Tokushima J. exp. Med. 43, 93~100, 1996

THYROTROPIN-RELEASING HORMONE RELEASE IN NORMAL AND HYPERTHYROID RATS AS MEASURED BY MICRODIALYSIS

YASUHIRO OKAUCHI, HIDEO TAKAHASHI, MIKI MIZOBUCHI, HIROSHI BANDO AND SHIRO SAITO*

The 1st Department of Internal Medicine, School of Medicine, The University of Tokushima, Tokushima 770, Japan.
(Accepted: November 1, 1996)

To measure the thyrotropin-releasing hormone (TRH) release from the hypothalamus in euthyroid and hyperthyroid states, we investigated the changes in TRH levels in the anterior pituitary of conscious male rats using an in vivo microdialysis technique. In the euthyroid rats (n=18), TRH levels in the extracellular dialysates of the anterior pituitary varied from 1.0 to 101.0 pg/ml in a pulsatile fashion: 15.9±13.9 (mean±SD) pg/ml with 5.8±1.5 pulses/24 h. In the hyperthyroid rats (n=5) who received L-thyroxine at 10 μg/100 g body weight for 7 days, TRH levels in the dialysates during 6 h was 3.6±1.7 pg/ml, and significantly lower ($P<0.05$) than those of the control rats (15.5±14.1 pg/ml); the pulse frequency was unchanged. These findings demonstrated for the first time the existence of pulsatile TRH release from the hypothalamus, and showed that thyroid hormone inhibits TRH release by the reduction of pulse amplitude, but not of pulse frequency.

Key words: Thyrotropin-releasing hormone (TRH)—Pulsatile secretion —Microdialysis—Hyperthyroidism

Thyrotropin (thyroid-stimulating hormone; TSH) is known to be secreted in a pulsatile fashion in the rat[27,32,42] under the control of hypothalamic thyrotropin-releasing hormone (TRH) and somatostatin (SRIF)[9,13,21,24,34,35]. TRH is a tripeptide [8,33], widely distributed in the central nervous system[4,15,16,26,40]. The synthesis and secretion of TRH in the rat brain have been investigated mainly by measuring the TRH levels in the tissues and hypophysial portal blood[11,14,23,30,38], as well as TRH messenger ribonucleic acid (mRNA) levels in the tissues[18,45,46]. However, since it has been impossible to measure TRH levels in the brain of a conscious rat until recently, the dynamics of TRH release have not been clarified.

A brain microdialysis was recently developed by Ungerstedt et al. and was first applied to the in vivo analysis of monoamines and their metabolites in the rat brain[39]. This technique is beneficial in that substances can be collected via a microdialysis membrane from freely-moving animals with negligible damage on the brain tissue. However, it was not easy to be used in the analysis of neuropeptides mainly due to the low recovery and the insensitivity of the assays. In the present study, we applied this technique to monitor TRH secretion from the hypothalamus of conscious rats, and we investigated TRH release in the euthyroid and hyperthyroid states.

* 岡内泰弘・高橋秀夫・溝渕　樹・板東　浩・齋藤史郎

Materials and Methods

Animals

Male Wistar rats weighing 200–350 g were purchased from Nippon SLC Co. Ltd. (Hamamatsu, Japan). The animals were housed individually in a temperature-(20–22°C) and humidity-(24–25%) controlled room under artificial illumination (light, 9:00–21:00 h; dark, 21:00–9:00 h), and given regular rat chow and water ad libitum.

Microdialysis system

Microdialysis was performed as reported previously[37]. In brief, a microdialysis probe with a polycarbonate (PC) membrane, 2 mm in length (CMA-12 probe, CMA Microdialysis, Stockholm, Sweden), was used. Perfusion was performed using a pulse-free microinfusion pump (EP-60, Eicom Co., Ltd., Kyoto, Japan) and Hamilton gas-tight syringe by the push method. The polyethylene inlet tube (0.28 mm in internal diameter, 1 mm in length) connected the syringe and probe. A teflon-coated outlet tube (0.1 mm in internal diameter; 50 cm in length) connected the probe and the sampling tube. These tubes were confirmed to absorb a negligible amount of TRH.

The influence of perfusion rates on TRH recovery was assessed by measuring relative recovery rates at the flow rates of 1, 2 and 4 μl/min, respectively. The influence of albumin concentration in the perfusion medium was assessed by measuring relative recovery rates using Ringer's solution containing 0, 0.1 and 1% bovine serum albumin (BSA) at a flow rate of 2 μl/min. The influence of the addition of anti-TRH antibody (diluted to 1:80,000) to the perfusate was also examined by measuring recovery rates at a flow rate of 2 μl/min.

For examining the effect of anti-TRH antibody(diluted to 1:80,000) to the perfusate was also examined by measuring recovery rates at a flow rate of 2 μl/min.

For examining the effect of anti-TRH antibody in the perfusate on the relative recovery rate, TRH was dissolved in Ringer's solution to the concentrations of 0.5, 1, 2, 4 and 5 ng/ml, and kept at 37°C. After the probe was inserted into each solution, the dialysate was collected every 30 min, and TRH concentrations were measured by radioimmunoassay (RIA). The relative recovery rate was estimated by the differences in TRH concentrations between the external and internal fluids.

To confirm the capability of this system to detect abrupt changes in TRH concentration in the external fluid, a probe was first inserted into Ringer's solution kept at 37°C for 1h, then transferred to Ringer's solution containing 5 ng/ml TRH kept at 37°C for 2h, and finally transferred to Ringer's solution kept at 37°C. The dialysate was collected every 30 min, and the TRH concentration in each dialysate was measured by RIA.

Surgery

The male rats were anesthesized with pentobarbital sodium (50 mg/kg body weight (BW), intraperitoneally), and placed in a stereotaxic apparatus (David Kopf Instruments, USA). A plastic guide cannula (22-gauge, 10 mm in length, CMA Microdialysis, Stockholm, Sweden) was implanted through a small burr hole in the skull, with its tip just dorsal to the anterior pituitary using the coordinates: 0.9 mm lateral to the midline, 6.0 mm posterior to the bregma, and 9.0 mm ventral to the dura, according to Paxinos and Watson's atlas[28]; the cannula was held in place with stainless cork screws and dental resin. A 22-gauge stainless obdurator was inserted into the end of the guide cannula until the start of brain microdialysis. The animals were allowed to recover for 7 days after he surgery, and given an injection of Cefotetan (20 mg/kg BW) to prevent infection.

Preparation of hyperthyroid rats

To induce hyperthyroidism, each animal was injected subcutaneously with L-thyroxine (T4, 10 μg/100 g BW in saline) daily for 7 days after the surgery. Control animals wre given the same volume of physiological saline for 7 days. Blood samples were drawn before and 7 days after T4 administration, to measure the plasma levels of thyroid hormone and TSH. The L-thyroxine administration ended 24 h before microdialysis.

In vivo microdialysis

Before each experiment, the microdialysis probe was soaked and perfused with Ringer's solution containing 0.1% BSA and rabbit anti-TRH antibody (1:80,000) at a flow rate of 2.0 μl/min for 12h. To proceed the microdialysis, the obturator was removed, and the probe was inserted gently through the guide cannula under ether anesthesia. Sampling was started 12 h after the probe was inserted to minimize the influence of the probe insertion on TRH secretion. Every dialysate was collected in ice-cooled polystyrene tubes every 30 min and stored frozen at −30°C until determination of TRH levels.

Hormone assays

Synthetic TRH was purchased from Peptide Institute, Inc. (Osaka, Japan) and anti-TRH rabbit antiserum was provided by Mitsubishi Yuka Bio-Clinical Laboratories, Inc. (Tokyo, Japan). TRH levels in the dialysates were determined by RIA, as described previously[1,19]. The sensitivity of the assay was 1 pg/ml, which corresponded to 95% of total binding. The intra-assay and inter-assay coefficients of variation at a concentration of 10 pg/ml level were 3.0 and 12.6%, respectively. In the present study, a perfusion medium containing anti-TRH antibody was used in the microdialysis, to provide a better estimate of TRH concentration in the dialysates, as Pich et al. reported recently[29]. The perfusate containing anti-TRH antibody was used for construction of the standard curve of TRH RIA.

Plasma rat TSH (rTSH) was determined by an RIA kit supplied by National Institute of Diabetes and Digestive, and Kidney Diseases (NIDDK, U.S.A.). This kit consists of rTSH antigen (NIDDK-rTSH-1–9), anti-rTSH rabbit antiserum (NIDDK-anti-rTSH-RIA-6), and rTSH reference preparation (NIDDK-rTSH-RP-3). Plasma T3 and T4 concentrations were determined by commercial RIA kits (SPAC T3 and T4 RIA Kits, Daiichi Radioisotope Laboratories, Ltd., Tokyo, Japan).

Histological examination

After the completion of microdialysis, the animals were anesthesized by pentobarbital sodium (50 mg/kg BW) and sacrificed by intracardiac perfusion of buffered 10% formaldehyde. Formalin-fixed brain sections (30 μm thick) were prepared and stained with Cresyl Violet to confirm the location of the microdialysis probe.

Statistical analysis

Data are presented as mean±SD. Analysis of variance followed by Students'*t* tests were used to assess significant differences. differences were considered to be significant at $P<0.05$.

Pulsatility of TRH secretion was examined by cluster analysis[4]. A cluster size of 2 by 2, and a *t* statistic of 2.28 were used as parameters for analysis of episodic hormonal data. A pulse was considered significant if the percent increase in the TRH levels from the nadir to the peak was greater than or equal to 2.28 times to the intraassay coefficient of variation, which limited the false positive rate to 2%.

Results

Recovery rate of TRH in vitro

The relative recovery rates at a flow rate

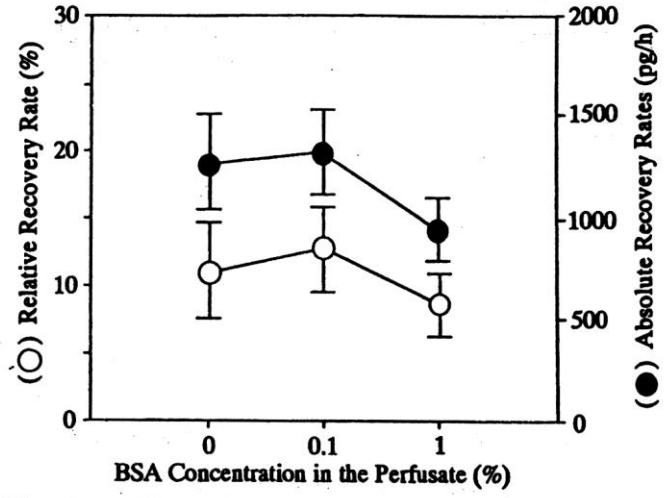

Fig. 1. Effect of the concentration of bovine serum albumin (BSA) in the perfusate on the relative recovery rate of TRH in vitro. The relative recovery rates (open circle) and the absolute recovery rates (closed circle) of TRH are plotted vs. BSA concentration in the perfusate. Values represent the means±SD of four measurements.

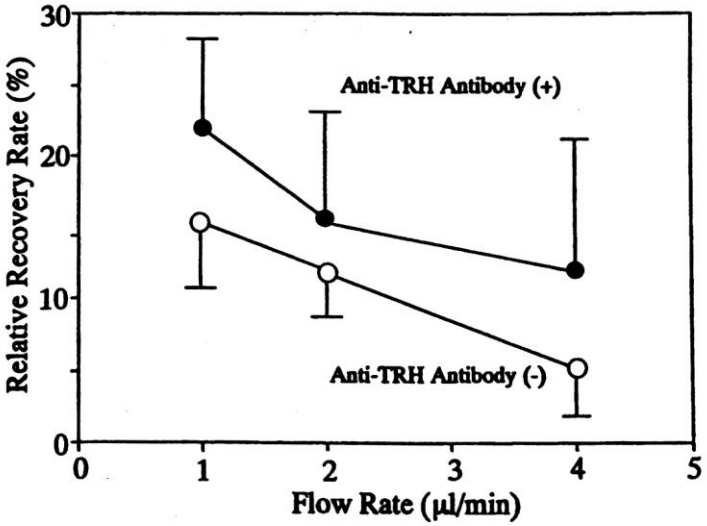

Fig. 2. Effect of anti-TRH serum (1:80,000) in the perfusate on the relative recovery rate in vitro. The relative recovery rates of TRH are plotted vs. flow rates in the absence of anti-TRH antibody (open circles), and in the presence of anti-TRH serum (closed circles). Values are represented as the means±SD of four measurements.

of 2 μl/min varied with the concentrations of BSA in Ringer's solution: 10.4±3.4% at 0% BSA, 11.9±3.0% at 0.1% BSA, and 8.2±2.1% at 1% BSA, respectively. The absolute recovery rates were 1250±410 pg/h at 0% BSA, 1430±360 pg/h at 0.1% BSA,

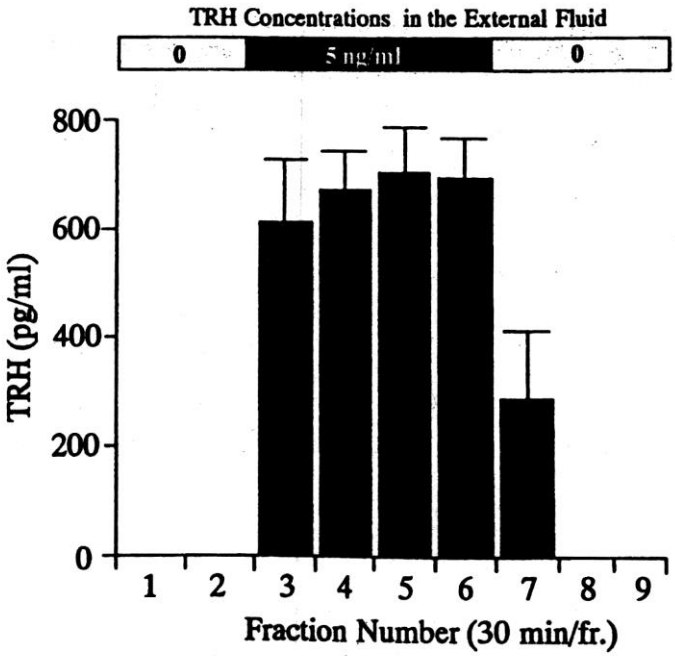

Fig. 3. In vitro recovery of TRH during rapid changes of TRH concentrations in the external fluids. Ringer's solution containing 0.1% BSA and anti-TRH serum (1:80,000) was perfused at a flow rate of 2.0 μl/min. Each fraction was collected every 30 min. Values represent the mean±SD of four measurements.

and 980±260 pg/h at 1% BSA, being maximal at 0.1% BSA (Fig. 1).

The relative recovery rates at the flow rates of 1, 2 and 4 μl/min in the absence of anti-TRH antibody were 15.1±3.2, 11.9±3.0 and 5.2±2.8%, respectively (Fig. 2). The absolute recoveries were 910±190, 1430±360 and 1240±670 pg/h, respectively, being maximal at 2 μl/min. The addition of anti-TRH antibody to the perfusion medium significantly improved TRH recovery at all of the three flow rates (Fig. 2).

The capability of our microdialysis system to detect rapid changes in the TRH concentration of the external fluid is shown in Fig. 3. The TRH concentrations in the dialysates corresponded quickly to the changes in TRH concentrations in the external fluid between 0 and 5 ng/ml. The slightly retarded increase in the TRH levels of the fractions 3 and 4 might be due to a time lag when the perfusate passed through the outlet tubing.

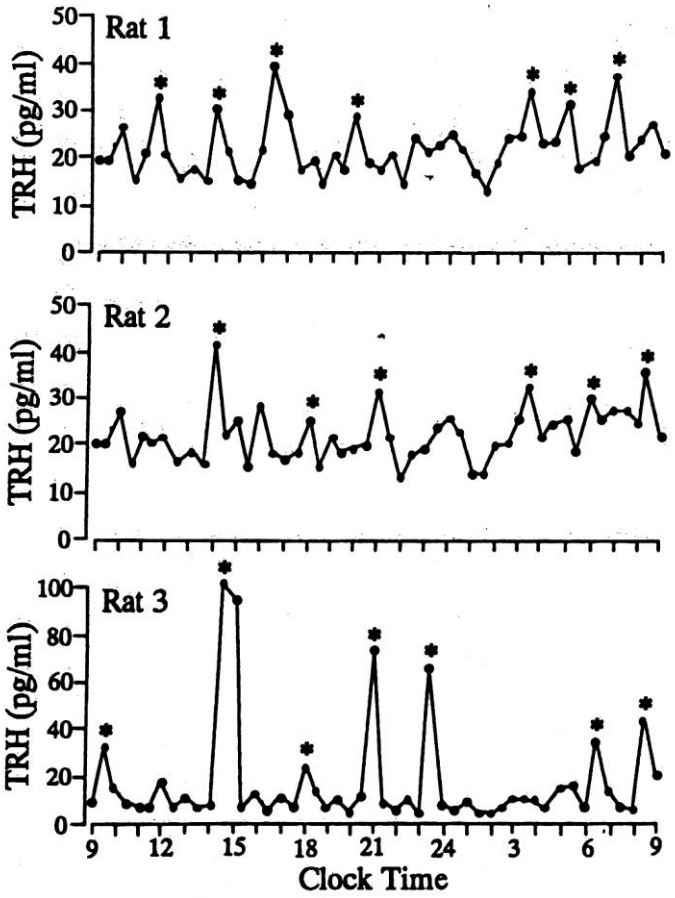

Fig. 4. Profiles of TRH release in a 24-h period in three representative euthyroid rats (rat 1–3). Closed circles represent the extracellular TRH concentrations in the anterior pituitary. Asterisks indicate the TRH pulses detected by cluster analysis.

Extracellular TRH levels in the rat anterior pituitary

Profiles of TRH release in three euthyroid rats are shown in Fig. 4. TRH levels in the dialysates varied in a pulsatile fashion with a frequency of 5.8±1.5 pulses/24 h (n=18). The peak levels were 25.7–103.7 pg/ml, and the nadir levels were 1.0–13.1 pg/ml. The mean TRH levels in euthyroid rats were 15.9±13.9 pg/ml (n=18).

Effects of T4 administration on TRH release

Plasma T3, T4 and TSH concentrations were 171.8±35.5 ng/ml, 7.93±1.96 μg/dl,

Table 1.
Plasma T_3, T_4 and TSH levels in rats treated with L-thyroxine

	T_3 (ng/ml)	T_4 (μg/dl)	TSH (ng/ml)
Control	66.2±11.4	3.24±0.78	2.28±0.93
T_4-Treated	171.8±35.5[a]	7.93±1.96[a]	0.72±0.39[a]

Each value represents the mean±S.D. (n=5).
[a] $P<0.05$ vs. control.

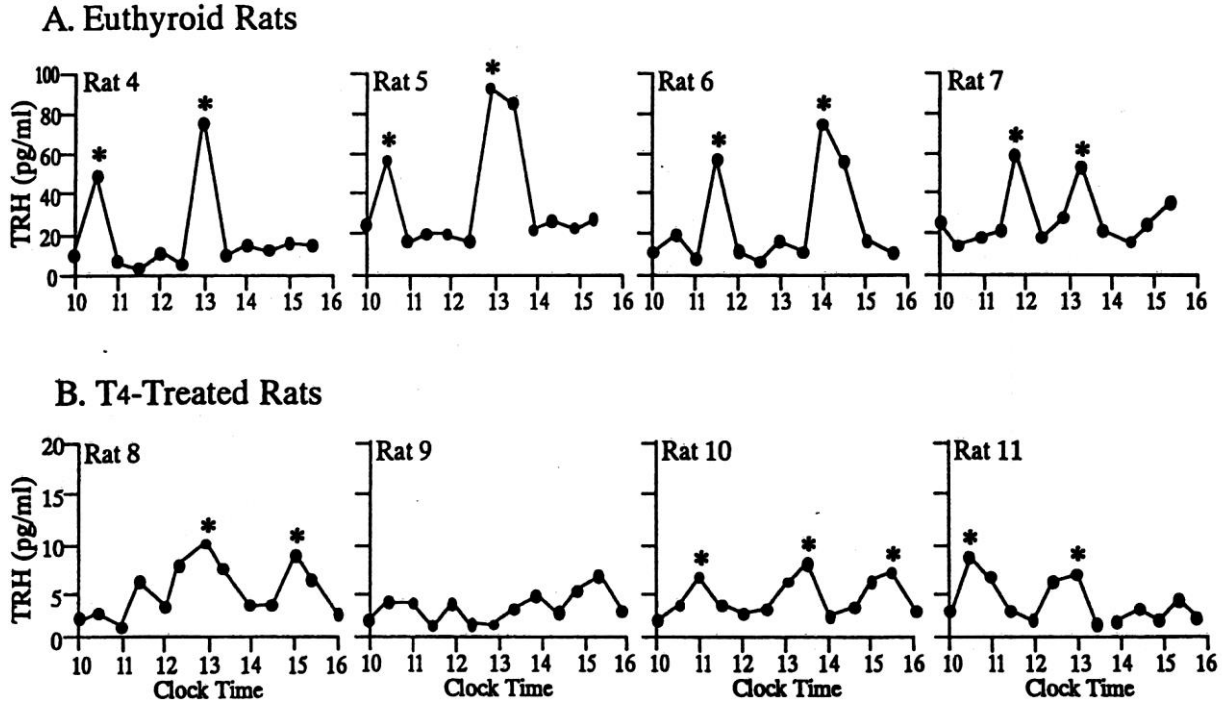

Fig. 5. Profiles of TRH release in a 6-h period in four euthyroid (A: rat 4–7) and hyperthyroid (B: rat 8–11) rats. Data represent extracellular TRH concentrations in the pituitary. Asterisks indicate the TRH pulses detected by cluster analysis.

Table 2.
The mean and peak TRH levels and pulse frequency in the dialysates of extracellular fluid of the anterior pituitary detected in euthyroid and hyperthyroid rats.

Thyroid status	Mean level (pg/ml)	Peak level (pg/ml)	Pulse frequency (pulse/6 h)
Euthyroid	15.5±14.1	52.9±49.0	1.4±0.53
Hyperthyroid	3.57±1.67[a]	6.26±2.81[a]	0.80±0.40

Each value represents the mean±SD (n=5).
[a]$P<0.05$ vs. euthyroid rats.

and 0.72±0.39 ng/ml, respectively in T4-treated rats, manifesting the hyperthyroid state (Table 1). Profiles of TRH release were studied during 6h in the light, on the basis that pulsatile release of TRH showed no circadian fluctuation in 24 h. Profiles of TRH release in four T4-treated rats shown in Fig. 5. TRH release could be detected with a frequency of 0.8±0.4 pulses/6h (n=5), which was not significantly different from the control value. The peak and mean TRH levels were 6.26±2.81 and 3.57±1.67 pg/ml (n=5), respectively, significantly lower than those of the control animals: 52.9±49.0 and 15.5±14.1 pg/ml (n=5), as shown in Table 2.

Discussion

Until recently, the application of the microdialysis technique was limited to monitoring the secretory profiles of small molecules[39]. Kendrick and Levine first applied this technique to estimate neuropeptide secretion, such as oxytocin and luteinizing hormone-releasing hormone[20),22)]. Recent technical advances in the microdialysis system have made it possible to detect the release of small peptides, including insulin-like growth factor-1, corticotropin-releasing hormone and somatostatin[29,37,47)]. Waterfall et al. used microdialysis to measure TRH levels in the rat hypothalamus for the first time[43)], and Okuda et al. demonstrated by microdialysis that the TRH concentration in the septum of the rat brain increased after withdrawal of 30% of the total blood volume[25)].

In the present study, we examined the optimal conditions of in vivo microdialysis for monitoring TRH release, concerning flow rate, perfusion medium and connecting tubes. As a result, the maximal recovery of TRH was obtained at a flow rate of 2 μl/min with a perfusion medium containing 0.1% BSA. The addition of anti-TRH antibody in the perfusion medium significantly improved the recovery rate. Because it has been supposed that TRH bound to anti-TRH antibody in the perfusion medium could not move across the microdialysis membrane, nor degradate easily. We used anti-TRH antibody at three different dilution rates (1:20,000, 1:40,000, and 1:80,000). TRH RIAs are most sensitive with anti-TRH antibody (diluted to 1:80,000) (data not shown). Therefore we decided to use anti-TRH antibody (diluted to 1:80,000). The connecting tubes and teflon-coated outlet tubes we used proved to be best due to the advantages of low perfusion resistance, little adsorption of peptides to the tubes and the minimal restraint of the rats (data not shown).

TRH is widely distributed in the rat brain, and TRH synthesized in the parvocellular subdivision of the hypothalamic paraventricular nucleus is released into the hypophyseal portal vein, resulting in TSH release from the anterior pituitary [4,15,16,26,40)]. We implanted the microdialysis probe in the anterior pituitary because of minimal damage on the TRH-synthesizing neurons in favor of the recovery of peptides[20),22)]. Histological examinations after the experiments confirmed that the microdialysis probes were placed precisely in the anterior pituitary with little damage or gliosis.

Hypophyseal TSH is secreted in a pulsatile fashion with a circadian rhythm[2,3,6,32)]. TRH is thought to play an important role in

the generation of pulsatile TSH secretions, because the administration of anti-TRH antibody attenuates the secretory profile of TSH[21,24,34,35]. However, there has been little direct evidence showing the pulsatile secretion of hypothalamic TRH. In the present study, we directly demonstrated for the first time that the TRH levels in the antrerior pituitary varied in a pulsatile fashion in conscious male rats. The pulse frequency of the TRH secretion was calculated by cluster analysis as 5.8±1.5 pulses/24 h, lower than that of the TSH pulse frequency (2.8 pulses/h)[6]. This difference may be derived from 1) the difference in sampling interval, 2) the effects of other factors on TSH secretion and 3) the difference in the pulse detection methods. TSH secretion is also known to be regulated by catecholamine and somatostatin, as well as TRH[9,12,13,24,30,44]. Catecholamine released from the hypothalamus mainly regulates the circadian rhythm of TSH secretion. In the present study, TRH secretion did not exhibit a circadian rhythm under the 12 h light and 12 h dark condition. Therefore, catecholamine may regulate TSH secretion independently of TRH. In addition, we detected a secretory profile of somatostatin from the hypothalamus with a circadian rhythm[36]. However, the mechanism of the interaction of these factors and their influence on TSH release remain to be studied.

Recent studies using immunohistochemistry and in situ hybridization demonstrated that pro-TRH mRNA and pro-TRH-derived peptides are exppressed in the anterior pituitary[5,7,1017]. The anterior pituitary cells in themselves have rhythmicity in their hormone secretion[42]. TRH-like peptides in the anterior pituitary may be involved in the secretory pulse of TSH. However, a previous study found that TRH concentration in the hypothalamic tissues were 255 pg/mg and peak TRH concentrations in the hypophyseal microdialysates (25.7–103.7 pg/ml) in the present study were much higher than that in the antrerior pituitary (2.2 pg/mg)[26]. Therefore, further studies are required to determine the significance of TRH-like peptides in the anterior pituitary on TRH release.

In the present study, in the T4-treated rats, the peak TRH levels in the intrahypophysial dialysates were decreased to 11% of those in the control animals, indicating the direct suppression of TRH secretion by thyroid hormone. Rondeel et al.previously reported that the TRH content of the hypothalamic tissues of the T4-treated rats was decreased to 45% of the content in the control animals[31], in accordance with our results. The peak levels of TRH in the intrahypophysial dialysates were reduced significantly in the T4-treated rats, but the pulse frequency was unchanged, suggesting that thyroxine inhibits TRH release mainly by suppressing the pulse amplitude but not the pulse frequency.

In preliminaly study, we administered 0.05% methimazole (MMI) in drinking water to each animal for 21 days to induce hypothyroidism, and performed in vivo microdialysis. TRH release could be detected with a frequency of 1.4±0.5 pulses/6h (n=5), which was not significantly different from the control value. These data were supported our results in this study. Meanwhile the peak and meaan TRH levels were not significantly higher than those of control animals. We assumed that stimulation of TRH release is attenuated by feedback of elevated TSH levels in MMI-induced hypothyroidism. We are studying further on TRH secretory profiles in various hypothyroid conditions.

In summary, we demonstrated, using the microdialysis technique, that TRH was released in a pulsatile fashion from the hypothalamus into the aanterior pituitary in conscious male rats, and that thyroid hormone inhibits the amplitude, but not the pulse frequency of TRH secretion.

Acknowledgements

We thank Mitsubishi Yuka Bio-Clinical Laboratories, Ltd. for a kind supply of anti-TRH rabbit antibody, the National Institute of Diabetes and Digestive, and Kidney Diseases for providing the materials for rTSH RIA, and Professor Y. Morita, Department of Physiology, School of Medicine, University of Tokushima, for valuable technical help. This work was supported in part by a Grant-in-Aid for Research on Intractable Diseases, from the Ministry of Health and Welfare of Japan, and by a grant from The Foundation of Growth Science.

References

1) Bassiri, R. M., & Utiger, R. D.: Endocrinology **90**: 722 (1972)
2) Brabant, G., Ranft, U., Ocran, K., Hesch, R. D., & von zur Mühlen, A.: Acta Endocrinol. **112**: 315 (1986)
3) Brabant, G., Prank, K., Hoang-Vu, C., Hesch, R. D., & von zur Mühlen, A.: J. Clin. Endocrinol. Metab. **72**: 145 (1991)
4) Brownstein, M., Palkovits, M., Saavedra, J. M., Bassiri, R. M., & Utiger, R. D.: Science **185**: 265 (1974)
5) Bruhn, T. O., McLean, D. B., Bolduc, T. G., & Jackson, I. M. D.: Endocrinology **129**: 556 (1990)
6) Bruhn, T. O., McFarlane, M. B., DEckey, J. E., & Jackson, I. M. D.: Endocrinology **131**: 2615 (1992)
7) Bruhn, T. O., Rondeel, J. M. M., Bolduc, T. G., & Jackson, I. M. D.: Endocrinology **134**: 85 (1994)
8) Burgus, R., Dunn, T. F., Desiderio, D., Ward, D. N., Vale, W., & Guillemin, R.: Nature **226**: 321 (1970)
9) Chihara, K., Arimura, A, Chihara, M., & Schally, A. V.: Endocrinology **103**: 1916 (1978)
10) Childs, G. V., Cole D. E., Kubek, M., Tobin, R. B., & Wilber J. F.: J. Histochem. Cytochem. **26**: 901 (1978)
11) Ching, M. C. H., & Utiger, r. D.: J. Endocrinol. Invest. **6**: 347 (1983)
12) Dahl, G. E., Evans, N. P., Thrun, L. A., & Karsch, F. J.: Endocrinology **135**: 2392 (1994)
13) Ferland, L., Labrie, F., Jobin, M., Arimura, A., & Schally, A.V.: Biochem. Biophys. Res. Commun. **68**: 149 (1976)
14) Fink, G., Koch, Y., & Aroya, N. B.: Brain Res. **243**: 186 (1982)
15) Jackson, I. M. D., & Reichlin, S.: Endocrinology **95**: 854 (1974)
16) Jackson, I. M. D., & Lechan, R. M.: Endocrinology **111**: 55 (1982)
17) Jackson, I. M. D., Bruhn, T. O., & Bolduc, T. G.: Clin. Res. **40**: 281A (Abstract) (1992)
18) Kakucska, I, Rand, W., & Lechan, R. M.: Endocrinology **130**: 2845 (1993)
19) Kamijo, K., Sato, M., Taniuchi, A., Watanabe, Y., Kurimoto, F., & Sakurai, H.: Nippon Naibunpi Gakkai Zasshi (in Japanese) **37**: 333 (1989)
20) Kendrick, K. M.: Methods Enzymol. **168**: 182 (1989)
21) Koch, Y., Goldhaber, G., Fireman, I., Zor, U., Shani, J., & Tal, E.: Endocrinology **100**: 1476 (1977)
22) Levine, J. E., & Powell, K. D.: Methods Enzymol. **168**: 166 (1989)
23) Mori, M., & Yamada, M.: J. Endocrinol. **114**: 443 (1987)
24) Morley, J. E.: Endocr. Rev. **2**: 396 (1981)
25) Okuda, C., Sawa, T., Harada, M., & Miyazaki, M.: Life Sci. **47**: 1997 (1990)
26) Oliver, C, Eskay, R. L., Ben-Jonathan, N., & Porter, J. C.: Endocrinology **95**: 540 (1974)
27) Ottenweller, J.E., & Hedge, G. A.: Endocrinology **111**: 509 (1982)
28) Paxinos, G., & Watson, C.: The rat brain in stereotaxic coordinates, 2nd ed., Academic Press, New York (1986)
29) Pich, E. M., Koob, G. G., Heilig, M., Menzaghi, F., Vale, W., & Weiss, F.: Neuroscience **55**: 695 (1993)
30) Rondeel, J. M. M., DeGreef, W. J., Van der Schoot, P., Karels, B., Klootwijk, W., & Visser, T. J.: Endocrinology **123**: 523 (1988)
31) Rondeel, J. M. M., de Greef, W. J., Klootwijk, W., & Visser, T. J.: Endocrinology **130**: 651 (1992)
32) Rousett, J. B., Perrin, F., Fournier, M., & Orgiazzi J.: Endocrinology **107**: 1245 (1980)
33) Schally, A.V., Redding, T.W., Bowers, C.Y., & Barrett, J.F.: J. Biol. Chem. **244**: 4077 (1969)
34) Szabo, M., & Frohman, L. A.: Endocrinology **101**: 1023 (1977)
35) Szabo, M., Kovathana, M., Gordon, K., & Frohman, L. A.: Endocrinology **102**: 799 (1978)
36) Takahashi, H., Shintani, Y., Okauchi, Y., Ishikawa, M., Bando, H., & Saito, S.: Nippon Naibunpi Gakkai Zasshi (in Japanese) **68**: 808 (Abstract) (1992)
37) Takahashi, H., Shintani, Y., Okauchi, Y., Ishikawa, M., Bando, H., Azekawa, T., Morita, Y., & Saito, S.: J. Neurosci. Methods **52**: 33 (1994)
38) Thomas, G. B., Cummins, J. T., Yao, B., Gordon, K., & Clark, I. J.: J. Endocrinol. **117** (1): 115 (1988)
39) Ungerstedt, U.: Measurement of Neurotransmitter Release in Vivo. pp. 81, John wiley & Son, New York (1984)
40) Utiger, R. D., & Winokur, A.: Science **185**: 265 (1974)
41) Veldhuis, J. D., & Johnson, M. L.: Am. J. Physiol. **250**: E486 (1986)
42) Veldhuis, J. D., Carlson, M. L., & Johnson, M. L.: Proc. Natl. Acad. Sci. USA **84**: 7686 (1987)
43) Waterfall, A. H., Clarke, R. W., & Bennett, G. W.: Proceedings of the Physiological Society, Nottingham Meeting, 19–20 April (1990) (Abstract)
44) Wong, C. C., Dohler, K. D., Atkinson, M. J., Geerings, H., Hesch, R. D., & von zur Muhlen, A.: J. Endocrinol. **102**: 377 (1983)
45) Yamada, M., & Wilber, J. F.: Neuropeptides **15**: 49 (1990)
46) Yamada, M., Satoh, T., Monden, T., Murakami, M., Iriuchijima, T., Wilber, J. F., & Mori M: Neuroendocrinology **55**: 317 (1992)
47) Yamaguchi, F., Itano, T., Miyamoto, O., Janjua, N. A., Ohmoto, T., Hosokawa, K., & Hatase, O.: Neurosci. Lett. **128**: 273 (1991)