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Hypothalamic Somatostatin and LH-RH after Hypophysectomy, in Hyper- or Hypo-Thyroidism, and during Anesthesia in Rats (40028)

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Implants of growth hormone (GH) in the rat median eminence decrease pituitary weight and GH content, suggesting a negative feedback effect of GH at the hypothalamic level (1). Demonstration of the presence of TSH in the hypothalamus also implies that TSH might affect hypothalamic activity (2). Recently Oliver *et al.* (3) reported that hypophysial portal blood not only flows from median eminence to the pituitary gland but, as well, there is retrograde circulation via the neurohypophysis to the primary capillary plexus. Further, portal plasma contains high concentrations of both neurohypophysial and adenohypophysial hormones. These results favor a view that the pituitary hormones might exert a direct feedback control on the hypophysiotropic hormones of the hypothalamus. On the other hand, thyroidectomy results in the degranulation of the pituitary acidophil cells in rats (4), but also decreases pituitary GH content (5), and impairs GH secretion (6). The mechanism by which T_4 and T_3 influence GH synthesis in the pituitary is still unknown, but these thyroid hormones appear to exert a direct effect on the pituitary gland (7).

Most anesthetics are known to change the rate of secretion of various pituitary hormones, probably by an effect mediated via the hypothalamus. The administration of sodium pentobarbital (Nembutal) into rats blocks the preovulatory surge of gonadotropin and ovulation (8). Nembutal increases serum GH and prolactin levels (9, 10), whereas urethane reduces GH and prolactin (11).

Our recent studies using antiserum to somatostatin indicated that this tetradecapeptide is involved in a regulatory mechanism of GH and TSH secretion in rats (12).

The following questions could be raised: 1. If a short feedback regulatory mechanism takes place for GH and TSH secretion, does the removal of GH and TSH by hypophysectomy affect the hypothalamic somatostatin? 2. Does the absence or the excess of circulating thyroid hormone affect the hypothalamic somatostatin? 3. Do Nembutal and urethane modify somatostatin content of the hypothalamus? Our studies were designed to answer some of these questions.

Materials and methods. Experimental procedures. Young adult male rats of CD strain from Charles River were used throughout the experiments. They were maintained at a constant temperature (24°) and illumination (lights on 0500–1900 hr) and were given free access to tap water and Purina laboratory chow.

In Exp. I, the rats were hypophysectomized when they were 35-days old and sacrificed by decapitation 24 days or 2.5 months after the operation. Intact age-matched rats served as controls.

In Exp. II, 70-day-old male rats, weighing 300–350 g, were divided into the following four groups: Group 1, intact control rats; Group 2, thyroidectomized rats. These two groups were injected with 0.2 ml of 0.9% saline subcutaneously (sc). Group 3, thyroidectomized rats were injected with 0.1 μg of L-thyroxine (T_4) sc; and Group 4, normal rats were injected with 10 μg of T_4 sc. All groups were injected every 2 days for 10 days.

In Exp. III, the rats were injected with 4.5 mg Nembutal/100 g body weight (bw) intraperitoneally (ip) or 150 mg urethane/100 g bw ip. They were sacrificed by decap-

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itation 5, 10, 30 min to 1 or 2 hr after the injection of the anesthetic. The blood was allowed to clot; the serum was separated by centrifugation and kept at −20° until assayed for GH and TSH.

Extracts of the hypothalami of rats. Immediately after decapitation, the hypothalamic tissue from the posterior margin of the optic chiasma to the mammillary body and laterally to the lateral boundary of the tuber cinereum and dorsally approximately 1 mm from the ventral surface, was dissected and immediately frozen. The hypothalamic tissue was then homogenized in 1 ml of ice-cold 2 *N* acetic acid, heated for 5 min in boiling water, chilled, and centrifuged at 3000 rpm for 15 min at 4°. Seven-tenths ml of the supernatant was lyophilized. The residue was dissolved in a diluent for radioimmunoassay (RIA) shortly before the assay. The inhibition curves of the hypothalamic extracts in the RIA for somatostatin and LH-RH were parallel to those of synthetic somatostatin and LH-RH, respectively. The results were expressed as ng of somatostatin or LH-RH per hypothalamus (ng/SEM) and per 100 g bw.

Radioimmunoassay. LH-RH was measured by RIA as described by Arimura *et al.* (12) using rabbit antiserum #743 at a final dilution of 1:28000. The sensitivity was 1 pg per tube. The antigenic determinant of this antiserum resided in the amino acid sequence from position 3 to 9. The RIA for LH-RH was quite specific and did not exhibit any significant cross-reaction with other hypothalamic and pituitary hormones.

Somatostatin was also determined by RIA as described previously (14) using rabbit antiserum to cyclic somatostatin #101 at a final concentration of 1:28000. *N*-Tyr-Somatostatin, a pentadecapeptide prepared by Dr. C.A. Meyers, was iodinated by ^{125}I using lactoperoxidase, purified on a CMC column, and used as a tracer. The standard curves obtained with ^{125}I-*N*-Tyr-Somatostatin and ^{125}I-Tyr^{1}-Somatostatin were exactly the same. ^{125}I-*N*-Tyr-Somatostatin was more stable than ^{125}I-Tyr^{1}-Somatostatin. After storage for 1.5 months at −50°, the former could be used for RIA without repurification. The cyclic somatostatin was used as the reference standard. The diluent for RIA was 0.25% human serum albumin (HSA)/0.1% gelatin/0.14 *M* NaCl/0.025 *M* EDTA/0.01 *M* phosphate buffer pH 7.5. The sensitivity of the assay was 1 pg per tube.

The serum TSH and GH levels were determined by radioimmunoassay (RIA), using the rat pituitary hormone RIA kits provided by NIAMDD. The data were analyzed using a computer program described by Duddleson (14).

Statistical analysis. The mean concentrations of somatostatin or LH-RH in the hypothalamus and in the various treatment groups were compared by using factorial analysis of variance, and the effect of each treatment was tested for a significant level. Mean serum GH, TSH, prolactin, and LH levels in various groups were also compared with one another by Duncan's new multiple range test (16).

Results. The effect of hypophysectomy on the hypothalamic somatostatin and LH-RH content. The mean hypothalamic somatostatin content in 58-day-old rats which were hypophysectomized 24 days previously was 15.1 ± 1.78 (SE) ng, whereas that of the age-matched intact rats was 49 ± 4.9 ng (Fig. 1). The mean somatostatin content of 117-day-old rats which were hypophysectomized 79 days before was 2.2 ± 0.5 ng, and that of the age-matched intact rats was 24.5 ± 2.4 ng.

Two by two factorial analysis of variance indicated that both hypophysectomy and aging affected hypothalamic somatostatin content, and that there was a significant interaction between these two factors. The somatostatin content in 117-day-old, hypophysectomized rats was significantly smaller than in any of the other groups. The hypothalamic somatostatin content of the older rats (117 days old) was significantly lower than that of the younger animals (58 days old) in both intact and hypophysectomized animals.

When the hypothalamic somatostatin was expressed as ng/100 g bw, the content in hypophysectomized 58-day-old rats was not significantly different from that in the age-matched animals, whereas the content in the hypophysectomized, 117-day-old rats

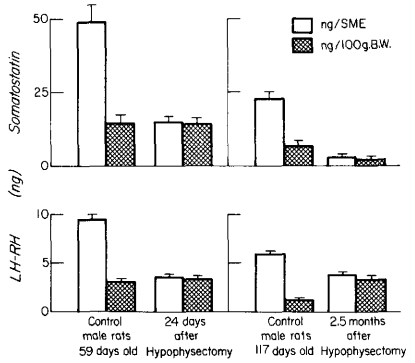

FIG. 1. The effect of hypophysectomy on the hypothalamic somatostatin and LH-RH contents. The vertical bars indicate ± SEM. The hypothalamic somatostatin and LH-RH contents (ng/SME) 24 days and 2.5 months after hypophysectomy were significantly lower than those of their respective control groups. Contents of the hypothalamic somatostatin and of LH-RH (expressed in ng/SME and ng/100 g BW) in 117-day-old rats were significantly lower than those in 59-day-old rats ($P < 0.05$). The hypothalamic somatostatin content (ng/100 g bw) 2.5 months after hypophysectomy was significantly lower than that in the age-matched intact rats ($P < 0.05$).

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The mean hypothalamic LH-RH contents in hypophysectomized 58- and 117-day-old rats were similar; 3.5 ± 0.3 ng/SME and 3.2 ± 0.3 ng/SME, respectively. On the other hand, the mean hypothalamic LH-RH contents of the age-matched intact rats were 9.1 ± 0.8 ng/SME and 6.0 ± 0.8 ng/SME, respectively, the difference being significant (Fig. 1). As in the case of somatostatin, both hypophysectomy and aging affected hypothalamic LH-RH as indicated by analysis of variance.

Effect of absence and excess of T_4 on the hypothalamic somatostatin, LH-RH, serum GH, and TSH levels in rats. As shown in Table I, there was no significant difference in the hypothalamic somatostatin and the LH-RH contents between the control rats (62.5 and 5.2 ng, respectively), the thyroidectomized rats (63.9 and 4.6 ng, respectively), the thyroidectomized rats treated with 0.1 μg of T_4 (65 and 3.3 ng, respectively), and the rats treated with 10 μg of T_4 (65.7 and 4.2 ng, respectively). The mean serum TSH concentration in the thyroidectomized rats (4.5 ± 0.4 μg/ml) and in those thyroidectomized and treated with 0.1 μg of T_4 (3 + 0.2 μg/ml) were significantly higher than the serum TSH level in the control rats (0.57 ± 0.04 μg/ml), whereas the serum TSH levels in the normal rats treated with 10 μg of T_4 (0.4 ± 0.05 μg/ml) were significantly lower than the control ($P < 0.05$). The GH levels were not significantly different between the groups, due to considerable variation in each group.

Effect of urethane and Nembutal anesthesia on hypothalamic somatostatin and LH-RH content. Five min after urethane injection, the hypothalamic content of somatostatin appeared to rise slightly, but this was not statistically significant. The somatostatin decreased significantly ($P < 0.05$) 10 min after injection and then showed a rebound. The serum GH levels fell rapidly immediately after injection of urethane and remained at a low level after 10 min.

On the other hand, the hypothalamic somatostatin content decreased significantly ($P < 0.05$) 5 min after injection of Nembutal, which was associated with an apparent rise of serum GH. It then rose gradually for 60 min after injection and then decreased. Although, due to a large variation of serum GH in each group, the difference in GH between the groups did not reach a significant level, the pattern of change in the hypothalamic somatostatin was close to a mirror image of the pattern of change in

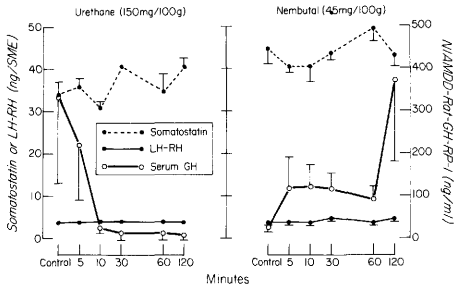

FIG. 2. The effect of urethane (150 mg/100 g) and Nembutal (4.5 mg/100 g) on hypothalamic LH-RH contents, somatostatin contents, and serum GH levels. The concentration of hypothalamic somatostatin 10, 30, and 120 min after urethane injection were significantly different from those of the control level ($P < 0.05$). The hypothalamic somatostatin contents 5, 10, and 60 min after Nembutal administration were significantly different from the control level ($P < 0.05$).

TABLE I. EFFECT OF ABSENCE OR EXCESS OF THYROID HORMONE ON HYPOTHALAMIC LH-RH AND SOMATOSTATIN CONTENTS AND SERUM PITUITARY HORMONE LEVELS

	Hypothalamic LH-RH (ng/SME)	Hypothalamic somatostatin (ng/SME)	Serum GH (ng/ml)	Serum TSH (μg/ml)
Control male rats	5.2 ± 0.4(8)[a]	62.5 ± 3(8)	21.0 ± 10(9)	0.57 ± 0.04(7)
Thyroidectomized rats ($\bar{T}$)	4.6 ± 0.3(8)	63.9 ± 4(7)	14.8 ± 10(7)	4.5 ± 0.4 (6)
$\bar{T}$ + 0.1 μg T_4	3.3 ± 0.5(6)	65.0 ± 4(6)	8.6 ± 5(5)	3.0 ± 0.2 (5)
Normal rats + 10 μg T_4	4.2 ± 0.3(9)	65.7 ± 4.3(10)	15.1 ± 14(9)	0.4 ± 0.06 (9)

[a] Mean ± SE. The number in parenthesis represents the number of animals in each group.

the serum GH levels. The hypothalamic content of LH-RH did not change after injection of these anesthetics.

Discussion. The present study indicates that hypophysectomy results in a decrease in both somatostatin and LH-RH contents of the hypothalamus in rats. In other words, the removal of the target organ, the pituitary gland, resulted in a decrease in both the inhibiting hormone for GH and TSH and the stimulating hormone for LH and FSH. The results are in agreement with findings by Baker and Yen (17) for somatostatin, and Baker and Dermodey (18) for LH-RH, as examined by immunocytochemical methods, and with the finding by Wakabayashi *et al.* (19) for somatostatin as examined by RIA. Bassiri and Utiger (20) reported that hypothalamic TRH also decreased after hypophysectomy. Reduction of the hypothalamic hormone content may be the result of an increased release and/or a decreased synthesis, or a greater rate of secretion than those of synthesis, regardless of their magnitudes. It is unknown which of these conditions resulted in posthypophysectomy decrease in hypothalamic hormones. Although Nallar and McCann (21) reported that bioassayable LH-RH in the plasma increased to a detectable level after hypophysectomy, we could not confirm their results (unpublished observation). Since hypophysectomy reduced the contents of both release inhibiting and release stimulating hormones in the hypothalamus, it is inconceivable that the reductions of these hypothalamic hormones resulted from a short feedback action provoked by the removal of pituitary hormones. Rather, these reductions appear to have been caused by decreased synthesis associated with extensive degeneration of the hypothalamic hypophysial nerve tract following surgery.

However, a significant difference between the changes in hypothalamic somatostatin and LH-RH was observed in long-term hypophysectomized rats. Somatostatin progressively decreased up to 2.5 months after hypophysectomy, whereas LH-RH did not decrease further after the 24th postoperative day. Hypothalamic TRH decreased to 54% of the control after 2 weeks but was restored to 74% of the control after 4 weeks, which could be the result of regeneration of the neural tissue (20). Baker and Yen (17) also reported that the greatest depletion of somatostatin in rats was observed in the medial caudal region of the hypothalamus, where the regeneration of the neural tissue had occurred. Therefore, the progressive decrease of somatostatin even after the neural tissue was generated in contrast to the changes in LH-RH and TRH should be more meaningful if any short feedback influence were to be considered. It is possible that the synthesis of somatostatin in the hypothalamus may require a certain level of GH and/or TSH in circulation, whereas synthesis of LH-RH and TRH may not need gonadotropins and TSH, respectively.

Although somatostatin appears to be involved in the physiological regulatory mechanism of TSH secretion (12, 22, 23), its level in the hypothalamus did not change by either excess or removal of the thyroid hormones. Thyroidectomy and excess T_4 did not modify the hypothalamic LH-RH content either, although such a condition is known to affect the pituitary gonadal function (4). Hypothalamic TRH does not change under similar conditions (20).

On the other hand, urethane and Nembutal modified the somatostatin content but

not the LH-RH content in the hypothalamus. The rapid change of hypothalamic somatostatin could reflect the rapid change in the turnover rate of this hormone. After urethane injection, the somatostatin remained at the same level or appeared to show a slight rise at 5 min, and decreased significantly at 10 min. The pattern resembled that of hypothalamic corticotropin releasing factor (CRF) after stress. The change of hypothalamic CRF after stress was interpreted as the result of a rapid increase of synthesis associated with an enhanced release since plasma ACTH rose rapidly (24). Since the apparent rise or the maintained level of hypothalamic somatostatin 5 min after urethane injection was associated with a rapid decrease of serum GH, this could also be interpreted as a rapid increase of synthesis associated with an enhanced release of somatostatin.

On the other hand, the hypothalamic somatostatin curve was almost the mirror image of the serum GH curve during Nembutal anesthesia. However, in this experiment the initial GH levels were low and GH rose slightly following injection of Nembutal. It is not clear whether this relationship is related to the change in the turnover of hypothalamic somatostatin and GH secretion.

Summary. Twenty-four days after hypophysectomy both hypothalamic somatostatin and LH-RH contents decreased to $^1/_3$ of the control values in male rats. The LH-RH remained unchanged thereafter, whereas the somatostatin continued to fall until 2.5 months after hypophysectomy. The hypothalamic somatostatin and LH-RH contents in 117 day old rats (24.5 and 6.0 ng, respectively) were significantly lower than those in 59-day-old rats (49 and 9.1 ng, respectively).

Thyroidectomy and excess of T_4 did not modify the hypothalamic somatostatin in LH-RH content. On the other hand, an injection of Nembutal or urethane altered the hypothalamic somatostatin content, but not the LH-RH content. After urethane injection the somatostatin content did not change at 5 min as compared with the level at 0 time, but showed a decrease at 10 min. This was associated with a rapid fall of serum GH level which remained low thereafter. The hypothalamic somatostatin then increased stepwise. Nembutal caused a decrease of the hypothalamic somatostatin content at 5 and 10 min and an increase at 60 min. The pattern of change in the hypothalamic somatostatin levels resembled a mirror image of the patterns of change in the serum GH levels.

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