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Effects of Thyroidectomy on Thyrotropin-Releasing Hormone (TRH) and Somatotropin Release-Inhibiting Factor (SRIF) Patterns in Intrahypophysial Microdialysates in Rats

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Abstract. The effects of thyroidectomy on patterns of TRH and somatotropin release-inhibiting factor (SRIF) release from the hypothalamus were investigated by using a microdialysis technique. Thyroidectomized and sham-operated rats underwent placement of a guide cannula into the anterior pituitary gland to obtain dialysates, or implantation of an intravenous cannula into the right atrium for blood sampling. Seven days postoperatively dialysates were collected at a flow rate of 2 μl/min every 1 h. TRH concentrations in dialysates from thyroidectomized rats (0.43 ± 0.22 pg/h) were significantly higher than those from control rats (0.17 ± 0.02 pg/h). In contrast, SRIF concentrations in dialysates from thyroidectomized rats (2.45 ± 0.05 pg/h) were significantly lower than those from control rats (3.80 ± 0.22 pg/h). In addition, plasma TSH concentrations in thyroidectomized rats (24.8 ± 0.5 ng/m*l*) were increased compared with those in control rats (2.5 ± 0.1 ng/m*l*), and plasma GH concentrations were decreased from 68.6 ± 6.4 ng/m*l* in control rats to 21.2 ± 0.6 ng/m*l* in thyroidectomized rats. These findings indicate that TRH and SRIF releases from the hypothalamus are detectable by microdialysis method, and directly show the increase in TRH secretion and the decrease in SRIF secretion from hypothalamus in the hypothyroid state.

Key words: Thyroidectomy, TRH, Somatotropin release-inhibiting factor (SRIF), Microdialysis, Adenohypophysis, Central feedback action

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THYROID hormones exert various effects on a number of organs to maintain physiological functions and metabolic homeostasis. In the hypothyroid state, growth and development are severely disturbed [1], at least partly due to impaired plasma GH release. Thyroid hormones contribute to GH messenger RNA (mRNA) transcription and protein biosynthesis [2]. Thyroid hormone treatment for hypothyroidism therefore restores both the GH secretion and pituitary GH content to normal. Somatotropin release-inhibiting factor (SRIF) and GH-releasing hormone (GHRH) also regulate GH secretion from the anterior pituitary. TSH secretion is controlled by thyroid hormone and TRH. Moreover, SRIF inhibits TSH secretion. TRH and SRIF are known to be widely distributed in the central nervous system, gastrointestinal tract and pancreas, etc. In previous studies, hormone levels of TRH and SRIF have been measured in various tissues [3] and hypophysial portal blood [4, 5], although it has been difficult to measure the release of these hormones from the hypothalamus in conscious animals. In the present study, we investigated the effects of thyroidectomy on TRH and SRIF patterns in intrahypophysial microdialysates from conscious, freely moving male rats.

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Materials and Methods

Experimental animals

Male Sprague-Dawley rats, weighing 300–350 g, were purchased from Nippon SLC Co., Ltd., Hamamatsu, Japan. The animals were individually housed in an air-conditioned room (22 ± 2 °C) under artificial illumination (light, 0900–2100 h: dark, 2100-0900 h) and given regular rat chow (Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water *ad libitum*.

Surgery for microdialysis

Rats were anesthetized with an intraperitoneal (i.p.) injection of 50 mg/kg of body weight (B.W.) of pentobarbital sodium. With a stereotaxic device (David Kopf Instruments, USA), as previously described [6], a guide cannula (22-gauge, 10 mm in length, CMA Microdialysis, Sweden) was implanted with its tip just dorsal to the anterior pituitary gland by using specific coordinates (0.9 mm lateral to the midline, 6.0 mm posterior to the bregma and 9.0 mm ventral to the dura), and fixed in position with dental resin (GC Reparisin, GC Shika, Kyoto). A 22-gauge stainless steel obturator was inserted into the end of the guide cannula until the start of the microdialysis experiment. Cefotetan (20 mg/kg B.W.) was injected postoperatively to prevent infection.

Surgery for blood sampling

Rats were anesthetized with pentobarbital sodium as described above. An intravenous cannula was implanted into the right atrium. A silicon tube (0.7 mm in diameter) was then inserted from the jugular vein into the right atrium (about 33 mm from the vein). The cannula was passed through a subcutaneous tunnel to below the nape of the neck at the middleline where the stainless steel spring was fixed to the skin by ligation. The cannula was passed through the inside of the spring, filled with physiological saline containing 50 U heparin per m*l*, and stoppered with a piece of thick fishing thread. Rats that had undergone surgery were housed in their individual cages. Heparin (50 U) and Cefotetan (20 mg/kg B.W.) were given daily *via* the cannula to prevent clotting and infection, respectively. All experiments were performed in accordance with protocols approved by the Institutional Animal Care and Use Committee of the University of Tokushima.

Experimental protocol

In the first group of rats for microdialysis, thyroidectomy or sham operation was performed after rats were anesthetized with pentobarbital sodium as described above. Subsquently the thyroidectomized or sham operated rats were operated on for microdialysis on the same day. One week after thyroidectomy, the microdialysis experiment was started at 0900 h.

In the second group of rats for blood sampling, thyroidectomy or sham operation was performed, followed by intravenous cannulation on the same day. Blood sampling was started at 0900 h one week after thyroidectomy. The blood samples were collected in heparinized tubes every 30 min during a 9-h period. Blood for transfusion was collected from the sham-operated and thyroidectomized rats, heparinized, then pooled one day before the experiment. A 0.3 m*l* aliquot of blood was immediately transfused into the rats after collecting 0.3 m*l* blood to keep the hematocrit value constant. Blood samples were immediately centrifuged, and the plasma was stored at −20 °C for TSH and GH assays.

Postoperatively, the thyroidectomized rats received drinking water containing 0.1% $CaCl_2$ to prevent hypocalcemia in both the first and second groups.

Microdialysis procedure

A polycarbonate (PC) membrane (2 mm long; CMA12, CMA Microdialysis, Sweden) was used as a microdialysis probe. Ringer's solution, containing 0.1% bovine serum albumin (BSA), was perfused at a flow rate of 2 μl/min with a pulse-free microinfusion pump (EP-60, Eicom, Kyoto, Japan) and a 2.5-m*l* Hamilton gas-tight syringe. Tubes made of fluoroethylenepropylene (FEP) were connected between the syringe and probe and between the probe and the sampling tube. Perfusion was carried out by push methods [6], at a rate of 2 μl/min. The relative recovery rates for TRH and

SRIF were 11.9 ± 3.0% (unpublished data) and 10.8 ± 0.6% [6], respectively.

After the microdialysis system was primed, the probe was inserted through the guide cannula into each animal in a conscious, freely moving state 12 h before sampling to minimize the influence of probe insertion on TRH and SRIF secretion. On the day of the experiment (one week after the thyroidectomy), sampling was started at 0900 h . The dialysates were collected in ice-cooled test tubes at 1-h intervals for 9 h at a perfusion rate of 2 μl/min and stored frozen at −20 °C for determination of TRH and SRIF levels. A 50 μl of 1 fraction of dialysates was measured for TRH RIA assay or SRIF RIA assay, respectively.

At the end of the experiment, the animals were deeply anesthetized with pentobarbital and fixed by the infusion of 10% formaldehyde. The probe position was then verified histologically.

Hormone assays

TRH concentrations in the dialysates were measured by RIA as reported previously [7, 8]. The sensitivity of the assay was 0.5 pg/ml in the present study. The intra- and interassay coefficients of variation (CVs) were less than 3% and 12.6%, respectively.

SRIF concentrations were measured by RIA as previously reported [9, 10]. The lowest detectable SRIF concentration was 15 pg/ml, and a dose-response was observed in 15–2000 pg/ml range. The intra- and interassay CVs of this assay were less than 9% and 14%, respectively. The cross-reactivity of SRIF(1–28)NH_2 with the SRIF antibody used in this study was 109.2 ± 4.5% (mean ± SD) on a molar basis. TRH and SRIF concentrations in dialysates are expressed as pg/h for data analysis.

Plasma TSH and GH concentrations were measured by RIA kits supplied by the NIDDK, USA. The sensitivity of TSH and GH assays were 0.2 ng/ml and 0.5 ng/ml, respectively. The intra- and interassay CVs of these two assays were less than 8% and 12%. Plasma T_3 and T_4 concentrations were measured with commercial RIA kits (SPAC T_3 RIA Kit, SPAC T_4 RIA Kit, Daiichi Radioisotope Institute, Tokyo, Japan).

Statistical analysis

The plasma TSH and GH profiles and the dialysate TRH and SRIF profiles of individual rats were analyzed by using the Cluster analysis program for endocrine pulse detection [11]. Briefly, a t statistic was selected to maintain a maximal false-positive rate of 1% or less by using cluster sizes of one or two in the nadir and peak. In data analysis, TRH and SRIF concentrations that fell below the concentration of detection for the assay were assigned a value equal to the sensitivity of the assay (0.5 pg/ml and 15 pg/ml, respectively). The significance of differences between the pairs of groups were tested by means of the unpaired Student's t test. Data are expressed as the mean ± SEM. A P value of <0.05 was considered statistically significant.

Results

Effects of thyroidectomy on body weight, plasma T3 and T4 concentrations

One week after the surgery, the body weight gains in sham-operated and thyroidectomized rats were 1.3 ± 0.5% and −9.7 ± 2.4% (mean ± SEM, $P<0.001$), respectively, plasma T_3 concentrations were 49.1 ± 3.7 ng/dl and <10 ng/dl (undetectable), respectively ($P<0.001$), and plasma T_4 concentrations were 3.4 ± 0.2 μg/dl and <2 μg/dl (undetectable), respectively ($P<0.001$).

Effects of thyroidectomy on plasma TSH and GH concentrations

Plasma TSH concentrations in thyroidectomized rats were noticeably higher than those in sham-operated rats. Mean plasma TSH concentrations in sham-operated and thyroidectomized rats were 2.5 ± 0.1 ng/ml and 24.8 ± 0.5 ng/ml, respectively ($P<0.001$). The pulse frequency in plasma TSH secretory profiles in sham-operated and thyroidectomized rats were 2.8 ± 0.4 pulses/9 h and 3.2 ± 0.4 pulses/9 h, respectively. The pulse amplitude of plasma TSH in thyroidectomized rats was significantly increased at 30.1 ± 0.6 ng/ml compared with 4.0 ± 0.2 ng/ml in sham-operated rats

($P<0.001$).

Plasma GH concentrations in thyroidectomized rats were noticeably lower than those in sham-operated rats. Mean plasma GH concentrations in sham-operated and thyroidectomized rats were 68.6 ± 6.4 ng/m*l* and 21.2 ± 0.6 ng/m*l* ($P<0.001$), respectively. The pulse frequency in plasma GH secretory profiles in sham-operated and thyroidectomized rats were 3 pulses/9 h and 1 pulse/9 h, respectively. The pulse amplitude of plasma GH in thyroidectomized rats was significantly reduced at 34.3 ± 2.1 ng/m*l* compared with 195.6 ± 13.1 ng/m*l* in sham-operated rats ($P<0.001$).

Figure 1 shows the plasma TSH and GH secretory profiles of three individual control rats (rats 1–3) and thyroidectomized rats (rats 6–8). In control rats, plasma TSH secretion was irregularly pulsatile. Plasma GH secretion was also pulsatile, with spontaneous GH secretory bursts, at approximately 3-h intervals, documented in three individual control rats. Again, in three individual thyroidectomized rats plasma TSH secretion was pulsatile, but both the mean concentration and pulse amplitude were higher than those values in control rats. In contrast, the mean level, pulse frequency, and pulse amplitude of plasma GH secretion were decreased in thyroidectomized rats, showing almost a complete loss of the spontaneous GH secretory burst.

TRH and SRIF concentrations in intrahypophysial microdialysates

During the entire duration of the experiment, each rat exhibited the full range of its normal activities, including drinking, feeding, grooming, and sleeping. Dialysate collected from most rats al-

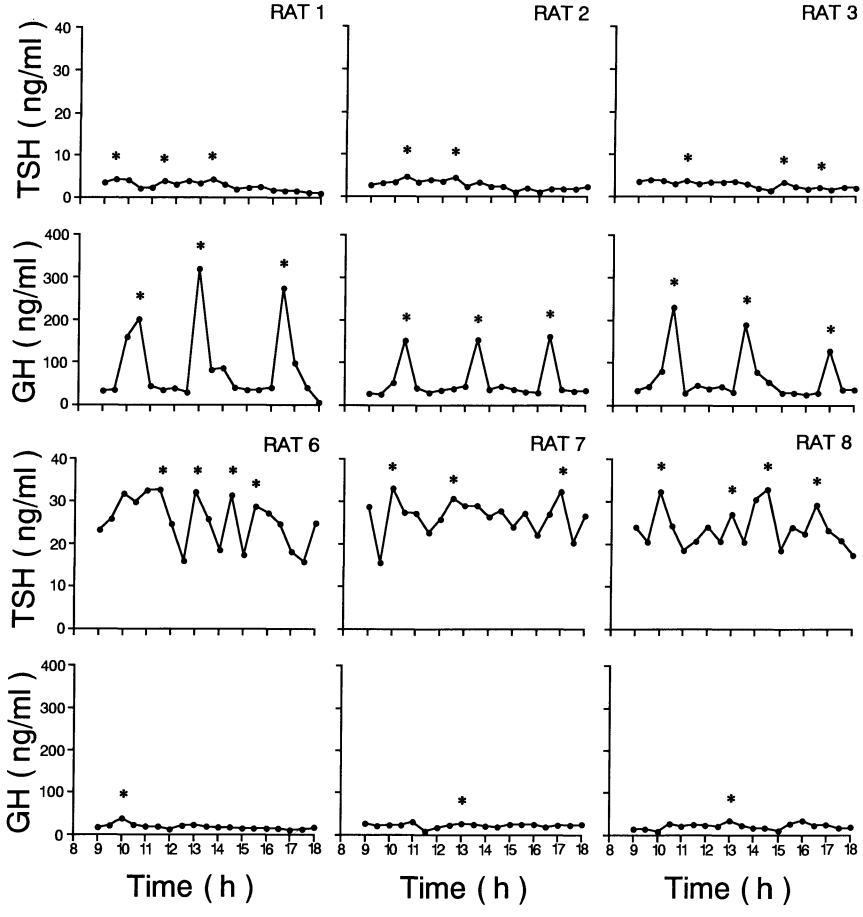

Fig. 1. Changes in plasma TSH and GH concentrations in three individual sham-operated rats (rats 1–3) and three individual thyroidectomized rats (rats 6–8). *, TSH and GH pulses were defined by Cluster analysis.

lowed us to measure TRH and SRIF concentrations but in some rats the position of insertion of the dialysis membrane (fitted into the tip of the probe) into the hypophysis could not be confirmed, and therefore these rats were excluded from the analysis.

TRH concentrations in the dialysates obtained from thyroidectomized rats were higher than those from sham-operated rats. Mean dialysate TRH concentrations in sham-operated rats and thyroidectomized rats were 0.17 ± 0.02 pg/h and 0.43 ± 0.22 pg/h, respectively ($P<0.001$). The pulse frequency in dialysate TRH secretory profiles in sham-operated and thyroidectomized rats were 1.4 ± 0.5 pulses/9 h and 1.8 ± 0.2 pulses/9 h, respectively. The pulse amplitude of dialysate TRH in thyroidectomized rats of 0.51 ± 0.05 pg/h was significantly greater than the value of 0.36 ± 0.02 pg/h in sham-operated rats ($P<0.05$).

SRIF concentrations in the dialysates obtained from thyroidectomized rats were noticeably reduced compared with those from sham-operated rats. Mean dialysate SRIF concentrations in sham-operated rats and thyroidectomized rats were 3.80 ± 0.22 pg/h and 2.45 ± 0.05 pg/h, respectively ($P<0.001$). The pulse frequency in dialysate SRIF secretory profiles in sham-operated was 1.2 ± 0.4 pulses/9 h. The pulse amplitude of dialysate SRIF from sham-operated rats was 5.4 ± 0.4 pg/h. Since the dialysate SRIF concentrations were undetectable in almost all of the thyroidectomized rats, the pulse frequency and amplitude of dialysate SRIF were not estimated.

Figure 2 shows the dialysate TRH and SRIF secre-

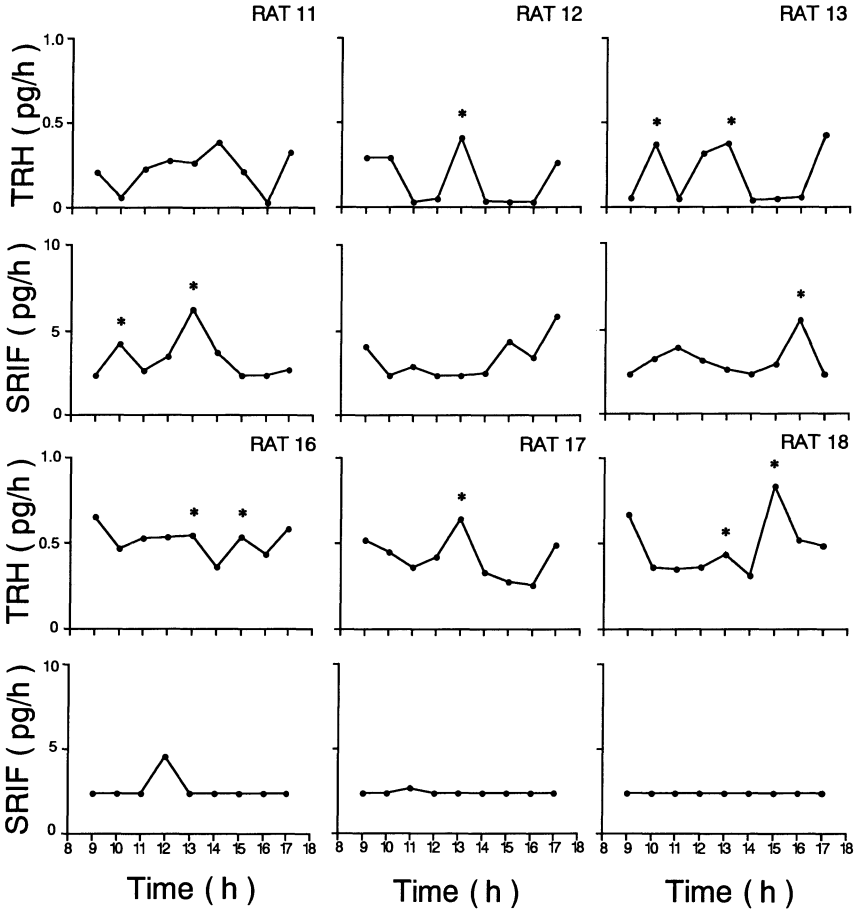

Fig. 2. Secretory profiles of TRH and SRIF in dialysates obtained from three individual sham-operated rats (rats 11–13) and three individual thyroidectomized rats (rats 16–18). Undetectable concentrations in samples represented the least detectable value. *, TRH and SRIF pulses were defined by Cluster analysis.

tory profiles of three individual control rats (rats 11–13) and three individual thyroidectomized rats (rats 16–18). In thyroidectomized rats TRH concentrations were increasing as compared with control rats and SRIF concentrations were undetectable in almost all of samples.

Discussion

Until now, *in vitro* perifusion experiments [12, 13], *in vivo* push-pull perfusion experiments [14], hypophysial portal blood sampling [4, 5, 15, 16] and determination of the expression of hypothalamic hormone mRNA [12, 17] have been performed in order to clarify the secretory profiles of hypothalamic hormones, but it is very difficult to study the secretory profiles of hypothalamic hormones in conscious, freely moving rats. Recently the releases of LHRH [18], corticotropin-releasing hormone (CRH) [19, 20], vasopression [21] and SRIF [6, 22, 23] have been measured by a microdialysis method in rat brain. We also have established a microdialysis method [6], for estimating the dynamics of TRH and SRIF releases under nearly physiological conditions. In the present study, we used a push method to obtain intrahypophysial microdialysate.

Plasma T_3 and T_4 concentrations became undetectable one week after thyroidectomy in thyroidectomized rats, indicating the hypothyroid state. Plasma TSH concentrations in the rats were high, as previously reported [24]. By means of Cluster analysis, a pulsatile pattern was revealed in plasma TSH but no difference was observed between sham-operated and thyroidectomized rats in pulse frequency. But there has been no direct evidence indicating whether the reduction in the thyroid hormone concentration acts directly at the level of the anterior pituitary or the hypothalamus.

In contrast, the mean concentration, pulse frequency and pulse amplitude of plasma GH were decreased in thyroidectomized rats, due to the inability of somatotrophs to secrete GH in the absence of thyroid hormone. Since the GH gene is known to be positively regulated by thyroid hormone [2], we attempted to clarify the participation of TRH and SRIF in the increase in plasma TSH, and the reduction in plasma GH in the hypothyroid state by means of intrahypophysial microdialysis.

The intrahypophysial TRH concentrations in thyroidectomized rats were twice those in sham-operated rats, indicating the increase in TRH release from the hypothalamus in the former. This proves that the negative feedback action of thyroid hormone on TSH secretion is mediated, at least in part, at the level of the hypothalamus. Previous studies suggested that thyroid hormone acts on the pituitary gland by a negative feedback mechanism, but not on TRH release from the hypothalamus. For example, in hypothyroid rats induced by thyroidectomy or antithyroid drug, TRH release into the hypophysial portal blood was not affected [4, 25, 26]. Furthermore, hypothalamic TRH content was unchanged after thyroidectomy [27, 28]. On the other hand, it was reported that uni- or bilateral thyroidectomy caused an increase in TRH in the median eminence 7 days after surgery [29], and thyroidectomy increases TRH in the hypophysial portal blood of rats [30] and sheep [31]. Moreover, the TRH mRNA level increased in the paraventricular nucleus (PVN) of hypothyroid rats where TRH neurons are located [32, 33]. Since TRH neurons have a thyroid hormone receptor [34], thyroid hormone should exert a direct action on TRH release from the hypothalamus. The results of our present study on TRH release in hypothyroid rats are consistent with those of other recent reports.

TRH release from the hypothalamus is reported to be pulsatile [35]. We also found a pulsatile change in the TRH concentration in intrahypophysial microdialysates, but the pulse frequency was less than that documented in a previous report [31] in which hypophysial portal blood was drawn from a ewe. If sampling was performed at shorter intervals, pulse frequency would increase, even in our experiment. Since the pulse amplitude increased, but the pulse frequency did not change, as to TSH and TRH secretion in our thyroidectomized rats, we hypothesize that the thyroid hormone concentration in the blood does not influence the pulse frequency of TSH and TRH secretion.

The SRIF levels in dialysate obtained from our thyroidectomized rats were noticeably reduced compared with those in sham-operated rats, indicating a decrease in SRIF release after thyroidectomy. There have been many reports on SRIF release or tissue content after surgical or

chemical thyroidectomy. SRIF concentrations in the hypophysial portal blood did not change in short-term hypothyroidism [5, 36], but hypothalamic SRIF content decreased in long-term thyroidectomized rats [13]. The SRIF mRNA level in the hypothalamus also decreased in thyroidectomized rats [37]. These findings suggest that the synthesis and secretion of SRIF is reduced in thyroidectomized rats.

The dialysate SRIF pattern was also pulsatile in control rats as reported previously [38] with push-pull perfusion methods. In our study, the pulse frequency and pulse amplitude of dialysate SRIF were not estimated because the dialysate SRIF concentrations were undetectable in almost all of the thyroidectomized rats. It is therefore, unclear whether thyroid hormone participates in pulsatile secretions of SRIF.

In hypothyroidism, the somatotrophs in the anterior pituitary show higher sensitivity to SRIF, but lower sensitivity to GHRH [39]. There have been many reports on the synthesis and secretion of GHRH in the hypothyroid state. Hypothalamic GHRH decreases [39], but GHRH mRNA expression increases in thyroidectomized rats [17], suggesting the participation of GHRH in GH secretion in thyroidectomized rats, but GHRH concentrations in intrahypophysial microdialysates cannot be measured at present because of low recovery rate of GHRH with the microdialysis method. We therefore need to improve microdialysis techniques for the study of GHRH release from the hypothalamus.

It was recently reported that GH exerts negative feedback on GHRH gene expression. GH excess has caused a decrease in the GHRH mRNA level [40], as well as GHRH content and secretion [41] in the hypothalamus. GH deficiency and hypophysectomy caused an increase in GHRH mRNA and a decrease in hypothalamic GHRH with a transient increase in GHRH secretion [42]. In contrast, GH increases SRIF release and mRNA levels in the rat hypothalamus [12]. In our study, plasma GH concentrations were lowered in thyroidectomized rats, although SRIF in dialysates was decreased. A decrease in dialysate SRIF in thyroidectomized rats may be due to a reduction in GH secretion from the pituitary gland.

In summary, TRH release from the hypothalamus is increased in thyroidectomized rats, resulting in the stimulation of TSH secretion. Thyroid hormone deficiency firstly impairs GH release from the anterior pituitary resulting in a decrease in SRIF release from the hypothalamus due to a reduction in GH secretion from the pituitary gland. The nature of the involvement of GHRH in this situation remains to be studied.

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