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Comparison of Pancreatic and Hypophysiotropic TRH Systems

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Summary

The thyrotropin-releasing hormone (TRH) is a molecule with widespread distribution through many organ systems. The function of TRH is probably not identical in each system so that TRH synthesis and secretion may be unique for each system under specific experimental conditions. The present study was designed to explore the common and diverse features of the regulation of TRH encoded with the same gene in two different organs: hypophysiotropic hypothalamus and pancreatic islets. During *in vitro* incubation, the TRH content in hypothalamic structures remained stable while that in isolated pancreatic islets increased sharply. In contrast to the pancreatic islets, exposure to different concentrations of D-glucose did not affect TRH release from the hypothalamic paraventricular nucleus or median eminence. This divergence in the regulation of the hypophysiotropic and pancreatic TRH systems may be related to differences in the role of TRH produced in these tissues.

Key words

TRH content • TRH release • Hypothalamus • Pancreas • *In vitro* • Glucose effects

Introduction

TRH has been isolated from mammalian hypothalamus (Burgus *et al.* 1969, Boler *et al.* 1969) on the basis of its ability to stimulate thyrotropin secretion (Greer 1951, Schreiber *et al.* 1961). TRH is present in various structures of the central nervous system (Oliver *et al.* 1974, Segerson *et al.* 1987, Merchenthaler *et al.* 1988), endocrine, gastrointestinal, and uropoietic systems (Morley *et al.* 1977, for review see Lechan 1993) and has many different functions. Even in the hypothalamus the distribution of TRH is not uniform. TRH-containing perikarya are located in the paraventricular nucleus (PVN), the suprachiasmatic portion of the preoptic nucleus, the dorsomedial nucleus and the lateral basal hypothalamus (Merchenthaler *et al.* 1988).

Individual TRH systems may behave differently in the same experimental situation, thus reflecting their diverse functions. It is therefore essential to study individually defined TRH systems in order to be able to make valid conclusions about their function. TRH involved in pituitary thyrotropin regulation is synthesized in the parvocellular part of the PVN and delivered by axonal transport for release at the primary plexus of the hypophysial portal system from nerve terminals in the median eminence (ME) (Aizawa and Greer 1981, Brownstein *et al.* 1982). A recently developed methodology has enabled a comparison of the TRH-secreting activity of the PVN and ME separately *in*

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vitro (Nikodémová and Štrbák 1995). It is of particular interest to compare hypophysiotropic TRH with a non-neural TRH system. We have chosen pancreatic TRH as a non-neural model, since it has a well-defined cellular distribution (Leduque *et al.* 1985, 1989, Kulkarni *et al.* 1995) and special ontogenetic dynamics (Martino *et al.* 1980, Giraud *et al.* 1984, Dutour *et al.* 1987). Moreover, hypothalamic and pancreatic TRH are encoded by the same gene in the rat (Dutour *et al.* 1987) and both are under negative feedback control of thyroid hormones (Giraud *et al.* 1984, Leduque *et al.* 1989, Taylor *et al.* 1990, Polk *et al.* 1991). The present study was designed to compare some features of the regulation of TRH expressed in these tissues.

Material and Methods

Male Wistar rats of 250-300 g body weight were kept under controlled temperature (22-24 °C) and a 12 h light/12 h dark cycle (light beginning at 06:00 h) and fed Purina Chow and tap water *ad libitum.*

Hypothalamic structures

After decapitation (09:00-11:00 h), the brain was rapidly removed and the ME and PVN area were dissected using an operating microscope (Nikodémová and Štrbák 1995). After a 30-min preincubation period, the ME (1 ME/tube) and PVN (1 pair from 1 animal/tube) were incubated at 37 °C for four successive 30-min periods in a 150 µl medium according to the sequence: 1) basal medium, 2) stimulating medium, 3) basal medium, 4) stimulating medium. The stimulating medium contained 56 mM KCl and the last period also served to validate survival of the tissue until termination of the experiment. There was no statistical difference in TRH release between the two basal incubation periods (periods 1 and 3) or the two periods with high KCl (periods 2 and 4). Therefore the results were pooled and are presented as TRH release under either basal or stimulated conditions.

Composition of media

Basal medium contained: 6 mM $NaHCO_3$, 128 mM NaCl, 5.6 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM D-glucose, 1.5 mM ascorbic acid, 2 mM HEPES, pH 7.4, osmolality 284 mosm/kg H_2O. To minimize degradation of released TRH, 30 mg bacitracin/100 ml medium was added. The stimulating (membrane depolarizing) medium was prepared from the basal medium by substituting KCl for NaCl to maintain physiological osmolarity (77.6 mM NaCl, 56 mM KCl).

Glucose effect

The effect of glucose in the medium was studied using two models: 1) D- and L-glucose were combined to give a final 20 mM concentration to discern biological osmotic effects, and 2) increasing concentrations (0-24 mM) of D-glucose were added to the medium. The osmolality of all media was measured cryoscopically before each experiment.

Pancreatic islets

Islets were isolated according to Lacy and Kostianovsky (1967). Briefly, the common bile duct was cannulated proximally near the hilus of liver under pentobarbital anesthesia, a suture was placed adjacent to the duodenum, and the pancreas was distended by injection of approximately 10 ml of Hank's Balanced Salt Solution. The pancreas was then dissected out, minced, and digested with 6 mg/ml collagenase (Type XI, Sigma, St Louis, Mo, USA) for 15 min at 37 °C. At the end of incubation, the tissue was disrupted by vigorous hand-shaking and the islets were separated from the remaining acinar tissue under a dissecting microscope. A 30-min preincubation period in Krebs Ringer bicarbonate medium (118 mM NaCl, 4 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$, 3 M D-glucose, 0.25% BSA, 0.3 mg/ml bacitracin in 10 mM HEPES, pH 7.4, 95% O_2, 5 % CO_2) at 37 °C was used prior to all experiments. Batches of 20 islets per tube were used in static incubation.

Basal incubation

Islets were incubated in Krebs Ringer bicarbonate medium for 3 h in a final volume of 300 µl. The medium was removed and stored at –20 °C until RIA analysis. Islets were sonicated and subsequently processed as described below.

Depolarizing KCl-induced TRH secretion

Islets were incubated for two subsequent 30-min periods in a total volume of 300 µl. The basal medium (4 mM KCl) was used during the first 30 min, then KCl concentration in the medium was changed to 40 mM. Incubations were performed at different concentrations of extracellular Ca^{2+}. The medium was removed, frozen and stored at –20°C until RIA.

TRH determination in the medium

TRH released into the medium was determined directly by specific RIA as described previously (Nikodémová and Štrbák 1995). The antibody, prepared in our laboratory, is highly specific for TRH and does not exhibit significant cross-reactivity (<0.1 %) with TRH free acid, His-Pro diketo-piperazine, TRH-Gly or Lys-Arg-Glu-His-Pro-Gly. The lower limit of detection was 2 pg. TRH standards were prepared in each medium employed. There was no difference between the standard curves in basal or stimulating medium. In one specified experiment, the TRH content in pancreatic islets was determined with a new antibody described elsewhere (Benický and Štrbák 2000).

TRH determination in the tissue

The TRH extraction method was described previously (Nikodémová and Štrbák 1995). The tissue was placed into 200 µl ice-cold water and sonicated; 200 µl 2 M acetic acid was added and the tubes were kept at –20 °C overnight. The samples were then centrifuged at 7000 *g* for 10 min. After addition of 50% methanol to the sediment and vortexing, the samples were again centrifuged. The supernatants from the first and second centrifugations were pooled and lyophilized. The lyophilizates were kept at –20 °C and reconstituted in the assay buffer on the day of RIA. The standards for the calibration curves were prepared in the same way and thus the correction for recovery was included.

Statistical analysis

Data (expressed as means ± S.E.M.) were compared by unpaired or paired Student's t-test and by analysis of variance followed by the Bonferoni test. $P<0.05$ was considered as significant.

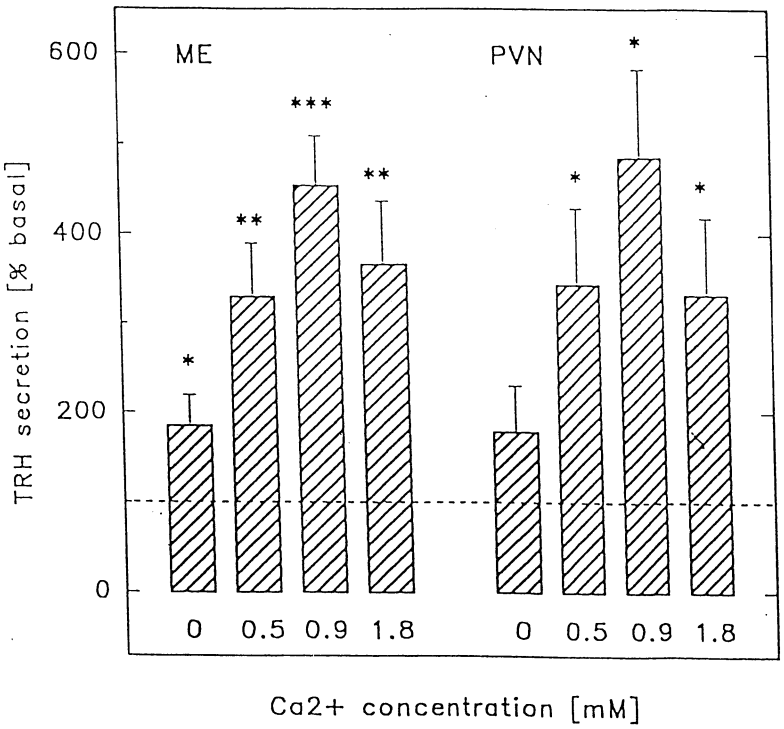

Fig. 1. *Effect of medium Ca^{2+} concentration on TRH secretion stimulated by depolarizing with 56 mM KCl from hypothalamic PVN and ME. TRH release is expressed as percentage of secretion under basal conditions (5.6 mM KCl) in the same tube. ME – median eminence, PVN – paraventricular nucleus. K^+ stimulation depends on the medium Ca^{2+} in both structures. Data are means ± S.E.M., n = 5-12 for each bar, combined from 3-4 experiments; * $P<0.05$, ** $P<0.01$ compared to basal secretion.*

Results

KCl-induced TRH release

Isolated pancreatic islets released TRH under basal conditions and the release was increased in response to depolarization with 40 mM KCl (Table 1). While the basal TRH release did not depend on Ca^{2+} concentration in the medium, KCl did not induce TRH release in Ca^{2+}-depleted media (Table 1). Both hypothalamic ME and PVN released TRH in response to each repetitive KCl depolarization. This response was positively correlated with the Ca^{2+} concentration in the medium (Fig. 1), the highest response amplitude being found at 0.9 mM Ca^{2+}.

Effect of in vitro incubation on TRH tissue content

There was a several-fold increase in the tissue content of TRH in the pancreatic islets from the

beginning to the end of incubation. In contrast, the TRH content in the ME and PVN did not change significantly during the incubation (Table 2).

Effect of medium glucose on TRH release

Various combinations of D- and L-glucose (giving a total concentration of 20 mM) in the medium did not affect either basal or KCl-stimulated TRH release from ME or PVN (Fig. 2). Similar results were found when basal and KCl-stimulated TRH release was evaluated with graded D-glucose concentrations up to 24 mM (Fig. 3).

Table 1. TRH secretion (pg TRH/20 islets/30min) by isolated pancreatic islets

Ca^{2+} concentration	Basal medium	40 mM KCl
0	6.0±0.5	7.7±1.2
1 mM	4.8±1.1	11.9±2.2*
2.5 mM	5.7±0.5	15.4±2.6**

*Data are means ± S.E.M. (n = 5). The islets at particular Ca^{2+} concentrations were incubated consecutively in basal and high KCl medium. Significantly different from the values obtained in basal medium: * $p<0.05$, ** $p<0.01$.*

Table 2. Changes of TRH content during incubation

TRH	Before incubation	After incubation
pg / PVN	367±28	261±41
pg / ME	438±44	564±67
pg / 20 islets	4.2±1.5	42.2±2.5***
pg / 20 islets[#]	14.4±2.2	39.7±2.0***

Data are means ± S.E.M. Hypothalamic structures (PVN and ME, n = 6-12) were incubated for 150 min, pancreatic islets (n = 6) were incubated for 180 min. [#] *independent experiment with a new antibody. Significantly different from the values obtained before incubation:* *** $p<0.001$.

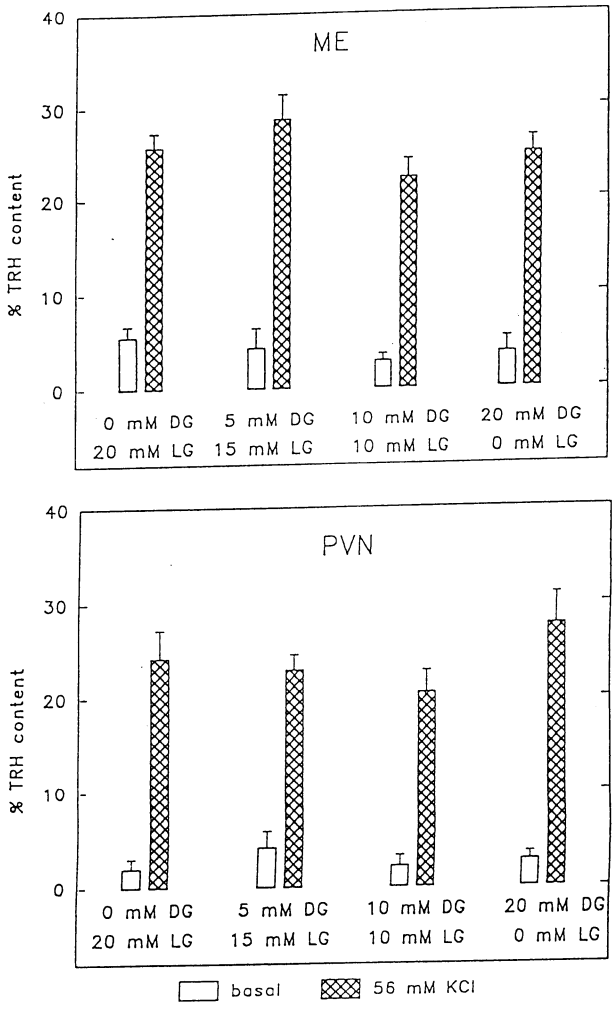

Fig. 2. *Lack of effect of different combinations of D-glucose (DG) and L-glucose (LG) on TRH release from ME and PVN under basal and depolarizing KCl-stimulated conditions. Release is expressed as percentage of TRH tissue content Data are means ± S.E.M., n = 6-12.*

Discussion

TRH subserves numerous biological functions both within the central nervous system and in extraneural locations. For comparison, we have chosen two anatomically well-defined systems: i) hypophysiotropic hypothalamic TRH which is synthesized in the parvocellular part of the PVN (Aizawa and Greer 1981, Brownstein *et al.* 1982) and released at nerve terminals in the ME to enter hypophysial portal blood, and ii) non-neural TRH synthesized in pancreatic B cells (Dutour *et al.* 1987, Leduque *et al.* 1989). These two systems also

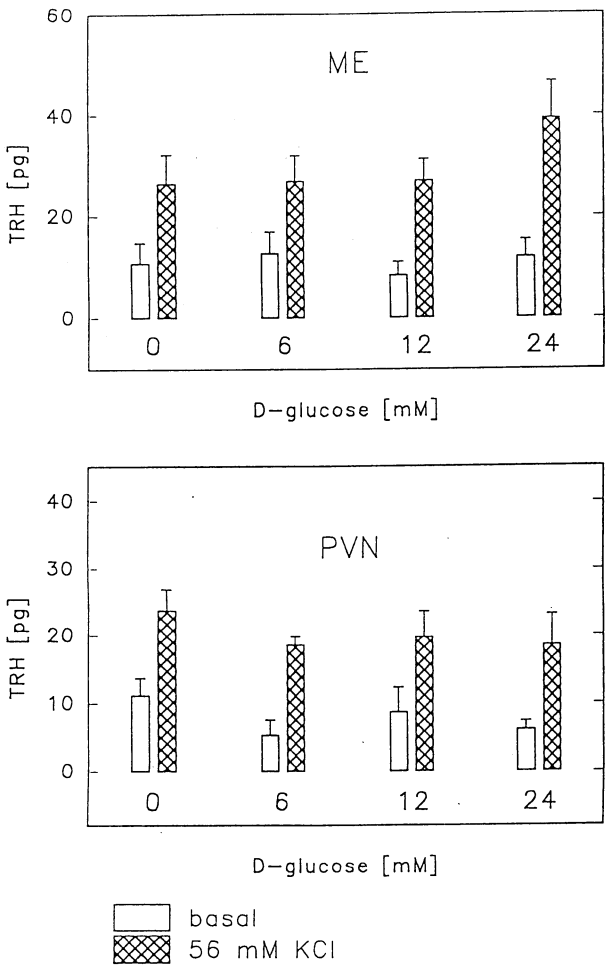

Fig. 3. *Lack of effect of different concentrations of D-glucose on ME and PVN TRH release under basal and KCl-stimulated conditions. Results are expressed as pg of TRH released. Data are means ± S.E.M., n = 10-12.*

have different ontogenic patterns in the rat (Martino *et al.* 1980). Hypothalamic TRH mRNA and TRH increase gradually after birth and remain high throughout life (Martino *et al.* 1980, Covarrubias *et al.* 1988). In contrast, pancreatic TRH mRNA and TRH content reach their peaks on the 1st and the 3rd postnatal day, respectively. They decrease thereafter to become very low for the remaining life (Martino *et al.* 1980, Giraud *et al.* 1984, Dutour *et al.* 1987). From this point of view, it is of considerable interest that in our experiments the TRH content of the islets increased dramatically during 2-3 h of *in vitro* incubation immediately following their isolation, whereas the TRH content of the hypothalamic structures remained relatively stable over the same period. Since the TRH content is very low in pancreatic islets at the beginning of the experiment, the relative amount of newly produced TRH is very high when considering the additional amount of neurohormone secreted. Possibly *in vivo* regulation of pancreatic TRH synthesis, in contrast to that in the hypothalamus, is under tonic inhibition in adult rats. This would be similar to the well-known tonic *in vivo* inhibition of pituitary prolactin and MSH secretion. An alternative explanation may be that relatively more TRH in the pancreatic islets is present in the precursor form and is processed during incubation. In fact, changes in the ratio of TRH-Gly (immediate TRH precursor) to TRH were found to be inversely related to tissue TRH concentrations in the hypothalamus and pancreas during ontogenesis (Fuse *et al.* 1991).

Secretion of TRH in both the hypothalamus and pancreatic islets was induced *via* a Ca^{+}-dependent process by membrane depolarization with high K^{+}, indicating regulated secretion. We used a slightly higher concentration of depolarizing K^{+} in the hypothalamic than in the pancreatic islet experiments. Nevertheless, both K^{+} concentrations were well above the threshold value required for inducing depolarization and there was no discernible difference in the K^{+} effects between these two tissue types. We found a substantial difference between hypothalamic PVN and ME and pancreatic islets in the effect of increasing medium glucose concentration. Secretion of TRH by pancreatic islets was stimulated by both 6 and 12 mmol D-glucose (Benický and Štrbák 1999), while that of PVN and ME was unaffected. Pancreatic TRH is located in the B cells of the islets of Langerhans in the same secretory granules as insulin (Leduque *et al.* 1989) and its role in glucoregulation has been hypothesized and repeatedly studied (Dolva *et al.* 1983, Giraud *et al.* 1984, Vara and Tamarit-Rodriguez 1988, Dutour *et al.* 1990, Ebiou *et al.* 1992a,b, Kulkarni *et al.* 1995). Recently Yamada *et al.* (1997) described permanent hyperglycemia and impaired insulin response to glucose in mice with targeted disruption of the TRH gene. It is of interest that glucose stimulates and insulin inhibits pancreatic TRH release (Benický and Štrbák 2000).

Cell volume can be a powerful link in the transduction chains regulating cellular function (Haussinger *et al.* 1994) including secretion of insulin (Blackard *et al.* 1975) and TRH (Benický *et al.* 1997, 1998, Nikodémová *et al.* 1997, 1998, 1999). It was therefore important to use an osmotically balanced medium to test the effect of D-glucose on TRH secretion. Biologically inactive L-glucose in reciprocal

concentrations was used to compensate the osmolarity in our experiments. The absence of the glucose effect on TRH secretion from PVN and ME in our experiments is at variance with Lewis *et al.* (1989) who reported inhibition by 1, 5 and 25 mM D-glucose (as compared to L-glucose as a control stimulus) of basal TRH release from hypothalamic halves. In our experiments, D-glucose, either with or without L-glucose in the medium to eliminate a non-specific osmotic effect, had no significant effect on TRH secretion from either PVN or ME. This difference from Lewis *et al.* (1989) might be explained by a greater sensitivity of their TRH assay (0.5 pg/tube). Another explanation could be that the relatively specific hypophysiotropic TRH studied in our experiments may react differently than the complex mixture of TRH systems contained in the hypothalamic halves employed by Lewis *et al.* (1989). In any case, there was no stimulation of TRH release from hypothalamic tissue by D-glucose in either their or our study, in contrast to our current findings in pancreatic islets. Both these studies are at variance with that of Yamaguchi *et al.* (1991) who reported a stimulating effect of glucose in the medium on the depolarization-induced TRH release from hypothalamic slices. However, there was no effect on basal TRH secretion. The reason for the difference might be due to the different design of the experiment (preincubation in a medium containing tested concentrations of D-glucose) and/or due to anatomically different hypothalamic slices.

The pattern of TRH release by pancreatic islets is dependent on the age of the donor rat as well as on the age and type of culture (Ebiou *et al.* 1992a). We therefore kept both systems (hypothalamic and pancreatic) as similar as possible. Animals of the same sex and age from the same breeding series were used and experiments were run in parallel within 1-2 weeks. Both systems were studied using short-term incubation immediately after removal of the tissue from the body. We found that acute exposure to glucose enhances TRH release from freshly isolated pancreatic islets. Similar results have been obtained in organ culture of neonate (Dutour *et al.* 1990) or adult (Dolva *et al.* 1983) rat pancreas. In contrast, Vara and Tamarit-Rodriguez (1988) found that glucose induced inhibition of TRH release during short-term incubation of adult rat islets. We do not know the reason for these differences. However, in our experiments, the presence of bacitracin in the medium was essential for the determination of TRH secretion because it effectively inhibited TRH degradation. Bacitracin does not affect the formation of TRH from prepro-TRH (Friedman *et al.* 1995).

In conclusion, we have found a divergence in the regulation of the hypophysiotropic and pancreatic TRH systems which may be related to a difference in the role of TRH produced in these tissues.

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References

AIZAWA T, GREER MA: Delineation of the hypothalamic area controlling thyrotropin secretion in the rat. *Endocrinology* **109:** 1731-1738, 1981.

BENICKÝ J, ŠTRBÁK V: Glucose stimulates and insulin inhibits release of pancreatic TRH in vitro. *Eur J Endocrinol* **142:** 60-64, 2000.

BENICKÝ J, GREER MA, ŠTRBÁK V: Hyposmolar medium and ethanol in isosmotic solution induce the release of thyrotropin-releasing hormone (TRH) by isolated rat pancreatic islets. *Life Sci* **60:** 865-872, 1997.

BENICKÝ J, NIKODÉMOVÁ M, ŠTRBÁK V: Ethanol drinking increases TRH content and release in rat pancreatic islets (Abstract). *Physiol Res* **47:** 15P, 1998.

BLACKARD WG, KIKUCHI M, RABINOVITCH A, RENOLD AE: An effect of hyposmolarity on insulin release in vitro. *Am J Physiol* **228:** 706-713, 1975.

BOLER J, ENZMANN F, FOLKERS K, BOWERS CY, SCHALLY AV: The identity of chemical and hormonal properties of the thyrotropin releasing hormone and pyroglutamyl-histidyl-proline amide. *Biochem Biophys Res Commun* **37:** 705-710, 1969.

BROWNSTEIN MJ, ESKAY RL, PALKOVITS M: Thyrotropin-releasing hormone in the median eminence is in processes of paraventricular nucleus neurons. *Neuropeptides* **2:** 197-201, 1982.

BURGUS R, DUNN TF, DESIDERIO D, GUILLEMIN R: Molecular structure of the hypothalamic hypophysiotropic TRF factor of ovine origin: mass spectrometry demonstration of the PCA-His-Pro-NH2 sequence (in French). *C R Acad Sci Hebd Seances Acad Sci D* **269:** 1870-1873, 1969.

COVARRUBIAS L, URIBE RM, MENDEZ M, CHARLI JL, JOSEPH-BRAVO P: Neuronal TRH synthesis: developmental and circadian TRH mRNA levels. *Biochem Biophys Res Commun* **151:** 615-622, 1988

DOLVA LO, NIELSEN JH, WELINDER BS, HANSSEN KF: Biosynthesis and release of thyrotropin-releasing hormone immunoreactivity in rat pancreatic islets in organ culture. Effects of age, glucose, and streptozotocin. *J Clin Invest* **72:** 1867-1873, 1983.

DUTOUR A, GIRAUD P, KOWALSKI C, OUAFIK L, SALERS P, ŠTRBÁK V, OLIVER C: Ontogenesis of TRH mRNA in the rat pancreas. *Biochem Biophys Res Commun* **146:** 354-360, 1987.

DUTOUR A, GIRAUD P, MALTESE JY, BECQUET D, PESCE G, SALERS P, OUAFIK LH, RENARD M, OLIVER C: Regulation of TRH release by the cultured neonate rat pancreas. *Peptides* **11:** 1081-1085, 1990.

EBIOU JC, BULANT M, NICOLAS P, ARATAN-SPIRE S: Pattern of thyrotropin-releasing hormone secretion from the adult and neonatal rat pancreas: comparison with insulin secretion. *Endocrinology* **130:** 1371-1379, 1992a.

EBIOU JC, GROUSELLE D, ARATAN-SPIRE S: Antithyrotropin-releasing hormone serum inhibits secretion of glucagon from isolated perfused rat pancreas: an experimental model for positive feedback regulation of glucagon secretion. *Endocrinology* **131:** 765-771, 1992b.

FRIEDMAN TC, LOH YP, CAWLEY NX, BIRCH NP, HUANG SS, JACKSON IM, NILLNI EA: Processing of prothyrotropin-releasing hormone (Pro-TRH) by bovine intermediate lobe secretory vesicle membrane PC1 and PC2 enzymes. *Endocrinology* **136**: 4462-4472, 1995.

FUSE Y, POLK DH, LAM RW, FISHER DA: Ontogeny of thyrotropin releasing hormone and precursor peptide in the rat. *Pediatr Res* **30:** 28-33, 1991.

GREER MA: Evidence of hypothalamic control of the pituitary release of thyrotropin. *Proc Soc Exp Biol Med* **77:** 603-608, 1951.

GIRAUD P, MALTESE JY, DUTOUR A, OUAFIK LH, SALERS P, ŠTRBÁK V, OLIVER C: Thyrotropin releasing hormone in neonatal rat pancreas. Regulation of biosynthesis and secretion. In*: Progress in Neuropeptide Research.* KD DOHLER, M PAWLIKOWSKI (eds), Birkhauser Verlag, Basel, 1984, pp 143-153.

HAUSSINGER D, LANG F, GEROK W: Regulation of cell function by the cellular hydration state. *Am J Physiol* **267:** E343-E355, 1994.

KULKARNI RN, WANG ZL, AKINSANYA KO, BENNET WM, WANG RM, SMITH DM, GHATEI MA, BYFIELD PG, BLOOM SR: Pyroglutamyl-phenylalanyl-proline amide attenuates hyrotropin-releasing hormone-stimulated insulin secretion in perifused rat islets and insulin-secreting clonal beta-cell lines. *Endocrinology* **136:** 5155-5164, 1995.

LACY PE, KOSTIANOVSKY M: Method for the isolation of intact islets of Langerhans from the rat pancreas. *Diabetes* **16:** 35-39, 1967.

LECHAN RM: Update on thyrotropin-releasing hormone. *Thyroid Today* **16:** 1-11, 1993.

LEDUQUE P, WOLF B, ARATAN-SPIRE S, DUBOIS PM, CZERNICHOW P: Immunocytochemical location of thyrotropin-releasing hormone (TRH) in the B-cells of adult hypothyroid rat pancreas. *Reguly Pept* **10:** 281-292, 1985.

LEDUQUE P, ARATAN-SPIRE S, SCHARFMANN R, BASMACIOGULLARI A, CZERNICHOW P, DUBOIS PM: Coexistence of thyrotropin-releasing hormone and insulin in cultured fetal rat islets: a light and electron microscopic immunocytochemical study during islet neoformation. *Biol Cell* **66:** 291-296, 1989.

LEWIS BM, DIEGUES C, HAM J, PAGE D, CREAGH FM, PETERS JR, SCANLON MF: Effects of glucose on thyrotropin-releasing hormone, growth hormone-releasing hormone, somatostatin and luteinizing hormone-releasing hormone release from rat hypothalamus *in vitro*. *J Neuroendocrinol* **1:** 437-441, 1989.

MARTINO E, SEO H, LERNMARK A, REFETOFF S: Ontogenetic patterns of thyrotropin-releasing hormone-like material in rat hypothalamus, pancreas, and retina: selective effect of light deprivation. *Proc Natl Acad Sci USA* **77:** 4345-4348, 1980.

MERCHENTHALER I, CSERNUS V, CSONTOS C, PETRUSZ P, MESS B: New data on the immunocytochemical localization of thyrotropin-releasing hormone in the rat central nervous system. *Am J Anat* **181:** 359-376, 1988.

MORLEY JE, GARVIN TJ, PEKARY AE, HERSHMAN JM: Thyrotropin-releasing hormone in the gastrointestinal tract. *Biochem Biophys Res Commun* **79:** 314-318, 1977.

NIKODÉMOVÁ M, ŠTRBÁK V: Different regulation of thyrotropin releasing hormone content and release in paraventricular nucleus (PVN) and median eminence (ME) of rat hypothalamus during in vitro incubation. *Life Sci* **56:** 1511-1521, 1995.

NIKODÉMOVÁ M, ŠTRBÁK V: Hyposmolarity stimulates and high Na^+ concentration inhibits TRH secretion from rat hypothalamus (Abstract). *Phys Res* **47:** 25P, 1998.

NIKODÉMOVÁ M, WEISMANN P, FILIPČÍK P, MRÁZ P, GREER MA, ŠTRBÁK V: Both iso- and hyperosmolar ethanol stimulate release of hypothalamic thyrotropin releasing hormone (TRH) despite opposite effect on neuron volume. *Neuroscience* **80:** 1263-1269, 1997.

NIKODÉMOVÁ M, GREER MA, ŠTRBÁK V: Hypo-osmolarity stimulates and high sodium concentration inhibits thyrotropin-releasing hormone secretion from rat hypothalamus. *Neuroscience* **88:** 1299-1306, 1999.

OLIVER C, ESKAY R.L, BEN-JONATHAN N, PORTER JC: Distribution and concentration of TRH in the rat brain. *Endocrinology* **95:** 540-546, 1974.

POLK DH, REVICZKY A, LAM RW, FISHER DA: Thyrotropin-releasing hormone in ovine fetus: ontogeny and effect of thyroid hormone. *Am J Physiol* **260:** E53-E58, 1991.

SCHREIBER V, KOČÍ J, ECKERTOVÁ A, FRANC Z, KMENTOVÁ V: The hypothalamic factor activating adenohypophysial acid phosphatases and TSH release *in vitro*: further purification by high-voltage electrophoresis. *Physiol Bohemoslov* **10:** 417-425, 1961.

SEGERSON TP, HOEFLER H, CHILDERS H, WOLFE HJ, WU P, JACKSON IM, LECHAN RM: Localization of thyrotropin-releasing hormone prohormone messenger ribonucleic acid in rat brain in situ hybridization. *Endocrinology* **121:** 98-107, 1987.

TAYLOR T, WONDISFORD FE, BLAINE T, WEINTRAUB BD: The paraventricular nucleus of the hypothalamus has a major role in thyroid hormone feedback regulation of thyrotropin synthesis and secretion. *Endocrinology* **126:** 317-324, 1990.

VARA E, TAMARIT-RODRIGUEZ J: Islet secretion of immunoreactive thyrotropin- releasing hormone and the 'paracrine-like' effects of its exogenous administration. *Acta Endocrinol* **118:** 429-436, 1988.

YAMADA M, SAGA Y, SHIBUSAWA N, HIRATO J, MURAKAMI M, IWASAKI T, HASHIMOTO K, SATOH T, WAKABAYASHI K, TAKETO MM, MORI M: Tertiary hypothyroidism and hyperglycemia in mice with targeted disruption of the thyrotropin-releasing hormone gene. *Proc Natl Acad Sci USA* **94:** 10862-10867, 1997.

YAMAGUCHI M, IRIUCHIJIMA T, MICHIMATA T, MORI M: Glucose affects the release of thyrotropin-releasing hormone from the rat hypothalamus. *Neuroendocrinology* **53:** 423-427, 1991.

Reprint requests

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