Histol Histopathol (2005) 20: 713-718

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

Thyrotropin-releasing hormone receptor expression in thyroid follicular cells: a new paracrine role of C-cells?

M. De Miguel[1], J.M. Fernández-Santos[1], J.C. Utrilla[1], A. Carrillo-Vico[2], J. Borrero[2], E. Conde[1], J.M. Guerrero[2] and I. Martín-Lacave[1]

[1]Departament of Normal and Pathological Histology, and

[2]Departament of Biochemistry and Molecular Biology, Faculty of Medicine, University of Seville, Seville, Spain

Summary. Thyrotropin-releasing hormone (TRH) synthesized in the hypothalamus has the capability of inducing the release of thyroid-stimulating hormone (TSH) from the anterior pituitary, which in turn stimulates the production of thyroid hormones in the thyroid gland. Immunoreactivity for TRH and TRH-like peptides has been found in some tissues outside the nervous system, including thyroid. It has been demonstrated that thyroid C-cells express authentic TRH, affecting thyroid hormone secretion by follicular cells. Therefore, C-cells could have a paracrine role in thyroid homeostasis. If this hypothesis is true, follicular cells should express TRH receptors (TRH-Rs) for the paracrine modulation carried out by C-cells. In order to elucidate whether or not C-cell TRH production could act over follicular cells modulating thyroid function, we studied TRH-Rs expression in PC C13 follicular cells from rat thyroid, by means of immunofluorescence technique and RT-PCR analysis. We also investigated the possibility that C-cells present TRH-Rs for the autocrine control of its own TRH production. Our results showed consistent expression for both receptors, TRH-R1 and TRH-R2, in 6-23 C-cells, and only for TRH-R2 in PC C13 follicular cells. Our data provide new evidence for a novel intrathyroidal regulatory pathway of thyroid hormone secretion via paracrine/autocrine TRH signaling.

Key words: TRH-R, Rat thyroid, C-cells, Follicular cells

Introduction

Thyrotropin-releasing hormone (TRH) is a tripeptide (pGlu-His-ProNH_2) synthesized from a larger polypeptide precursor which yields five copies of TRH and seven non-TRH peptides (Lechan et al., 1986). This hormone was originally isolated from mammalian hypothalamus and has the capability of inducing the release of thyroid-stimulating hormone (TSH) from the anterior pituitary. TRH binds to a specific receptor, the TRH receptor (TRH-R), which belongs to the large family of G protein-coupled receptors with seven transmembrane domains.

Two subtypes of TRH-Rs have been cloned, TRH-R1 and TRH-R2. The first one was originally cloned from a mouse thyrotropic pituitary tumor (Straub et al., 1990) and then from rat (Zhao et al., 1992; De la Pena et al., 1992; Sellar et al., 1993), human (Matre et al., 1993; Duthie et al., 1993; Yamada et al., 1993), chicken (Sun et al., 1998), bovine (Takata et al., 1998), lower vertebrate (Harder et al., 2001), and amphibians (Bidaud et al., 2002) tissues. TRH-R2 has been cloned from rat spinal cord complementary DNA (cDNA) library (Cao et al., 1998), rat brain cDNA libraries (Itadani et al., 1998; O'Dowd et al., 2000), mouse brain (Harder et al., 2001), and frog brain (Bidaud et al., 2002). TRH-R1 and TRH-R2 bind agonists with indistinguishable affinities and both signal via the phosphoinositide pathway (Harder et al., 2001). In contrast, the two receptor subtypes exhibit different tissue expression (Cao et al., 1998; Heuer et al., 2000; O'Dowd et al., 2000) and TRH-R2 exhibits a much higher basal activity than TRH-R1 does (Wang and Gershengorn, 1999). TRH-R1 is found in high abundance in the anterior pituitary and is mainly involved in the signaling of TRH within neuroendocrine brain areas, but it exhibits only a very limited mRNA expression pattern in other regions of the central nervous system (CNS) (Calzá et al., 1992; Zabavnik et al., 1993; Heuer et al., 1998). Northern blot analysis and *in situ* hybridization data reveal that TRH-R2 is expressed exclusively in rat brain and spinal cord (Cao et al., 1998; Heuer et al., 2000); although it has also been detected in pituitary (O´Dowd et al., 2000). Recently, a novel subtype of TRH receptor, TRH-R3, has been identified in *Xenopus laevis* brain (Bidaud et al., 2002); although a posterior study suggests that this TRH-R3 is a receptor for a ligand other than TRH (Lu et al., 2003).

Offprint requests to: Inés Martín-Lacave, Dpto. Citología e Histología Normal y Patológica, Facultad de Medicina, Universidad de Sevilla, Avda. Sánchez-Pizjuán s/n, 41009 Sevilla, Spain. e-mail: ilacave@us.es

Besides stimulation of TSH secretion at the pituitary, additional endocrine functions of TRH have been demonstrated; its strong effect as a prolactin secretagogue and its ability to stimulate the secretion of growth hormone (GH) under certain, mainly pathological, conditions. On the other hand, numerous morphological and pharmacological studies have implicated TRH as playing a role as neurotransmitter or neuromodulator in the CNS affecting behavior, temperature regulation, food intake, and nociception (Griffiths, 1985; Horita et al., 1986; Kelly, 1995; Cao et al., 1998; Horita, 1998; Nillni and Sevarino, 1999).

Although TRH have been tested in hypothalamus (over 70% of the total brain) and extrahypothalamic brain areas (Prasad, 1984), TRH or TRH-like immunoreaction for this hormone have been found in some tissues outside the nervous system such as prostate, pancreas, testis, adrenal gland, and thyroid (Morley, 1979; Jackson, 1982; Pekary et al., 1983, 1986; Iversen et al., 1984; Montagne et al., 1997, 1999). Moreover, in the case of thyroid, it has been well established that C-cells express true TRH at both mRNA and protein levels (Gkonos et al., 1989).

It has also been demonstrated that TRH inhibits the release of thyroid hormones and the increase of cAMP induced by TSH from dog thyroid (Delbeke et al., 1983; Iversen and Laurberg, 1985). Although the effect of TRH on thyroid hormone secretion could be due to hypothalamic TRH, C-cells could have a paracrine role in thyroid homeostasis by virtue of their own TRH synthesis. This paracrine role of C-cells could also explain the fact that this endocrine population has reached up to the center of the thyroid lobe along its phylogenetic evolution, establishing close contact with follicular cells in a "parafollicular" position (Kalisnik et al., 1988). Curiously, the central regions of the thyroid lobes present higher follicular cell activity than the periphery of the lobes. If this hypothesis is true, follicular cells should express TRH-Rs for the suggested paracrine modulation of thyroid hormone secretion carried out by C-cells. Supporting this idea, similar models have been recently described outside the nervous system and pituitary gland, such as testis, adrenal gland, thymus and other lymphoid tissue, where the expression of TRH-Rs has been demonstrated, suggesting a paracrine regulation by TRH in these organs (Satoh et al., 1994; Mellado et al., 1999; Montagne et al., 1999).

In order to elucidate whether or not C-cell TRH production could act over follicular cells modulating thyroid function, we investigate TRH-Rs expression in PC C13 follicular cells from rat thyroid, by means of immunofluorescence technique and RT-PCR analysis. We also study the possibility that C-cells present TRH-Rs for the autocrine control of its own TRH production.

Materials and methods

Cell culture

PC C13 cells (generously provided by Dr. Massimo Santoro, Centro di Endocrinologia e Oncologia Sperimentale di C.N.R., Naples) were grown in Coon's modified Ham's F12 medium (Sigma) supplemented with 5% calf serum (Gibco) and a mixture of six growth factors (10 nM thyrotropin, 10 nM hydrocortisone, 20 mg/ml glycyl-hystidyl-lysine, 10 nM hydrocortisone, 5 mg/ml transferrin, 5 nM somatostatin, and 100 nM insulin), as previously reported (Florio et al., 2001). Cell line 6-23 (rat thyroid C-cells; Clone 6, LGC Promochem-ATCC) was cultured with Dulbecco's modified Eagle's medium with 85% glucose (4.5 g/L), and 15% horse serum. Cultures were maintained at 37°C in a humidified atmosphere with 5% CO_2.

RNA Isolation and Reverse Transcription (RT)-PCR

Total RNA from cultured cells was isolated with *Tripure Isolation Reagent* (Roche Diagnostics, Germany). RNA concentration was determined spectrophotometrically. AMV reverse transcriptase and random primers from Roche Diagnostics (Germany) were used for RT-PCR. Five micrograms of RNA were reverse transcripted with *1st Strand cDNA Synthesis Kit* for RT-PCR (AMV) (Roche Diagnostics, Germany).

Four microlitres from the transcription reaction were amplified by PCR. A typical 35 cycles amplification was performed with 1 min denaturation step at 94ºC, 1 min annealing step at 57°C, and 1 min extension step at 72°C. Primers for amplification were the following: rTRH-R1-forward, 5'-AGATGTTTCAACAGCACCGT TTC-3'; rTRH-R1-reverse, 5'-TCTGTGCTAAAGCG GTCTGACTC-3'; rTRH-R2-forward, 5'-GGTTCTTC CTGGTGGATCTCAAT-3'; and rTRH-R2-reverse, 5'-GAGCAGTACCAGTGTGCGGTAAG-3' (Basmaciogullari et al., 2000). Primers were located in different exons to avoid amplification of contaminated genomic DNA. The products of amplification, 344 bp for TRH-R1 and 350 bp for TRH-R2, were separated on a 2% agarose gel. Each experiment was repeated three times. PCR products were direct sequenced at NBT (www.newbiotechnic.com) and compared with the appropriated TRH-R mRNA rat sequences (GeneBank Accession numbers: NM_013047 for TRH-R1, and AF149717 for TRH-R2). cDNA from rat brain total RNA was used as positive control for RT-PCR of both receptors. Negative controls consisted of omission of cDNA in the PCR mixture and of the reverse transcriptase enzime during retrotranscription.

Rat ß-actin mRNA was amplified as RT-PCR positive control (sense primer: 5'-TTGTAACCAACTG GGACGATATGG-3' and antisense primer: 5'-GATCTT GATCTTCATGGTGCTAGG-3'; leading a fragment of 746 bp).

Immunofluorescence TRH-R detection

Cells were trypsinized and transferred to a slide-flask (Labtek slide chamber; Nunc, USA) and incubated for 24 h. Afterwards, cells where starved in serum-free and growth factor-free medium for 24 h before

experiments. For immunofluorescence, slides were washed three times in PBS and quickly fixed in cold methanol at -20°C for 6 min. After PBS washing, slides were blocked with horse serum for 30 min at room temperature in a humidity chamber. Afterwards, polyclonal antibodies against the amino terminus of TRH-R1 (E-16) and TRH-R2 (D-21) of rat origin (diluted 1:50; Santa Cruz Biotechnology, Inc.) were added for overnight incubation at 4ºC in a humidity chamber. DAPI was added to the primary antibody for nucleus counterstaining. PBS-washed slides were incubated with donkey anti-goat antibody labeled with cyanine-2 (Cy2) (diluted 1:100, Jackson Immunoresearch Laboratories) for 45 min at room temperature in a humidity chamber. After PBS washing, slides were mounted in 90% glycerol, 2% n-propylgallate (Sigma), 1xPBS, and observed under epi-fluorescence microscope (Olympus BX50). Negative control reactions for TRH-Rs were carried out by omitting primary antibodies.

Results

The RT-PCR analysis of PC C13 cell line resulted in the amplification of the specific 350 bp band corresponding to TRH-R2, but did not yield any band with the primers for TRH-R1 (Fig. 1A). In the case of 6-23 C-cells, TRH-R1 and TRH-R2 mRNAs were demonstrated by this technique with the appearance of the corresponding 344 bp and 350 bp bands, respectively (Fig. 1A). The intensities of TRH-Rs PCR bands were similar to those of rat brain total mRNA, used as positive controls. The direct sequencing of the PCR products ensured that amplicons corresponded to rat TRH-Rs (data not shown).

Immunofluorescence technique was performed in order to further localize the TRH-R proteins within individual follicular cells. TRH-R1 immunostainning, as expected, rendered no fluorescence signals in PC C13 thyroid cells (Fig. 1B). In contrast, TRH-R2 was detected in the PC C13 cell line as cytoplasmic immunoreactivity (Fig. 1C).

Discussion

TRH is synthesized by peptidergic neurons of supraoptic and paraventricular nuclei of the hypothalamus, and then axonally transported to be stored in the median eminence. When secreted in the bloodstream, it reaches the anterior pituitary where it stimulates the production of TSH which in turn stimulates the production of thyroxin (T_4) and triiodothyronine (T_3) in the thyroid gland.

The effects of TRH on thyroid hormone secretion do not take place only at the pituitary, but also at the thyroid. It has been demonstrated that high concentrations of TRH inhibit TSH induced iodothyronine release and cAMP increase from dog thyroid, suggesting that TRH in the thyroid participates in the regulation of thyroid hormone secretion as an antagonist to TSH (Delbeke et al., 1983; Iversen and Laurberg, 1985).

In addition to this point, Rausell et al. (1999) found that the levels of TRH and TRH-like peptides in the thyroid were strongly influenced by thyroid status. The concentrations of TRH in the hyperthyroid and hypothyroid rats were higher than in the euthyroid rats. The possibility that TRH and TRH-like peptides act within the thyroid is also supported by other reports where exogenous TRH exerted a direct effect on thyroid hormone release in vitro (Raspe et al., 1991) and administration of TRH to hyperthyroid patients with very low levels of TSH resulted in decreased levels of circulating thyroid hormones (Sato et al., 1995).

Although there is evidence that the thyroid gland accumulates some TRH from the blood (Steiner et al., 1974), it is also known that TRH immunoreactivity is observed in the thyroid, correlating with true TRH gene expression (Gkonos et al., 1989). This last alternative opens the possibility of a paracrine regulation of follicular cell secretion through the releasing of TRH by neighbor C-cells.

If the hypothesis of TRH having an additional role as local regulator of thyroid hormones secretion is true, follicular cells must contain receptors for TRH. The present study shows, for the first time, the presence of TRH-R2 at both mRNA and protein levels in the rat thyroid follicular cell line PC C13.

The expression of TRH-R2 in thyroid follicular cells could explain the influence of thyroidal status on TRH secretion by C-cells (Rausell et al., 1999). The increased levels of TRH in experimental hyperthyroid and hypothyroid rats could be the response to increased levels of T_3 and TSH, respectively. This is also in accordance with Iversen and Laurberg (1985), who suggested that TRH in the thyroid participates in the regulation of thyroid hormone secretion as an antagonist to TSH. Moreover, the presence of TSH receptors in thyroid C-cells has been demonstrated by immunocytochemistry (Tamir et al., 1990). As it occurs with several regulatory peptides in the pituitary (Pazos-Moura et al., 2003), synthesis of TRH by C-cells seems to be under hormonal control which is in agreement with the proposed biological role of local regulators, namely to modulate or fine-tune the response of the tissue to hormonal regulation.

On the other hand, we also described the presence of TRH-R1 and TRH-R2 in a cell line from rat thyroid C-cells. It is interesting to consider the possibility of an autocrine regulation, common in endocrine cells, in the secretion of TRH by C-cells in the thyroid. Moreover, the presence of TRH-Rs in rat thyroid C-cells could explain the calcitonin release from thyroid C-cells after stimulation with TRH and calcium injection separately (Oishi et al., 1992).

TRH-R1 and TRH-R2 exhibit similar binding affinities for a number of TRH analogs and similar activation of the phosphoinositide-calcium signal transduction pathway by TRH (Harder et al., 2001). In

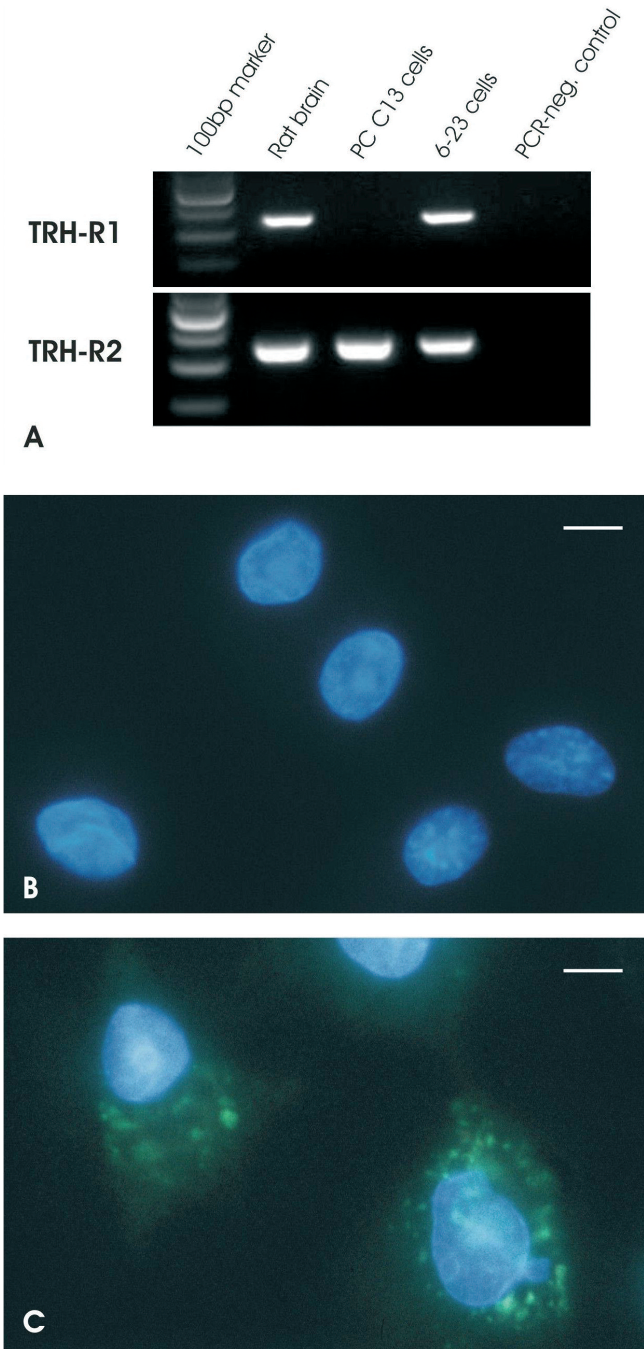

Fig. 1. TRH-Rs expression in rat thyroid cells at mRNA and protein levels. **A.** RT-PCR for TRH-R1 and TRH-R2 in PC C13 follicular cells and 6-23 C-cells. Rat brain cDNA was used as positive control for both receptors, and water instead of cDNA as negative control. **B.** PC C13 cells immunonegative for TRH-R1 antibody. **C.** Some TRH-R2 immunopositive PC C13 cells revealing the cytoplasmatic distribution of the receptor. Bar: 10 µm.

contrast, TRH-R2 is more rapidly internalized, more markedly downregulated, and exhibits greater basal signaling activity after binding TRH than TRH-R1 (Wang and Gershengorn, 1999; O'Dowd et al., 2000). Moreover, both receptors present distinct regulation and anatomic distribution in the rat. The extensive distribution of TRH-R2 in the brain suggests that it mediates many of the known functions of TRH that are not transduced by TRH-R1 (O'Dowd et al., 2000), and proposes important functional and structural differences between the two receptors. In the rat thyroid, C-cells express both TRH-Rs while follicular cells only express TRH-R2. Although it is only speculation, TRH-R2 from follicular cells could activate only when exposed to a high concentration of TRH, as suggested by Iversen and Laurberg (1985), and this may be due to its higher basal signaling. TRH for this activation process would be locally provided by C-cells TRH secretion, since hypothalamic TRH from the blood stream is present at a very low concentration in the thyroid (Steiner et al., 1974). Studies in order to elucidate the role of the specific TRH-Rs expression in each of the thyroid cell subtypes were not intended for the purposes of this paper.

In conclusion, we have demonstrated that rat thyroid follicular cells express TRH-R2 at mRNA and protein level. On the other hand, rat thyroid C-cells express both receptors, TRH-R1 and TRH-R2. Although further studies are needed to evaluate the significance of the different TRH-Rs expression in thyroid cells and the paracrine/autocrine effects of TRH in the thyroid, our data provide new evidence for a novel intrathyroidal regulatory pathway of thyroid hormone secretion via paracrine/autocrine TRH signaling.

Acknowledgements. This study was supported by grants from the Ministerio de Ciencia y Tecnología (ref. BFI2003-0606 and P2003-1339), and the Secretaría General de Universidad e Investigación of the Junta de Andalucía (CTS-439 and CTS-0160), Spain.

References

Basmaciogullari A., Cras-Meneur C., Czernichow P. and Scharfmann R. (2000). Pancreatic pattern of expression of thyrotropin-releasing hormone during rat embryonic development. J. Endocrinol. 166, 481-488.

Bidaud I., Lory P., Nicolas P., Bulant M. and Ladram A. (2002). Characterization and functional expression of cDNAs encoding thyrotropin-releasing hormone receptor from *Xenopus laevis*. Eur. J. Biochem. 269, 4566-4576.

Calzá L., Giardino L., Ceccatelli S., Zanni M., Elde R. and Hökfelt T. (1992). Distribution of thyrotropin-releasing hormone receptor messenger RNA in the rat brain: an in situ hybridization study. Neuroscience 51, 891-909.

Cao J., O'Donnell D., Vu H., Payza K., Pou C., Godbout C., Jakob A Pelletier M., Lembo P., Ahmad S. and Walker P. (1998). Cloning and characterization of cDNA encoding a novel subtype of rat thyrotropin-releasing hormone receptor. J. Biol. Chem. 273, 32281-

32287.

De la Pena P., Delgado L.M., Del Camino D. and Barros F. (1992). Cloning and expression of the thyrotropin-releasing hormone receptor form GH3 rat anterior pituitary cells. Biochem. J. 284, 891-899.

Delbeke D., Van Sande J., Cochaux P., Decoster C. and Dumont J.E. (1983). Effect of thyrotropin-releasing hormone on dog thyroid in vitro. Biochim. Biophys. Acta 761, 262-268.

Duthie S.M., Taylor P.L., Anderson L., Cook J. and Eidne K.A. (1993). Cloning and functional characterization of the human TRH receptor. Mol. Cell Endocrinol. 95, R11-R15.

Florio T., Arena S., Thellung S., Iuliano R., Corsaro A., Massa A., Pattarozzi A., Bajetto A., Trapasso F., Fusco A. and Schettini G. (2001). The activation of the phosphotyrosine phosphatase eta (r-PTP eta) is responsible for the somatostatin inhibition of PC Cl3 thyroid cell proliferation. Mol. Endocrinol. 15, 1838-1852.

Gkonos P.J., Tavianini M.A., C-C. Liu and Roos B.A. (1989). Thyrotropin-releasing hormone gene expression in normal thyroid parafollicular cells. Mol. Endocrinol. 3, 2101-2109.

Griffiths E.C. (1985). Thyrotropin releasing hormone: Endocrine and central effects. Psychoneuroendocrinology 10, 225-235.

Harder S., Dammann O., Buck F., Zwiers H., Lederis K., Richter D. and Bruhn T.O. (2001). Cloning of two thyrotropin-releasing hormone receptor subtypes form a lower vertebrate (*Catostomus commersoni*): Functional expression, gene structure, and evolution. Gen. Comp. Endocrinol. 124, 236-245.

Harder S., Lu X., Wang W., Buck F., Gershengorn M.C. and Bruhn T.O. (2001). Regulator of G protein signaling 4 suppresses basal and thyrotropin releasing-hormone (TRH)-stimulated signaling by two mouse TRH receptors, TRH-R1 and TRH-R2. Endocrinology 142, 1188-1194.

Heuer H., Ehrchen J., Bauer K. and Schäfer M.K.H. (1998). Region-specific expression of thyrotropin-releasing hormone-degrading ectoenzyme in the rat central nervous system and pituitary gland. Eur. J. Neurosci. 10, 1465-1478.

Heuer H., Schäfer M.K.H., O'Donnell D., Walker P. and Bauer K. (2000). Expression of thyrotropin-releasing hormone receptor 2 (TRH-R2) in the central nervous system of rats. J. Comp. Neurol. 428, 319-336.

Horita A. (1998). An update on the CNS actions of TRH and its analogs. Life Sci. 62, 1443-1448.

Horita A., Carino M.A. and Lai H. (1986). Pharmacology of thyrotropin-releasing hormone. Annu. Rev. Pharmacol. Toxicol. 26, 311-332.

Itadani H., Nakamura T., Itoh J., Iwaasa H., Kanatani A., Borkowski J., Ihara M. and Ohta M. (1998). Cloning and characterization of a new subtype of thyrotropin-releasing hormone receptors. Biochem. Biophys. Res. Commun. 250, 68-71.

Iversen E., Weeke J. and Laurberg P. (1984). TRH immunoreactivity in the thyroid gland. Scand. J. Clin. Lab. Invest. 44, 703-709.

Iversen E. and Laurberg P. (1985). Thyrotropin-releasing hormone (TRH) and hormone secretion from the follicular and C-cells of perfused dog thyroid lobes. Acta Endocrinol. (Copenh.) 109, 499-504.

Jackson I.M.D. (1982). Thyrotropin-releasing hormone. N. Engl. J. Med. 306, 145-155.

Kalisnik M., Vraspir-Porenta O., Kham-Lindtner T., Logonder-Mlinsek M., Pajer Z., Stiblar-Martincic D., Zorc-Pleskovic R. and Trobina M. (1988). The interdependence of the follicular, parafollicular, and mast cells in the mammalian thyroid gland: a review and a synthesis. Am. J. Anat. 183, 148-157.

Kelly J.A. (1995). Thyrotropin-releasing hormone-basis and potential for its therapeutic use. Essays Biochem. 30, 133-149.

Lechan R.M., Wu P., Jackson I.M.D., Wolf H., Cooperman S., Mandel G. and Goodman R.H. (1986). Thyrotropin-releasing hormone precursor: Characterization in rat brain. Science 231, 159-161.

Lu X., Bidaud I., Ladram A. and Gershengorn M.C. (2003). Pharmacological studies of thyrotropin-releasing hormone (TRH) receptors from Xenopus leavis: Is xTRHR3 a TRH receptor? Endocrinology 144, 1842-1846.

Matre V., Karlsen H.E., Wright M.S., Lundell I., Fjeldheim A.K., Gabrielsen O.S., Larhammar D. and Gautvik K.M. (1993). Molecular cloning of a functional human thyrotropin-releasing hormone receptor. Biochem. Biophys. Res. Commun. 195, 179-185.

Mellado M., Fernandez-Agullo T., Rodriguez-Frade J.M., San Frutos M.G., de la Pena P., Martinez-A C. and Montoya E. (1999). Expression analysis of the thyrotropin-releasing hormone receptor (TRHR) in the immune system using agonist anti-TRHR monoclonal antibodies. FEBS Lett. 451, 308-314.

Montagne J-J., Ladram A., Grouselle D., Nicolas P. and Bulant M. (1997). Thyrotropin-releasing hormone immunoreactivity in rat adrenal tissue is localized in mast cells. J. Histochem. Cytochem. 45, 1623-1627.

Montagne J-J., Ladram A., Nicolas P. and Bulant M. (1999). Cloning of thyrotropin-releasing hormone precursor and receptor in rat thymus, adrenal gland, and testis. Endocrinology 140, 1054-1059.

Morley J.E. (1979). Extrahypothalamic thyrotropin-releasing hormone (TRH)- its distribution and its functions. Life Sci. 25, 1539-1550.

Nillni E.A. and Sevarino K.A. (1999). The biology of pro-thyrotropin-releasing hormone-derived peptides. Endocrine Rev. 20, 599-648.

O'Dowd B.F., Lee D.K., Huang W., Nguyen T., Cheng R., Liu Y., Wang B., Gershengorn M.C. and George S.R. (2000). TRH-R2 exhibits similar binding but distinct regulation and anatomic distribution compared to TRH-R1. Mol. Endocrinol. 14, 183-193.

Oishi S., Yamauchi J., Fujimoto Y., Hamasaki S., Umeda T. and Sato T. (1992). Calcitonin release from medullary thyroid carcinoma by thyrotropin-releasing hormone: comparison with calcium injection. Acta Endocrinol. 126, 325-328.

Pazos-Moura C.C., Ortiga-Carvalho T.M. and Gaspar de Moura E. (2003). The autocrine/paracrine regulation of thyrotropin secretion. Thyroid 13, 167-175.

Pekary A.E., Richkind M. and Hershman J.M. (1983). Thyrotropin-releasing hormone and related peptides in canine tissues. J. Endocrinol. 98, 299-306.

Pekary A.E., Reeve J.R. Jr. and Smith V.P. (1986). Evidence for thyrotropin-releasing hormone (TRH) biosynthesis in the rat prostate. Life Sci. 39, 2565-2570.

Prasad C. (1984). Thyrotropin-releasing hormone. In: Handbook of neurochemistry. Lajtha A. (ed). Plenum Press. New York. pp 175-200.

Raspe E., Laurent E., Andry G. and Dumont J.E. (1991). ATP, bradykinin, TRH, and TSH activate the Ca^{++}-phosphatidylinositol cascade of human thyrocytes in primary culture. Mol. Cell. Endocrinol. 81, 175-183.

Rausell V., Fraser H.M., Tobaruela M., Del Rio-Garcia J. and Smyth D.G. (1999). Identification of the TRH-like peptides pGlu-Glu-Pro amide and pGlu-Phe-Pro amide in rat thyroid: regulation by thyroid status. Regul. Pept. 81, 55-60.

Sato A., Yamada T., Aizawa T., Ichikawa K., Komiya I., Takasu N. and Takemura Y. (1995). Effect of thyrotropin-releasing hormone on

serum thyroid hormones: a study in the patients with untreated and treated Graves' disease and subacute thyroiditis. J. Clin. Endocrinol. Metab. 80, 2173-2177.

Satoh T., Feng P., Kim U.J. and Wilber J.F. (1994). Identification of thyrotropin-releasing hormone receptor in the rat testis. Neuropeptides 27, 195-202.

Sellar R.E., Taylor P.L., Lamb R.F., Zabavnik J., Anderson L. and Eidne K.A. (1993). Functional expression and molecular characterization of the thyrotropin-releasing hormone receptor from the rat anterior pituitary gland. J. Mol. Endocrinol. 10, 199-206.

Steiner H., Hünzi H. and Studer R.O. (1974). Accumulation of tritiated thyrotropin releasing hormone in different organs, especially in the thyroid. Experientia 30, 1096-1098.

Straub R.E., Frech G.C., Joho R.H. and Gershengorn M.C. (1990). Expression cloning of a cDNA encoding the mouse pituitary thyrotropin-releasing hormone receptor. Proc. Natl. Acad. Sci. USA 87, 9514-9518.

Sun Y-M., Millar R.P., Ho H., Gershengorn M.C. and Illing N. (1998). Cloning and characterization of the chicken thyrotropin-releasing hormone receptor. Endocrinology 139, 3390-3398.

Takata M., Shimada Y., Ikeda A. and Sekikawa K. (1998). Molecular cloning of bovine thyrotropin-releasing hormone receptor gene. J. Vet. Med. Sci. 60, 123-127.

Tamir H., Liu K.P., Hsiung S.C., Adlersberg M., Nunez E.A. and Gershon M.D. (1990). Multiple signal transduction mechanisms leading to the secretion of 5-hydroxytryptamine by MTC cells, a neurectodermally derived cell line. J. Neurosci. 10, 3743-3753.

Wang W. and Gershengorn M.C. (1999). Rat TRH receptor type 2 exhibits higher basal signaling activity than TRH receptor type 1. Endocrinology 140, 4916-4919.

Yamada M., Monden T., Satoh T., Satoh N., Murakami M., Iriuchijima T., Kakegawa T. and Mori M. (1993). Pituitary adenomas of patients with acromegaly express thyrotropin-releasing hormone receptor messenger RNA: cloning and functional expression of the human thyrotropin-releasing hormone receptor gene. Biochem. Biophys. Res. Commun. 195, 737-745.

Zabavnik J., Arbuthnott G. and Eidne K.A. (1993). Distribution of thyrotropin-releasing hormone receptor messenger RNA in rat pituitary and brain. Neuroscience 53, 877-887.

Zhao D., Yang J., Jones K.E., Gerald, C., Suzuki Y., Hogan P.G., Chin W.W. and Tashjian A.H. (1992). Molecular cloning of a complementary deoxyribonucleic acid encoding the thyrotropin-releasing hormone receptor and regulation of its messenger ribonucleic acid in rat GH cells. Endocrinology 130, 3529-3536.

Accepted February 1, 2005