plasma GH levels. Simultaneous determination of hypothalamic GHRH/SS-like immunoreactivity (LI) and GHRH/SS mRNA levels did not reveal any difference in 14-day-old hypothyroid rats when compared with age-matched controls. In contrast, 21-day-old hypothyroid rats had decreased GHRH-LI content and a striking rise in GHRH mRNA levels, whereas SS-LI content and SS gene expression remained unaltered. These data indicate that in infant hypothyroid rats, changes in the functional activity of the GHRH neuronal system occur later than changes in GH secretion and are probably dependent on the GH deficiency. The functional activity of SS neurons was apparently unaltered in these hypothyroid rats, pointing to a lesser sensitivity of this system to the perturbation of the pituitary-thyroid axis.** [P.S.E.B.M. 1990, Vol 193]

Hypothyroidism markedly affects growth hormone (GH) secretion. In fact, thyroid hormone deficiency is associated with reduced basal and stimulated GH release (1–5), diminished pituitary GH content (1–6), and decreased GH mRNA levels (7). Thyroid hormone treatment of hypothyroid rats restores both GH secretion and pituitary GH content to normal (1–6) and increases rGH gene transcription (7).

Concerning the effects of thyroid hormone deficiency on central nervous systems GH regulatory mechanisms, it has been shown that, in adult rats, induction of hypothyroidism is associated with decreased hypothalamic growth hormone-releasing hormone (GHRH) content and increased GHRH mRNA levels, changes largely reversed by thyroxine treatment (8, 9). The effects of hypothyroidism on the other GH regulatory neuropeptide, i.e., somatostatin (SS), are not so well clarified. In fact, in adult hypothyroid rats, hypothalamic SS content was found alternatively to be diminished (10) or unaltered (4, 11). Moreover, to our knowledge, data on SS gene expression in the hypothyroid status have yet to be reported. All of these studies refer to adult rats. In infant hypothyroid rats, the existence of an impaired secretion (diminished basal and stimulated GH secretion and reduced pituitary GH content) has also been ascertained (12–14). Data on the effects of hypothyroidism on GH regulatory neuropeptides are, however, still scanty.

In neonatal rats, Walker and Dussault (14) reported reduced hypothalamic SS concentrations and a recent study from our group (15) has shown in 10-day-old hypothyroid rats unchanged mediobasal hypothalamus-GHRH concentrations in front of a clear-cut decrease in both plasma GH levels and pituitary GH content. We are unaware of other reports on GH regulatory neuropeptides in neonatally induced hypothyroidism.

The present study was designed to get further insight into the functional activity of GHRH and SS neurons in neonatally induced thyroid hormone deficiency. For this purpose we investigated in 14- and 21-day-old hypothyroid rats: (i) plasma GH titers and pituitary GH content; (ii) hypothalamic GHRH-like

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(GHRH-LI) and SS-like (SS-LI) immunoreactivity; and (iii) hypothalamic GHRH and SS mRNA levels.

Materials and Methods

Animals. Pregnant Sprague-Dawley rats were obtained from a commercial supplier (Charles River, Calco, Italy) on the 13th day of gestation. They were housed in individual cages under controlled conditions (22 ± 2°C, 65% humidity and artificial light from 0600 to 2000 hr) and assigned to either the treatment or the control group. The dams of the treatment group received 0.05% propylthiouracil (PTU) in the drinking water beginning on the day of parturition (Day 0). Their pups were sacrificed respectively on days 14 and 21 of life. In all 72 14-day-old rats (38 from the PTU and 34 from the control group) and 74 21-day-old rats (39 from the PTU and 35 from the control group) were examined. Data reported refer to pooled samples obtained from rats of either sex.

Experimental Procedure. On the day of the experiment infant rats, which had been isolated in a soundproof room for 1 hr, were killed by decapitation. Pituitaries and brains were rapidly removed, blood was collected into EDTA-containing tubes and plasma was separated and stored at −20°C until radioimmunoassay (RIA) determination of GH and thyroid-stimulating hormone (TSH). Pituitary glands were weighed and homogenized in 0.5 ml of 0.01 *M* $NaHCO_3$. The pellets were separated by centrifugation (2500*g* for 30 min) and the supernatant was diluted 1:100 with 0.01 *M* phosphate-buffered saline containing 0.05 M EDTA and 1% bovine serum albumin (pH 7.6) and kept frozen until RIA.

Immediately after brain removal, the hypothalami were carefully dissected, as described previously (16), and used for either RIA determination of GHRH/SS-LI or evaluation of 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, the hypothalami from each experimental group were collected in pools of four samples (about 120 mg of tissue), immediately frozen on dry ice, and stored at −70°C until used.

Radioimmunoassay: GH and TSH. Plasma and pituitary GH content and plasma TSH levels were determined by double-antibody RIA using reagents provided by NIADDK. For GH, results were expressed in nanograms/milliliter (plasma) or micrograms/pituitary in terms of the NIADDK standard rat GH-RP-1, the potency of which is 0.6 IU/mg. The sensitivity of the assay was 0.5 ng/ml; intraassay variability was 6%. For TSH, results were expressed in nanograms/milliliter (plasma) in terms of the NIADDK standard rat TSH-RP-2 (AFP-5135B), the potency of which is 35 IU/mg. The sensitivity of the assay was 0.1 ng/ml; intraassay variability was less than 5%. To avoid possible interassay variations, all of the samples were determined in the same RIA.

GHRH-LI. Rat GHRH-LI concentrations in the hypothalamus were evaluated by an homologous RIA, previously described and validated (17). Results were expressed in nanograms/hypothalamus. To avoid possible interassay variations, all samples were assayed in the same RIA.

SS-LI. Rat SS-LI concentrations were determined by an homologous RIA, described previously (18). Results were expressed in picomoles/milligram protein. To avoid possible interassay variations, all samples were assayed in the same RIA.

GHRH and SS Gene Expression. Total RNA was isolated from pooled hypothalamic tissue by the guanidinium thyocyanate/CsCl method (19, 20). Poly(A)$^+$ RNA was then purified by chromatography on oligo (dT)-cellulose (Pharmacia type 7; Pharmacia, Uppsala, Sweden) (21). Ultraviolet absorbance analysis was utilized for RNA quantitation. Starting from 120 mg of frozen tissue, routinely 50–60 μg of total RNA and 2–3 μg of poly(A)$^+$ RNA were obtained. Poly(A)$^+$ RNA samples (1 μg each sample) were spotted onto nitrocellulose sheets (BA85, 0.45 μm; Schleicher & Schuell, Dassel, West Germany) (prewetted with ×5 SSC) 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 (22) and the other with the plasmid containing the rat SS cDNA sequence, pSR-1 (23).

To determine the range of linearity of the autoradiographical signal, known dilutions (8, 1.6, 0.32, 0.064, 0.012 ng) of pBr 322 were used and processed similarly as the RNA samples. Control of the amount of the RNA blotted was performed by reprobing the slot blots with oligo(dA)·oligo(dT) 12-18 (Pharmacia) elongated via terminal transferase with [α-^{32}P]dTTP (Amersham, Little Chalfont, U.K.).

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 described previously (24). Specificity of hybridization was tested by Northern analysis which showed that the mRNA species hybridized with the prGRF-2 and pSR-1 corresponded respectively to the size of GHRH and SS mRNA (25–27).

For quantitative measurements of slot blots, autoradiograms were subjected to densitometry with an LKB Ultroscan XL Laser Densitometer. The individual densitometric values were normalized to the level of poly(A)$^+$ RNA present in each sample, averaged for each experimental group, and expressed as arbitrary densitometric units (ADU).

Statistical Analysis. Hormonal concentrations in the different experimental groups were compared by Dunnett's *t* test preceded by analysis of variance

(ANOVA). Densitometric values from scanning densitometry of slot blots were compared by Student's t test. In both instances, $P < 0.05$ was taken to be statistically significant.

Results

Plasma TSH Levels. Baseline plasma TSH levels were significantly higher in hypothyroid than in controls (14 days, 5.7 ± 0.2 vs 0.8 ± 0.1 ng/ml, $P < 0.01$; 21 days, 6.8 ±1.1 vs 0.9 ± 0.2 ng/ml, $P < 0.01$) (data not shown).

Plasma GH Levels. Baseline plasma GH levels were significantly lower in 14- and 21-day-old hypothyroid rats than in age-matched controls (14 days, 11.5 ± 2.2 vs 20.7 ± 5.3 ng/ml, $P < 0.01$; 21 days, 13.8 ± 1.2 vs 27.8 ± 3.5 ng/ml, $P < 0.01$) (Fig. 1).

Pituitary GH Concentrations. Pituitary GH concentrations were significantly lower in 14-day-old hypothyroid rats than in controls (3.2 ± 0.4 vs 13.6 ± 1.5 μg/gland, respectively, $P < 0.01$); in 21-day-old hypothyroid rats, a further decrease in pituitary GH concentrations was evident (0.6 ± 0.1 vs 15.8 ± 1.3 μg/gland in age-matched control rats, $P < 0.01$) (Fig. 2).

Hypothalamic GHRH-LI Concentrations. No significant difference in hypothalamic GHRH-LI concentrations was present between 14-day-old hypothyroid rats and controls (1.3 ± 0.1 vs 1.63 ± 0.1 ng/hypothalamus, respectively, P = NS); in contrast, in 21-day-old hypothyroid rats, hypothalamic GHRH-LI concentrations were significantly lower than those in controls (1.2 ± 0.1 vs 1.9 ± 0.1 ng/hypothalamus, respectively, $P < 0.01$) (Fig. 3).

GHRH mRNA Levels. No significant difference in hypothalamic GHRH mRNA levels was found between hypothyroid and controls at 14 days of age (0.14 ± 0.04 vs 0.13 ± 0.04 ADU, respectively, P = NS); in contrast, GHRH mRNA levels were markedly increased in 21-day-old hypothyroid rats (1.03 ± 0.1 vs 0.17 ± 0.1 ADU in age-matched controls, $P < 0.01$) (Fig. 4).

Hypothalamic SS-LI Concentrations. No significant differences in hypothalamic SS-LI concentrations were found between experimental and controls, either at 14 or 21 days of age (14 days, 3.5 ± 0.3 vs 3.8 ± 0.3 pmol/mg protein P = NS; 21 days 4.3 ± 0.3 vs 4.2 ± 0.3 pmol/mg protein, P = NS) (Fig. 5).

SS mRNA Levels. Hypothyroid rats at both 14 and 21 days of age had hypothalamic SS mRNA levels not different from those of age-matched controls (14 days, 0.15 ± 0.01 vs 0.17 ± 0.01 ADU, P = NS; 21 days, 0.14 ± 0.01 vs 0.16 ± 0.01 ADU, P = NS) (Fig. 6).

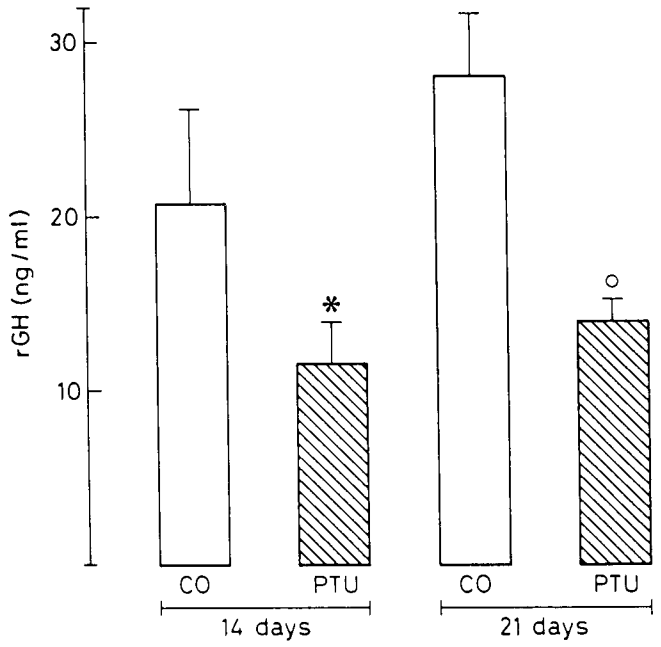

Figure 1. Plasma GH levels (ng/ml) in 14- and 21-day-old control and hypothyroid rats. Each point is the mean and SE of 20–25 determinations. *$P < 0.01$ vs 14-day-old controls; °$P < 0.01$ vs 21-day-old controls (ANOVA and Dunnett's t test).

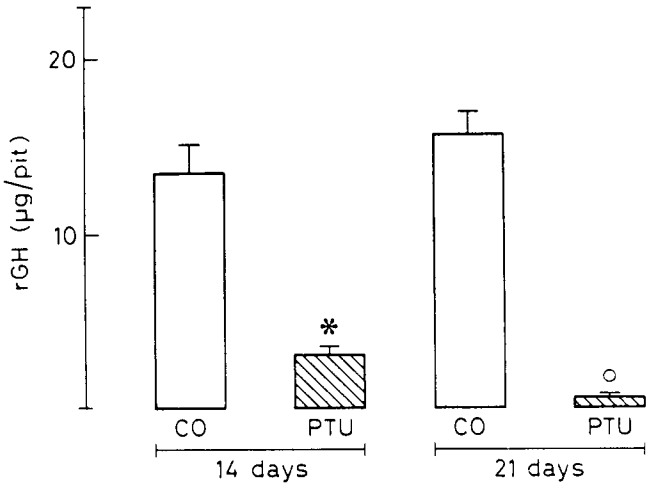

Figure 2. Pituitary GH concentrations (μg/pit) in 14- and 21-day-old control and hypothyroid rats. Each point is the mean and SE of 20–25 determinations. *$P < 0.01$ vs 14-day-old controls; °$P < 0.01$ vs 21-day-old controls (ANOVA and Dunnett's t test).

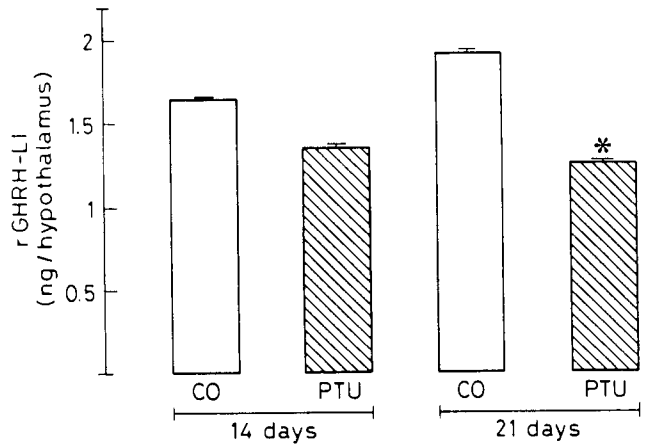

Figure 3. Hypothalamic GHRH-LI concentrations (ng/hypothalamus) in 14- and 21-day-old control and hypothyroid rats. Each point is the mean and SE of 10–15 determinations. *$P < 0.01$ vs 21-day-old controls (ANOVA and Dunnett's t test).

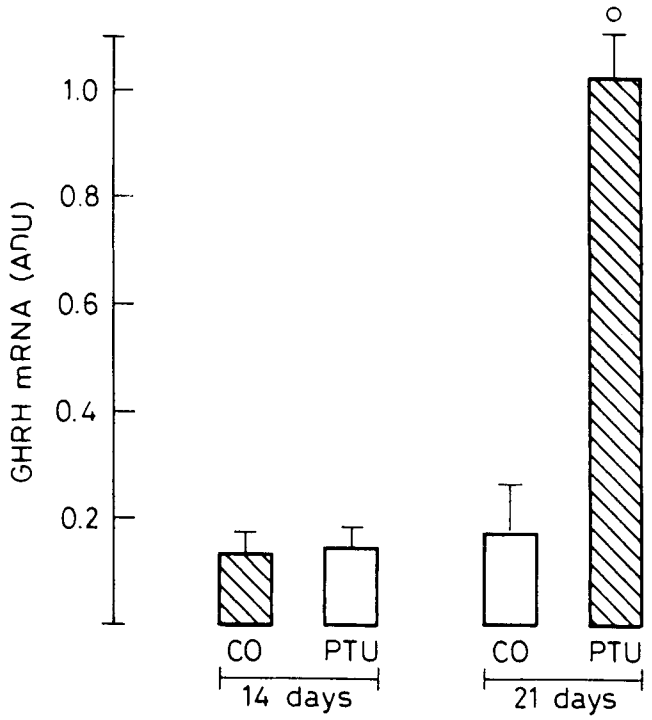

Figure 4. Hypothalamic GHRH mRNA levels, expressed as arbitrary densitometric units (ADU), in 14- and 21-day-old control and hypothyroid rats. Each point is the mean and SE of five determinations. °$P < 0.01$ vs 21-day-old controls (Student's t test).

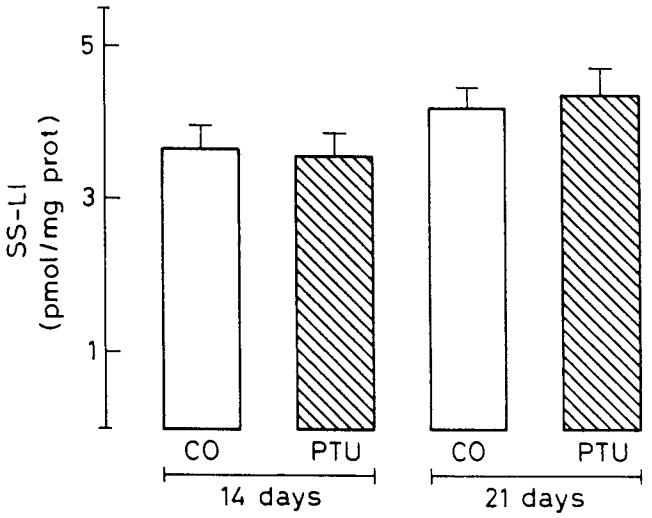

Figure 5. Hypothalamic SS-LI concentrations (pmol/mg protein) in 14- and 21-day-old control and hypothyroid rats. Each point is the mean and SE of 8–10 determinations. P = NS.

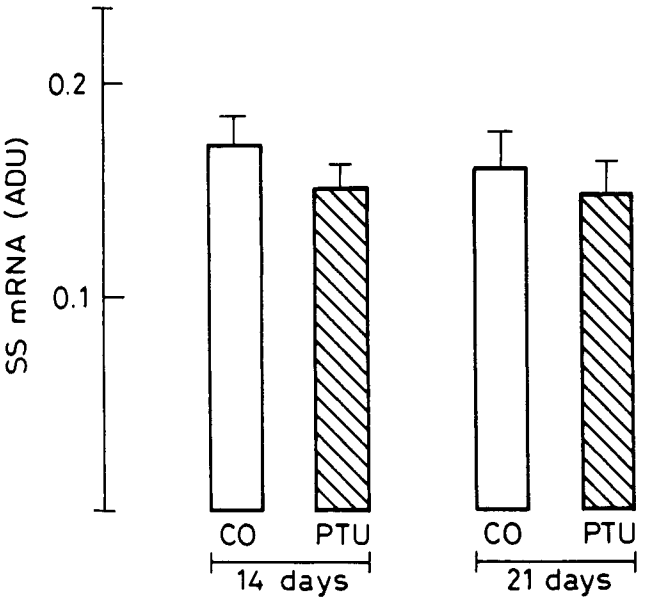

Figure 6. Hypothalamic SS mRNA levels, expressed as arbitrary densitometric units (ADU), in 14- and 21-day-old control and hypothyroid rats. Each point is the mean and SE of five determinations. P = NS.

Discussion

In a previous report, we found in 10-day-old rats made hypothyroid by the same procedure used in this study a striking decline in plasma GH levels and pituitary GH content and a reduced number of pituitary somatotrophs. Evaluation of hypothalamic GHRH by both radioimmunoassay and immunohistochemistry did reveal significant differences between hypothyroid and euthyroid infant rats in either the mediobasal hypothalamus GHRH-LI concentrations or morphologic aspect of hypothalamic GHRH-LI-secreting structures (15). In the present study, we have evaluated the hypothalamic-pituitary somatotropic function of hypothyroid rats at a later stage of postnatal development (14 and 21 days), and we have also considered the inhibitory GH regulatory neuropeptide, SS. It is now well recognized that simple measurements of tissue peptide levels do not provide reliable information on the functional activity of a peptidergic neuronal system (28). Thus, simultaneous evaluation of hypothalamic GHRH/SS-LI concentrations and GHRH/SS mRNA levels was performed.

In 14-day-old hypothyroid rats, plasma GH titers and pituitary GH content were significantly lower than in age-matched controls. At this stage of postnatal development, however, the functional activity of GHRH and SS neurons appeared to be unchanged, as shown by the similar GHRH/SS-LI concentrations and GHRH/SS mRNA levels present in hypothyroid and euthyroid controls. In 21-day-old hypothyroid rats, a further decrease in pituitary GH content occurred and it was associated with decreased GHRH-LI concentrations and a striking rise in GHRH mRNA levels.

The temporal changes occurring in GH secretion and in GHRH-LI content and gene expression indicate that deprivation of thyroid hormones probably acts primarily to depress somatotroph function and the alteration in GHRH neuronal activity is a secondary phenomenon. In fact, the seemingly discordant findings of reduced hypothalamic GHRH-LI concentrations in the presence of increased mRNA levels were also observed in hypophysectomized rats (24, 29), i.e., animals deprived of circulating GH. The observed changes, thus probably reflect an enhanced peptide synthesis overrid-

den by an increased rate of release, due to partial suppression of the inhibitory feedback of GH on hypothalamic GHRH-secreting structures (24). In keeping with this view it has been shown that both basal and K^+-evoked GHRH release are enhanced in the hypothalami of adult thyroidectomized rats, 2 weeks postsurgery, with respect to those of control rats (5, 9).

Supporting a role for GH deficiency to stimulate hypothalamic GHRH gene transcription are preliminary data showing that GH treatment of hypothyroid rats induced a reduction of hypothalamic GHRH mRNA to levels indistinguishable from those of euthyroid rats (authors' unpublished results).

Previous studies in adult rats have shown that hypothalamic GHRH-LI concentrations were reduced at 2 and 4 weeks postsurgery (9, 5). These data thus agree with our own (15 and this study) in showing changes in GHRH occurring later after the impairment of GH secretion has been established and not before a 2-week interval from the induction of hypothyroidism. However, in a recent report, published in abstract form, Downs *et al.* (9) found hypothalamic GHRH gene expression to be significantly enhanced in adult thyroidectomized rats as early as 1 week postsurgery with maximal increase occurring at 4 weeks. Differences in the temporal appearance of changes in hypothalamic GHRH gene expression in neonatal and adult rats may reflect an ontogenetic difference in GHRH sensitivity to GH feedback inhibition.

In contrast to GHRH, there were no significant changes in both hypothalamic SS-LI concentrations and SS-mRNA levels either in 14-day- or in 21-day-old hypothyroid rats. Unaltered hypothalamic SS-LI concentrations had also been reported in 5-week thyroidectomized adult rats (4), while reduction in hypothalamic SS-LI was referred only at 6 and 12 weeks postsurgery (5, 10). Concerning the early postnatal period, a reduction in SS-LI content has been reported in 12-day-old rats made hypothyroid by giving dams PTU, beginning on the third day of gestation (14). Studies of basal and K^+-stimulated SS-LI release *in vitro* in adult hypothyroid rats did not reveal any change at 8 weeks postthyroidectomy (5) while a significant decrease occurred only 12 weeks postsurgery (10).

In essence, our findings of unchanged SS neuronal activity in 14- and 21-day-old hypothyroid rats do agree with previous results pointing to a late reduction of hypothalamic SS-LI content (4) and release (5) in adult hypothyroid rats. Decrease in SS-LI secretion (5, 10, 14) in long-lasting thyroid hormone deficiency is consonant with the concomitant time-related reduction in GH secretion (1, 3, 15), a hormone which stimulates SS secretion (30) and gene expression (31). It may be that hypothyroidism of longer duration than 21 days results also in infant rats in decreased SS-LI concentrations and SS gene expression.

From the overall evaluation of data of the literature and our own it is tempting to suggest that the delayed occurrence of an impaired SS function in hypothyroid rats is related to changes occurring earlier in GHRH secretion. It is known that GHRH exerts a stimulatory effect on SS secretion (32) and gene expression (33). Thus, in hypothyroidism an enhanced GHRH activity, secondary to GH deficiency, may trigger this ultrashort feedback mechanism and in this way may temporally mask the tendency of SS neurons to decrease their functional activity.

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