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Thyrotropin-Releasing Hormone (TRH) Changes in the Pancreas, Gastrointestinal Tract, Hypothalamus and Plasma of Streptozotocin Diabetic Rats

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Summary. **Thyrotropin-releasing hormone (TRH) was measured in the pancreas, gastrointestinal tract and hypothalamus of streptozotocin diabetic rats. While glucagon concentrations in the pancreas were similar between normal and diabetic rats, TRH and insulin were remarkably reduced in the diabetic pancreas, suggesting TRH presence in B-cells of the pancreatic islets. In diabetic rats, plasma T_3 and T_4 levels were remarkably reduced. However, TRH concentrations in the gastrointestinal tract, hypothalamus and plasma were similar for diabetic and normal animals.**

INTRODUCTION

In a previous work, we examined the spontaneous motor activity and the contractility of intestinal smooth muscles of the streptozotocin (STZ) diabetic rats to acetylcholine and substance P, and found a decreased spontaneous motor frequency and decreased responses to both agents.[1,2] We therefore measured the substance P and somatostatin contents of the gastrointestinal tract of the STZ diabetic rats, and discovered some changes in their contents.[3]

Thyrotropin-releasing hormone (TRH) occurs in the central nervous system and also in the gastrointestinal tract.[4-6] TRH is suggested to contribute to the gastrointestinal motor activity either directly or indirectly via the central nervous system or via the myenteric plexus.[7-9] Diabetic gastrointestinal disorders are well known.[10-12] However, little is known about TRH changes in the gastrointestinal tract of diabetic rats. It has been reported that diabetic rats suffer from hypothyroidism,[13-16] and it has been suggested that the reduction of hypothalamic TRH release is a primary cause of pituitary-thyroid alteration.[14-16] However, the hypothalamic TRH content has been reported to be unchanged[16] or decreased.[14,15] Therefore we are interested in changes in the TRH concentration, if any, in plasma, in the central nervous system, and in the gastrointestinal tract of STZ diabetic rats.

MATERIALS AND METHODS

Materials and methods were similar to those reported in our previous studies.[1-3] Male Wistar rats of 7 weeks' age were obtained from a commercial source. They were injected with 60 mg/kg STZ (Sigma, U.S.A.) intravenously dissolved in citrate buffer (50 mM, pH=4.4), under light ether anesthesia. Controls were injected with only the vehicle, the citrate buffer of the equivalent volume. The animals were maintained with free access to food and water.

At 10 days, 1 month and 3 months after STZ injection, the animals were decapitated and the blood from the trunk was collected into a heparinized beaker kept in ice-water. Two ml aliquot was used for determination of the blood glucose, 3,3'5-triiodothyronine (T_3) and thyroxine (T_4). The remaining blood was transferred into a chilled glass tube and mixed with 1/20 volume of 500 mM EDTA and 100 mM FOY for the determination of plasma TRH. Plasma was separated by centrifugation at 3000 rpm at 4℃ for 20 min. Separated plasma was stored at −30℃ until use.

The hypothalamus was dissected according to the methods by J. Glowinski and L. L. Iversen.[17] The gastrointestinal tract was divided into the gastric fundus, the remaining portion of the stomach, duode-

num, jejunum, ileum, proximal colon and the caecum. The pancreas was also dissected out, adipose tissue thereby being removed as much as possible. The tissues were quickly rinsed with Krebs-Ringer solution and frozen in a dry-ice acetone mixture. Thereafter their wet weight was measured and stored at −30℃ until extraction.

TRH, insulin and glucagon were extracted by 10 volumes of 80% cold acetone by means of homogenization and centrifugation at 3000 rpm at 4℃ for 20 min. Supernatant was defatted with petroleum ether and evaporated to dryness in a centrifugal freeze dryer.

Thyroid hormones, insulin and glucagon were measured by using commercial radioimmunoassay (RIA) kits, Amersham (England), Daiichi radioisotope Institute and Eiken Kagaku (Japan), respectively. The methods of TRH, insulin and glucagon RIA were similar to those in previous studies[18-20] Highly specific TRH antiserum was kindly provided by Dr. S. Saito.[21] Extracted TRH from each tissues was confirmed to be identical to synthetic TRH by demonstrating the parallelism of tissue extracts to the TRH standard curve in RIA and the elution time through the Sephadex G-10 column and RIA of each fractions, as shown in Figs. 1 and 2.

Statistical comparison between two groups was made by Student's t-test.

RESULTS

Body weight, plasma glucose, T_3, T_4 and TRH levels are listed in Table 1. Plasma T_3 and T_4 levels were statistically lower than those in normal rats. However, plasma TRH levels were similar between dia-

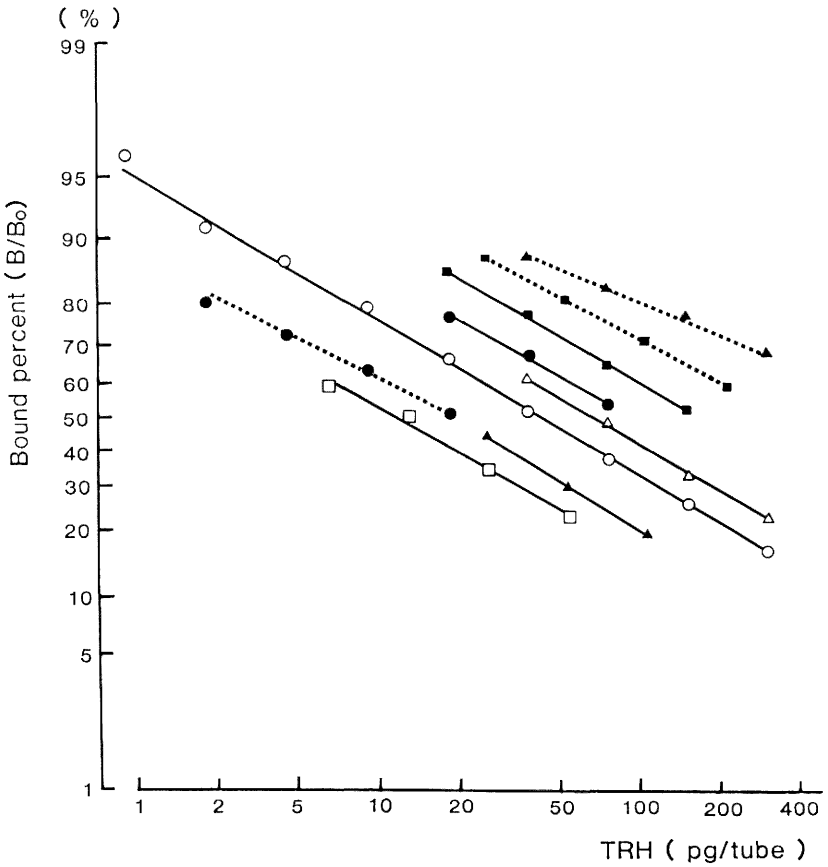

Fig. 1. A typical standard curve for TRH (○——○) and radioimmunoassay of serial dilutions of extracts of plasma (●——●), hypothalamus (△——△), extrahypothalamic brain (▲——▲), pancreas (□——□), stomach (■——■), small intestine (●·····●), large intestine (■·····■), and caecum (▲·····▲).

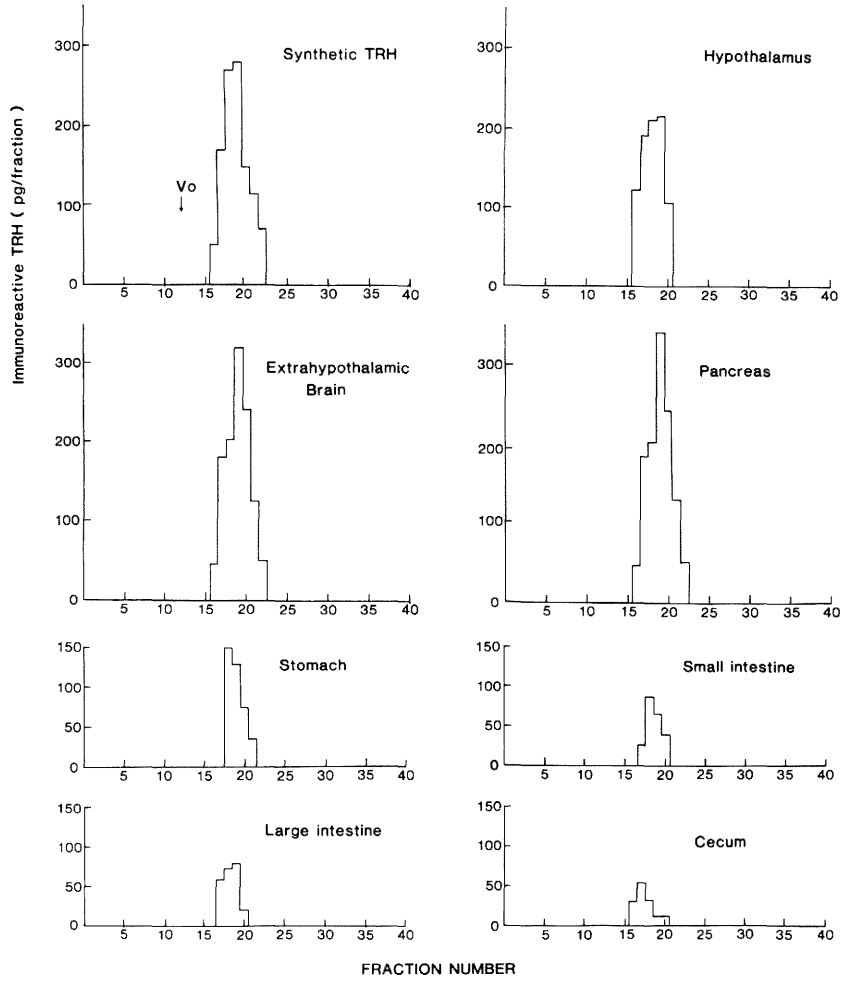

Fig. 2. Elution patterns of synthetic TRH and tissue extracts on the Sephadex G-10 column. A 1.1×56 cm column was eluted with 0.01M phosphate buffer containing 0.14 M NaCl and 0.1% bovine serum albumin, pH=7.4. Forty drops were collected, and the TRH in each fraction was determined by radioimmunoassay. The void volume (Vo) is shown on the diagram.

betic and normal animals.

Distension of the large and small intestines and the presence of pasty unform stools were noticed upon opening the abdomen in the diabetic rats. Wet weights of the various parts of the gastrointestinal tract are given in Table 2. Pancreatic weight decreased at 10 days after STZ administration. The wet weight of the diabetic pancreas recovered thereafter and remained similar to that of normal rats. Generally speaking, the wet weight of the gastrointestinal tract gradually increased over 3 months. A remarkable increase in wet weight was found in the caecum and the duodenum at already 10 days after STZ administration. As for the stomach, enlargement was found not in the fundus but rather in the other remaining portion of the stomach.

Insulin, glucagon and TRH concentrations of the pancreas are listed in Table 3. Insulin and TRH concentrations dramatically decreased concomitantly in the diabetic pancreas as early as 10 days after STZ administration. Glucagon concentrations were similar between diabetic and normal rats.

TRH concentrations of various gastrointestinal parts and the brain are shown in Table 4. There were no statistical differences in TRH concentrations between diabetic and normal gastrointestinal tracts and brains.

Table 1. Body weight (BW), blood glucose (BG) and plasma T_3, T_4 and TRH levels of diabetic (DM) and age-matched controls (Normal)

		Normal	DM
BW (g)	10 days	370± 8.4	320± 9.5**
	1 month	462±17.7	316± 9.8***
	3 month	538±19.3	301±15.0***
BG (mg/dl)	10 days	157±2.6	710±44.4***
	1 month	155±2.6	752±55.7***
	3 months	141±3.1	768±26.8***
T_3 (ng/dl)	10 days	48.0±4.20	26.0±1.40**
	1 month	46.2±2.67	35.6±3.17*
	3 months	54.6±4.29	43.2±1.63*
T_4 (μg/dl)	10 days	9.2±0.62	3.0±0.44***
	1 month	9.4±0.47	4.1±0.77***
	3 months	9.0±0.41	2.7±0.53***
TRH (pg/ml)	10 days	5.4±1.16	5.3±1.49
	1 month	———	———
	3 months	5.2±1.05	5.6±1.13

Values are expressed as the mean±SE in each group of 5 rats. Statistical differences between diabetic and age-matched control rats are shown by *p<0.05, **p<0.01, ***p<0.001.

DISCUSSION

TRH concentrations in the brain and gastrointestinal tract as well as the pancreas vary, according to the references so far reported.[4-6,22] Such disparity does not seem easily settled or compromised at once. Perhaps, the deviating results may be derived from different extraction methods (90% methanol, 90% ethanol, 1 N acetic acid, phosphate buffer and perchloric acid) and different sensitivities to the TRH antibodies used. It seems worthy of note that our values of TRH concentration in the rat hypothalamus, extrahypothalamic brain and gastric fundus extracted with 80% acetone are similar to those reported by Engler et al.[22] who used 1 N acetic acid extraction.

High TRH contents of the pancreas have been reported,[4,5] with especially high values in neonatal or developing rat pancreas.[22,23] The marked decrease in the TRH content in the pancreas of STZ diabetic rats suggests its presence in the pancreatic islets of Langerhans and, more precisely, in beta cells with RIA[6,24] or with immunohistochemistry.[25,26] We were able to confirm this view from the present observation of the associated marked reduction of insulin and TRH in the pancreas.

It is well known that hypothyroidism, low plasma T_3, T_4 and thyroid-stimulating hormone (TSH), are

Table 2. Table 2. The wet weight of the pancreas and the various parts of gastrointestinal tract

		Normal	DM
Pancreas (g)	10 days	1.58±0.051	1.33±0.049**
	1 month	1.50±0.093	1.68±0.096
	3 months	1.45±0.060	1.58±0.186
Stomach fundus (mg)	10 days	368±22	340±9
	1 month	372±17	332±19
	3 months	332±14	388±25
Stomach extrafundus (g)	10 days	1.09±0.051	1.09±0.066
	1 month	1.14±0.052	1.24±0.019
	3 months	1.19±0.019	1.47±0.095*
Duodenum (mg)	10 days	444±13	580±21***
	1 month	440±28	660±9***
	3 months	420±25	640±60**
Jejunum (mg)	10 days	372±21	484±12**
	1 month	340±25	588±42
	3 months	328±14	392±20
Ileum (mg)	10 days	276±18	352±34
	1 month	304±7	384±28
	3 months	276±27	372±36
Proximal colon (mg)	10 days	432±15	472±16
	1 month	412±27	532±23
	3 months	388±14	588±47*
Caecum (g)	10 days	1.06±0.064	1.68±0.067***
	1 month	1.10±0.067	2.85±0.186***
	3 months	1.07±0.059	3.17±0.335**

Values are expressed as the mean±SE in each group of 5 rats. Statistical differences between diabetic and age-matched control rats are shown by *p<0.05, **p<0.01, ***p<0.001. Wet weights of the duodenum, jejunum, ileum and proximal colon are those of 3 cm segments.

Table 3. TRH, insulin and glucagon concentrations in the pancreas

		Normal	DM
TRH (pg/g, ww)	10 days	750±116.0	87±12.5**
	1 month	880±160.0	80±5.8**
	3 months	1100±111.8	133±21.0**
Insulin (ng/g, ww)	10 days	398±40.6	5±1.6**
	1 month	283±66.2	7±4.5*
	3 months	408±31.8	1±0.8**
Glucagon (ng/g, ww)	10 days	518±19.5	619±41.0
	1 months	655±95.7	655±122.5
	3 months	580±110.0	642±183.5

Values are expressed as the mean±SE in each group of 5 rats. Statistical differences between diabetic and age-matched control rats are shown by *p<0.05, **p<0.001. ww: wet weight

Table 4. TRH concentrations in the various parts of gastro-intestinal tract., hypothalamus and extrahypothalamic brain

		Normal	DM
Stomach fundus (pg/g, ww)	10 days	470±27 (5)	440±54 (5)
	1 month	320±54 (4)	———
	3 month	330±36 (5)	290±54 (5)
Stomach extrafundus (pg/g, ww)	10 days	110±10 (5)	140±13 (5)
	1 month	120±10 (5)	120±10 (5)
	3 months	86± 7 (5)	74±18 (5)
Duodenum (pg/g, ww)	10 days	250±51 (5)	210±44 (5)
	1 month	69± 9 (5)	64± 9 (5)
	3 months	61±16 (5)	38± 6 (5)
Jejunum (pg/g, ww)	10 days	97±10 (5)	85±13 (5)
	1 month	120±23 (5)	65±23 (5)
	3 months	150± 7 (5)	130±10 (4)
Ileum (pg/g, ww)	10 days	210±27 (5)	180± 9 (5)
	1 month	210±36 (5)	240±13 (5)
	3 months	330±22 (5)	290±63 (5)
Caecum (pg/g, ww)	10 days	160±13 (4)	110±19 (5)
	1 month	42± 7 (5)	46± 4 (5)
	3 months	52± 8 (5)	37± 4 (5)
Proximal colon (pg/g, ww)	10 days	92±32 (4)	86±24 (5)
	1 month	110±35 (5)	68±11 (5)
	3 months	92±17 (5)	97±15 (5)
Hypothalamus (pg/mg, ww)	10 days	86±10 (5)	93± 8 (4)
	1 month	———	100± 9 (5)
	3 months	105± 6 (5)	114± 5 (5)
Extrahypothalamic brain (pg/mg, ww)	10 days	5.1±0.41 (5)	5.0±0.16 (5)
	1 month	5.9±0.47 (5)	6.7±0.37 (5)
	3 months	4.9±0.34 (5)	5.8±0.58 (5)

Values are expressed as the mean±SE in each group of 4-5 rats. Values between normal and diabetic rats are all statistically insignificant. The number of animals is indicated in parentheses. ww: wet weight

associated with STZ diabetic rats[13-16] as in human diabetes, with low T_3 and T_4 and decreased both basal and stimulated TSH secretion.[27,28] However, it has been reported that hypothalamic TRH is lower[14,15] or unchanged[16] in STZ diabetic rats. It has also been reported that plasma TRH is unchanged[15] or reduced.[16] In this respect, we found no differences in TRH concentrations of the plasma or hypothalamus between diabetic and age-matched control rats. Our results do not support the suggestion that the reduction of hypothalamic TRH release is a primary cause of pituitary-thyroid alteration[14-16] in diabetic rats, but we can not rule out the possibility of impaired hypothalamic TRH release. Hypothalamic TRH release seems to be impaired *in vitro* in STZ diabetic rats.[29] Hypothalamic lesions in the paraventricular nucleus (PVN) of rats resulted in decreased basal TSH secretion as well as TSH secretion in response to hypothyroidism.[30] In addition, changes in thyroid hormone levels have been shown to inversely and specifically alter TRH mRNA levels in the PVN, as measured by *in situ* hybridization histochemistry.[31,32] These investigations suggest that the PVN of hypothalamus plays an important role in hypothalamic-pituitary-thyroid axis regulation; therefore, microdissection technique, but not en bloc dissection, may reveal TRH changes in specific nuclei of the hypothalamus in diabetic rats.

TRH has been localized in gastrin cells in the antral mucosa and in neurons of the myenteric plexus in the gastrointestinal tract,[33] and has been suggested to be involved in the gastrointestinal activity directly[8] or indirectly via the myenteric plexus or peripheral nerves in the gastrointestinal tract[34] or via the central nervous system[7,9] Diabetic gastrointestinal disorders such as constipation and diarrhea are well known.[10-12] However, the present study shows that TRH concentrations in the diabetic gastrointestinal tract are not significantly changed from those of age-matched controls, unlike the case for somatostatin or VIP.[35,36] TRH in the gastrointestinal tract may not play important roles in diabetic gastrointestinal disorders.

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REFERENCES

1) Karakida T, Ito S, Homma S: In vitro motor activity of intestinal segments of streptozotocin diabetic rats. *J Autonom Nerv Syst* **26**: 43-50, 1989.
2) Liu HS, Karakida T, Homma S: Acetylcholine and substance P responsiveness of intestinal smooth muscles in streptozotocin diabetic rats. *Jap J Physiol* **38**: 787-797, 1988.
3) Karakida T, Ito S, Homma S: Changes in substance P and somatostatin content in gastrointestinal tract of streptozotocin diabetic rats. *J Physiol Soc Jap* **50**: 597, 1988.
4) Morley JE, Garvin TJ, Pekary AE, Hershman JM: Thyrotropin-releasing hormone in the gastrointestinal tract. *Biochem Biophys Res Comm* **79**: 314-318, 1977.

5) Leppaluoto J, Koivusalo F, Kraama R: Thyrotropin-releasing factor: Distribution in neural and gastrointestinal tissues. *Acta Physiol Scand* **104**: 175-179, 1978.
6) Martino E, Lernmark A, Seo H, Steiner DF, Referoff S: High concentration of thyrotropin-releasing hormone in pancreatic islets. *Proc Nat Acad Sci USA* **75**: 4265-4267, 1978.
7) Smith JR, La Hann TR, Chestnut RM, Carino MA, Horita A: Thyrotropin-releasing hormone: Stimulation of colonic activity following intracerebroventricular administration. *Science* **196**: 660-662, 1977.
8) Tonoue T, Furukawa K, Nomoto T: The direct influence of thyrotropin-releasing hormone (TRH) on the smooth muscle of rat duodenum. *Life Sci* **25**: 2011-2016, 1979.
9) Tonoue T, Nomoto T: Effect of intracerebroventricular administration of thyrotropin-releasing hormone upon the electroenterogram of rat duodenum. *Eur J Pharmacol* **58**: 369-377, 1979.
10) Katz LA, Spiro HM: Gastrointestinal manifestation of diabetes. *New Eng J Med* **275**: 1350-1361, 1966.
11) Hosking DJ, Bennett T, Hampton JR: Diabetic autonomic neuropathy. *Diabetes* **27**: 1043-1055, 1977.
12) Schmidt RE, Nelson JS, Johnson EM: Experimental diabetic autonomic neuropathy. *Amer J Pathol* **103**: 210-225, 1981.
13) Zaninovich AA, Brown TJ, Boado R, Bromage NR, Matty AJ: Thyroxine metabolism in diabetic rats. *Acta Endocrinol (Copenhagen)* **86**: 336-343, 1977.
14) Gonzalez C, Montoya E, Jolin T: Effect of streptozotocin diabetes on the hypothalamic-pituitary-thyroid axis in the rat. *Endocrinology* **107**: 2099-2103, 1980.
15) Mitsuma T, Nogimori T: Effects of streptozotocin-induced diabetes mellitus on hypothalamic-pituitary-thyroid axis in rats. *Endocrinol Jap* **29**: 695-700, 1982.
16) Wilber JF, Banerji A, Prasad C, Mori M: Alterations in hypothalamic-pituitary-thyroid regulation produced by diabetes mellitus. *Life Sci* **28**: 1757-1763, 1981.
17) Glowinski J, Iversen LL: Regional studies of catecholamines in the rat-I. The disposition of [^{3}H] norepinephrine, [^{3}H] dopamine and [^{3}H] DOPA in various regions of the brain. *J Neurochem* **13**: 655-669, 1966.
18) Fuwano S, Matsui N, Miyashita O, Sakai M, Yaginuma H, Togashi S, Wachi M, Okuda M, Itoh N: Decreased plasma TRH-like immunoreactivity in depressed patients. IInd report—Radio-immunoassay for measuring plasma TRH-like immunoreactivity and its clinical application. *Jap J Neuropsychopharmacol* **9**: 615-624, 1985.
19) Itoh N, Matsui N, Fuwano S, Yaginuma H, Miyashita O, Sakai M: Serial DST, TRH test, and TRH-like immunoreactivity measurements in major affective disorders. *Biol Psychiatry* **22**: 559-572, 1987.
20) Ito S, Iwasaki Y, Momotu T, Takai K, Shibata A, Matsubara Y, Muto T: Neurotensin and substance P and dumping syndrome. *Tohoku J Exp Med* **135**: 11-21, 1981.
21) Saito S, Musa K, Yamamoto S, Oshima I, Funato T: Radioimmunoassay of thyrotropin releasing hormone in plasma and urine. *Endocrinol Jap* **22**: 303-310, 1975.
22) Engler D, Scanlon MF, Jackson IMD: Thyrotropin-releasing hormone in the systemic circulation of the neonatal rat is derived from the pancreas and other extraneural tissues. *J Clin Invest* **67**: 800-808, 1981.
23) Koivusalo F, Leppaluoto J: High TRH immunoreactivity in purified pancreatic extracts of fetal and newborn rats. *Life Sci* **24**: 1655-1658, 1979.
24) Aratan-Spire S, Wolf B, Portha B, Bailbe D, Czebichow P: Streptozotocin treatment at birth induces a parallel depletion of thyrotropin-releasing hormone and insulin in the rat pancreas during development. *Endocrinology* **114**: 2369-2373, 1984.
25) Koivusalo F, Leppaluoto J, Krip M, Rajaniemi H: Presence of TRH immunoreactivity marginal islet cells in rat pancreas. *Acta Endocrinol (Copenhagen)* **97**: 398-404, 1981.
26) Kawano H, Daikoku S, Saito S: Location of thyrotropin-releasing hormone-like immunoreactivity in rat pancreas. *Endocrinology* **112**: 951-955, 1983.
27) Saunders J, Hall SEH, Sonksen PH: Thyroid hormones in insulin requiring diabetes before and after treatment. *Diabetologia* **15**: 29-32, 1978.
28) Radetti G, Drei F, Franzellin F, Pasquino B, Mengarda G: Thyroid function in type I juvenile diabetes mellitus: Tendency to the low T_3 syndrome. *Helv Paediat Acta* **40**: 461-466, 1985.
29) Bestetti GE, Reymond MJ, Boujon CE, Lemarchand-Beraud T, Rossi GL: Functional and morphological aspects of impaired TRH release by mediobasal hypothalamus of STZ-induced diabetic rats. *Diabetes* **38**: 1351-1356, 1989.
30) Izawa T, Green M: Delineation of the hypothalamic area controlling thyrotropin secretion in the rat. *Endocrinology* **109**: 1731-1738, 1981.
31) Segerson TP, Kauer J, Wolfe HC, Mobtaker H, Wu P, Jackson IM, Lechan RM: Thyroid hormone regulates TRH biosynthesis in the paraventricular nucleus of the rat hypothalamus. *Science* **238**: 78-80, 1987.
32) Koller KJ, Wolff RS, Warden MK, Zoeller RT: Thyroid hormones regulate levels of thyrotropin-releasing hormone mRNA in the paraventricular nucleus. *Proc Nat Acad Sci* **84**: 7329-7333, 1987.
33) Tsuruo Y, Hokfelt T, Visser TJ, Kimmel JR, Brown JC, Verhofstadt A, Walsh J: TRH-like immunoreactivity in endocrine cells and neurons in the gastrointestinal tract of the rat and guinea pig. *Cell Tiss*

Res **253**: 347-356, 1988.
34) Furukawa K, Nomoto T, Tonoue T: Effects of thyrotropin-releasing hormone (TRH) on the isolated small intestine and taenia coli of the guinea pig. *Eur J Pharmacol* **64**: 279-287, 1980.
35) Patel YC, Cameron DP, Bankier A, Malaise-Lage F, Ravazzola M, Studer P, Orci L: Changes in somatostatin in pancreas and other tissues of streptozotocin diabetic rats. *Endocrinology* **103**: 917-923, 1978.
36) Belai A, Lincoln J, Milner P, Burnstock G: Progressive changes in adrenergic, secrotonergic, and peptidergic nerves proximal colon of streptozotocin diabetic rats. *Gastroenterology* **95**: 1234-1241, 1988.