0163-769X/99/$03.00/0
Endocrine Reviews 20(5): 599–648

Printed in U.S.A.

The Biology of pro-Thyrotropin-Releasing Hormone-Derived Peptides*

EDUARDO A. NILLNI AND KEVIN A. SEVARINO

Division of Endocrinology (E.A.N.), Department of Medicine, Brown University School of Medicine, Rhode Island Hospital, Providence, Rhode Island 02903; and Yale University School of Medicine and Connecticut Veterans Affairs Healthcare System (K.A.S.), Department of Psychiatry, Research 151, West Haven, Connecticut 06516

I. Introduction
II. Biosynthesis of TRH and Other pro-TRH-Derived Peptides
  A. Biosynthesis and processing of pro-TRH
  B. Intracellular sites of pro-TRH processing
  C. Tissue-specific processing of pro-TRH
  D. The role of PCs and CPs
  E. Neuropeptide and catecholamine regulation of Pro-TRH biosynthesis and processing
  F. Glucocorticoids modulate the biosynthesis and processing of pro-TRH
  G. Leptin regulates pro-TRH biosynthesis
III. Function of TRH
  A. The HPT axis
  B. Extrahypophysiotropic TRH
IV. Function of non-TRH pro-TRH-Derived Peptides
  A. prepro-$TRH_{160–169}$ (pST_{10})
  B. prepro-$TRH_{178–199}$ (pFE_{22})
  C. prepro-$TRH_{178–185}$ and prepro-$TRH_{186–199}$ (pFQ_7 and pSE_{14})
  D. prepro-$TRH_{53–74}$ (pFT_{22})
  E. prepro-$TRH_{83–106}$ (pEH_{24}) and prepro-$TRH_{208–255}$
  F. TRH-Gly
V. Non-TRH pro-TRH-Derived Peptides Outside of the CNS
VI. TRH and Other pro-TRH-Derived Peptide Receptors
  A. The TRH receptor
  B. The prepro-$TRH_{160–169}$ (pST_{10}) receptor
VII. TRH Degradation
VIII. Concluding Remarks

I. Introduction

A SIGNIFICANT body of research has led to the conclusion that peptides are important regulatory components of nervous system function. Initially, the "peptidergic neuron" concept was reserved for the neurosecretory cells in the hypothalamus that released oxytocin and vasopressin directly into the circulation from their nerve terminals in the posterior pituitary. The idea of neurosecretion in the hypothalamus can be traced back to the work of Scharrer and Scharrer (1) as early as the late 1920s. Later work by Harris (2) specified that the hypothalamic substances secreted into the portal vessels were pituitary specific and led to the concept of "releasing factors" whose purpose was to initiate a cascade of events resulting in the release of peripherally active hormones (3). The discovery and chemical characterization of the first identified hypothalamic releasing factor, TRH (pyro-Glu-His-$ProNH_2$, also known as thyroliberin, and herein referred to as TRH) by Guillemin and colleagues (4) and Schally and colleagues (5) provided ultimate confirmation for the founding principles of neuroendocrinology and was followed by the discovery of other peptide-releasing factors (6, 7).

TRH, produced in the paraventricular nucleus of the hypothalamus (PVN),[1] stimulates the biosynthesis and secretion of TSH from the anterior pituitary (8, 9). TSH, in turn,

Address reprint requests to: Dr. Eduardo A. Nillni, Division of Endocrinology, Rhode Island Hospital, 593 Eddy Street, Providence, Rhode Island 02903 USA. E-mail: Eduardo_Nillni@Brown.edu

* This work was supported by National Science Foundation Grant IBN-9507952 (to E.A.N.) and the National Institute on Drug Abuse Grant 1-R01-DA-10762 (to K.A.S.).

[1] Abbreviations: iTRH, immunoreactive TRH; TRH-OH, deamidated TRH; CHP, cyclo (His-Pro); PTU, propylthiouracil; HPT, hypothalamic-pituitary-thyroid; DVC, dorsal vagal complex; DMN, dorsal motor nucleus of the vagus; NTS, nucleus tractus solitaris; IML, intermediolateral column of the spinal cord; SP, substance P; NPY, neuropeptide Y; CGRP, calcitonin gene-related peptide; CT, calcitonin; IL1, interleukin 1; VIP, vasoactive intestinal peptide; OAG, the diacylglycerol analog 1-oleoyl-2-acetyl-sn-glycerol; CCK, cholecystokinin; NT, neurotensin; THC, tetrahydrocannabinol; ACh, acetylcholine; ECS, electroconvulsive shock treatment; NE, norepinephrine; E, epinephrine; DA, dopamine; 5-HT, serotonin; NO, nitric oxide; NMDA, *N*-methyl-D-aspartate; 6-HODA, 6-hydroxydopamine; 5,7-DHT, 5,7-dihydroxytryptamine; GABA, γ-aminobutyric acid; EOPs, endogenous opioid peptides; CA, catecholamines; ENK, enkephalins; DYN, dynorphin; SRIF, somatostatin; CP, carboxypeptidases; CPE, carboxypeptidase E; CSF, cerebrospinal fluid; NAc, nucleus accumbens; VTA, ventral tegmental area; PVN, periventricular nucleus of the hypothalamus; RPa, nucleus raphe pallidus; RMg, nucleus raphe magnus; ROb, nucleus raphe obscurus; icv, intracerebroventricular; sc, subcutaneous, ic, intracerebral; SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto rats; MCA, middle cerebral artery; ALS, amyotrophic lateral sclerosis; RMN, Rolling mouse Nagoya model of ataxia; CNS, central nervous system; EEG, electroencephalogram; PAG, periaqueductal gray; RPGi, nucleus reticularis paragigantocellularis; MAF, mesencephalic reticular formation; POA; preoptic nucleus of the hypothalamus; LC, locus coeruleus; LPGi, nucleus paragigantocellularis lateralis; LS, long-sleep; SS, short-sleep; PBMC, peripheral blood monocyte; ME, median eminence; RER, rough endoplasmic reticulum; PCs, proconverting enzymes; RSP, regulated secretory pathway; GC, Golgi complex; SG, secretory granules; TGN, trans-Golgi network; ICC immunocytochemistry; TRE, thyroid response element; CRE, cAMP response element.

stimulates thyroid hormone biosynthesis and release (10). TRH is central in regulating the hypothalamic-pituitary-thyroid (HPT) axis. TRH influences the release of other hormones, including PRL, GH, vasopressin, and insulin (11–13), and the classic neurotransmitters noradrenaline and adrenaline (14). Further, TRH is present in many brain loci outside of the hypothalamus, supporting a potential role as a neuromodulator or neurotransmitter outside of traditional HPT axis function (15, 16). For example, TRH is implicated as a modulator of seizure activity (17) and gastrointestinal function (18). TRH has been also found outside the central nervous system (CNS) in the gastrointestinal tract, pancreas, reproductive tissues including placenta, ovary, testis, seminal vesicles, and prostate, retina, and blood elements (19). The widespread distribution of TRH within and outside the CNS supports a diverse range of roles for this molecule, roles likely to involve many functions outside of the traditional HPT axis.

Of the many peptide products derived from the TRH precursor (pro-TRH described below), until recently only TRH itself was studied extensively. In the last few years, a new wave of research has identified many other products derived from pro-TRH and suggests potential biological functions for these non-TRH peptides. The immediate precursor to TRH, TRH-Gly, independent of conversion to TRH, stimulates gastric acid secretion in a dose-dependent manner, although it is 100-fold less potent than TRH (18). Other peptides derived from pro-TRH, originally referred to as cryptic peptides because their roles are incompletely understood, are now being studied. In the hypothalamus, prepro-$TRH_{160-169}$ (pST_{10}, also known as Ps4) and prepro-$TRH_{178-199}$ (pFE_{22}), peptides that lie between the third and fourth and the fourth and fifth progenitor sequences for TRH in the TRH precursor, respectively, are released from perifused rat hypothalamic slices and the median eminence (20). [The reader should note that in Fig. 1, pro-TRH-derived peptides are named by "p" for peptide followed by the single letter amino acid designation for the first and last amino acid of the peptide, along with the peptide length in subscript. Where these peptides are first mentioned, they are followed by the longer prepro-TRH name that describes their amino acid residue positions within the precursor.] prepro-$TRH_{160-169}$ potentiates TSH release from anterior pituitary and stimulates TSHβ gene promoter activity (21). This peptide also potentiates TRH-induced gastric acid secretion when microinjected into the dorsal motor nucleus of the vagus (22). Thus, prepro-$TRH_{160-169}$ acts in concert with TRH both within and outside traditional HPT roles. prepro-$TRH_{178-199}$ is proposed to be a CRH-inhibiting factor (Refs. 23 and 24 but see Ref. 25).

Both prepro-TRH_{25-50} (pYE_{26}) and prepro-TRH_{53-74} (pFT_{22}) are released from the median eminence (ME) and isolated anterior pituitary cells in response to depolarizing concentrations of potassium (26). In general, pro-TRH-derived peptides are present in the ME in much higher levels than other regions of the brain, consistent with complete processing of pro-TRH in the ME (E. A. Nillni, unpublished data). *In vivo* studies demonstrate that pro-TRH processing is regulated (27). During opiate withdrawal in rats, prepro-TRH mRNA is induced in the periaqueductal gray (PAG). The N-terminal peptides prepro-TRH_{53-74} and propro-TRH_{83-106} (pEH_{24}) are increased (see *Section IV*), whereas the level of TRH remains unaltered (27, 28). In addition, prepro-$TRH_{178-199}$ and its processed forms are increased in the PVN during suckling (see *Section IV*). Thus, levels of various products derived from pro-TRH can be independently regulated under altered physiological conditions.

Substantial progress has been made in the last few years in understanding the biosynthesis and processing of prohormone and neuropeptide precursors. For pro-TRH (Fig. 1), we have developed a model of its processing to mature peptides (29–31). Like many other secreted peptides, processing of the primary translation product, prepro-TRH, begins with removal of the signal peptide during its passage into the lumen of the rough endoplasmic reticulum (RER). From our current knowledge, processing of pro-TRH takes place within the regulated secretory pathway (RSP) (for full definition see *Section IIA*). Two recently discovered serine proteases, which are members of the family of prohormone convertases (PCs), PC1 (SPC3) and PC2 (SPC2), related to subtilisin and the yeast-processing enzyme Kex 2 (32–34), are the primary PCs involved in posttranslational processing of pro-TRH (31, 35–37).

Recent contributions to the understanding of pro-TRH biosynthesis and processing provide a useful framework for uncovering the diversity of function displayed by pro-TRH-derived peptides and the way that this diversity is generated. We will review the current knowledge of pro-TRH processing to the most studied biological end product, TRH, and importantly, other pro-TRH-derived peptides. The importance of understanding pro-TRH processing is then underscored by a comprehensive review of the wide range of potential biological roles subsumed by non-TRH products derived from pro-TRH. Although data are limited, where possible we summarize where these peptides are produced and how their levels might be regulated. We then review the HPT axis, with emphasis of its effects on pro-TRH processing. This is followed by a comprehensive review of the function of extrahypophysiotropic TRH. A review of TRH and other pro-TRH-derived peptide receptors then explains mechanisms of pro-TRH peptide signal transduction. Finally, TRH degradation provides an additional way by which peptide levels are tightly controlled, and this area is reviewed. The degradation of other pro-TRH-derived peptides is not yet understood.

II. Biosynthesis of TRH and Other pro-TRH-Derived Peptides

A. Biosynthesis and processing of pro-TRH

In recent years considerable research has focused on the expression of neuropeptide genes and their tissue-specific regulation. However, it has become clear that the peptides derived from these genes play significant neuromodulatory roles in the control of the CNS neurotransmitters. Even more astounding is the discovery that multiple neuropeptides with distinct physiological functions arise from the processing of single polypeptide precursors (36, 38–40). Thus, to fully understand the biology of a neuropeptide, one must understand the processing of the preprohormone gene prod-

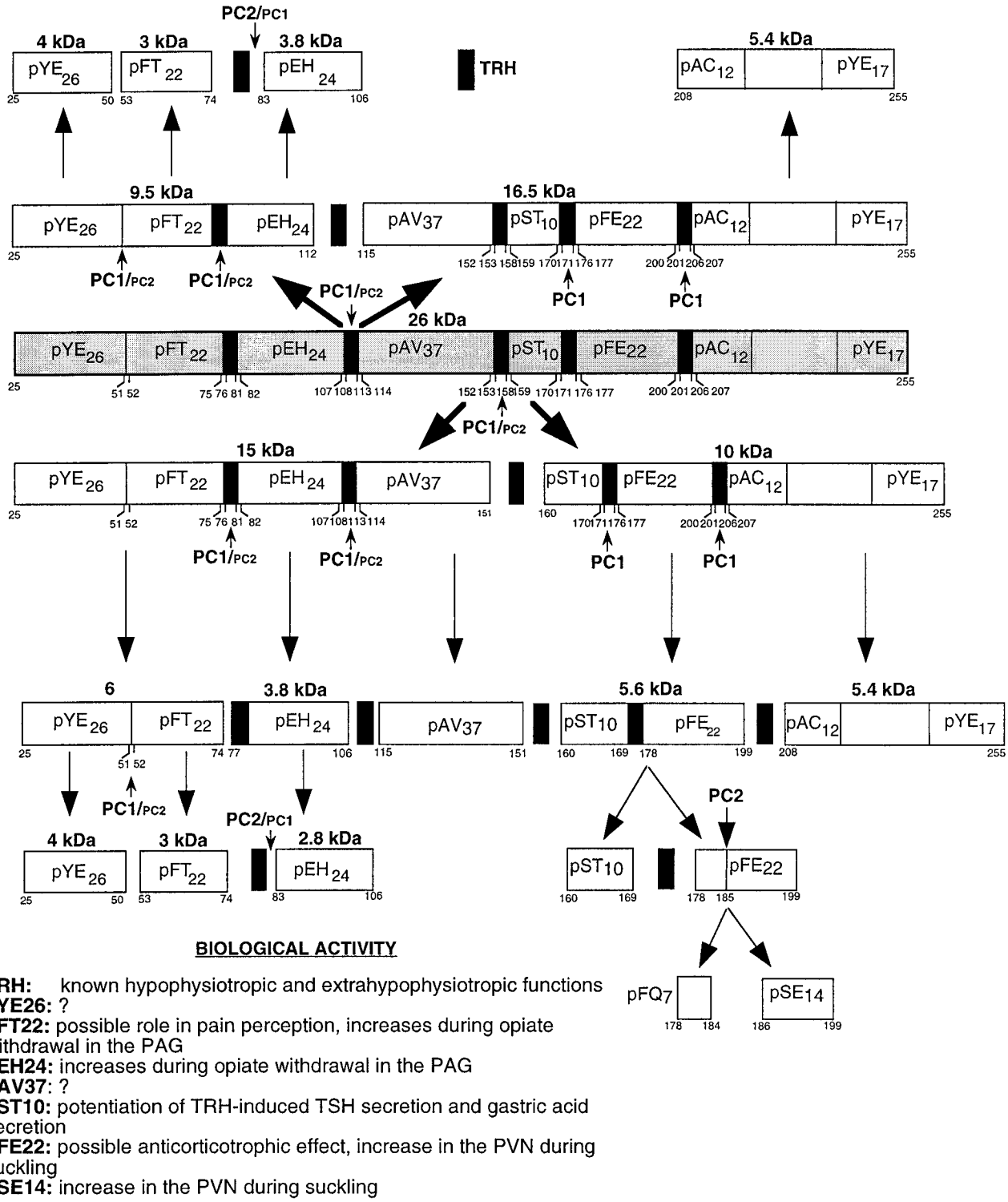


FIG. 1. Diagrammatic representation of the proposed processing model of rat pro-TRH to non-TRH peptides and TRH, and the postulated involvement of PC1 and PC2 as determined from our studies (for further details see *Section II.A–D*). The *small arrows* indicate PC1 and PC2 activity. Cleavage sites and direction of the processing cascade are indicated with *longer arrows*. *Thicker arrows* indicate that most of the initial cleavage of the intact precursor was produced at this site. The positions of paired basic residues are indicated by numbers. Non-TRH peptides are indicated in the *shaded* pro-TRH molecule, and TRH is indicated by a *black rectangle*.

uct, as well as the regulation of the gene's transcription. How do cells produce different levels of one peptide with respect to another when both derive from the same protein sequence? This is achieved through differential processing and degradation by the action of specific enzymes acting in specific cellular and extracellular compartments. Posttransla-

tional processing of hormone precursor proteins is a critical mechanism by which cells increase their biological and functional diversity, such that two or more peptides with different biological functions originate from the same precursor. It is through differential posttranslational processing mechanisms that cells selectively produce specific peptides for secretion.

Even though the amino acid structures of vasopressin and insulin had been elucidated in the 1950s (41), it was not until the early 1960s that the mechanisms of protein biosynthesis began to be understood and the genetic code was fully defined. In 1964, Sachs and Takabatake (42) provided the first evidence that the biosynthesis of vasopressin can be inhibited with puromycin, a protein synthesis inhibitor, and that newly synthesized vasopressin could not be detected in tissues until more than 1 h after pulse labeling. In these studies, Sachs and Takabatake demonstrated that before vasopressin becomes a biologically active peptide, it exists in a modified, or proform, state. Posttranslational modification was then required to convert the proform into an active peptide.

While the structure of insulin peptides was described early on (41), it was difficult to envision how the combination of A and B chains was attained in β-cells of the pancreas. Experiments initiated by Steiner in 1965 using tritiated leucine and phenylalanine to label proinsulin and insulin represented a landmark in prohormone theory. Using a pancreatic insulinoma derived from a patient, it was possible to determine that insulin could be derived *in vitro* from a single molecule that was converted to the A and B chains by trypsin treatment (43). Studies done in rat islets subsequently demonstrated conversion of proinsulin to insulin, in a relatively slow process taking approximately 40 min (44). During the same period, work done by Howell and Taylor (45) on insulin biosynthesis showed that newly synthesized insulin was released several hours after its biosynthesis. The emerging view of these findings was that some sort of orderly vectorial transport occurred involving the RER, the Golgi complex (GC), and secretory granules (SG). These data (46), along with the major contributions of other investigators who established that the biosynthesis of serum albumin, PTH, and glucagon also originate from larger precursors, formed the basis of the prohormone theory. This theory states that synthesis of peptide hormones and neuropeptides begins with mRNA translation into a large, inactive precursor peptide, followed by limited posttranslational proteolysis to release bioactive end products. Chretien and Li (47) also made an important contribution to the prohormone theory when they determined the amino acid sequences of β-lipotropin (β-LPH), γ-LPH, and β-melanotropin (β-MSH). They observed that β-MSH was part of the β-LPH sequence, providing evidence that β-MSH was a conversion product of β-LPH. They also observed that cleavage occurred at the C-terminal side of paired basic lysine or arginine residues. More definitive evidence for a precursor/product was provided with the cloning of POMC (29 kDa), which revealed that the ACTH and β-LPH sequences were present within the N terminus of POMC (38).

In summary, the biochemical processing of neuropeptides, peptide hormones, and other secreted proteins begins with limited posttranslational proteolysis of larger inactive precursors. Prohormones generally have their hormone sequences flanked by a single, a pair or four (tetra) basic amino acids where subtilisin-like processing enzymes produce their initial endoproteolytic cleavage (48, 49). This endopreoteolytic cleavage is produced at the C-terminal side of the single or paired basic amino acid residue(s) which is followed by removal of the basic residue(s) by carboxypeptidase enzymes (CP) (50, 51). Further modifications can occur in the form of N-terminal acetylation, pyroglutamate formation, or C-terminal amidation, which confers bioactivity to many peptides (52).

At the intracellular level, hormone precursors are synthesized on membrane-bound ribosomes, by which they are translocated into the lumen of the RER via a signal recognition peptide. During vectorial transport through the GC and beyond, the newly synthesized proteins are subjected to posttranslational modifications including glycosylation, phosphorylation, amidation, acetylation, and proteolytic conversion (48). Ultimately, partially processed proteins reach the last compartment of the GC, the *trans*-Golgi Network (TGN). At the TGN, unprocessed or partially processed products are sorted to the RSP (29, 53, 54) to be stored in immature secretory granules (ISG). Upon maturation, electron-dense SGs containing sorted products can fuse with the plasma membrane in response to an extracellular stimulation in a calcium-dependent manner, thereby releasing their contents into the external milieu (55). Two pathways of unstimulated release are proposed for AtT_{20} cells: constitutive (nongranular) secretion and basal release from compartments that form after sorting into the RSP (56). The mechanism whereby constitutive and regulated proteins are differentially sorted into separate vesicles after budding from the TGN is still under intensive investigation (57, 58).

Two hypotheses have been proposed to explain how proteins are selectively targeted from the TGN to the RSP. In the first hypothesis, proteins are sorted by passive aggregation, in which the proteins condense within forming ISG, thereby excluding other proteins from entering in the granule. This process occurs under acidic pH and high calcium concentrations (59, 60). Support for the aggregation hypothesis comes from studies done with chromogranin A (61, 62), chromogranin B (62), carboxypeptidase E (CPE) (63), and prohormone convertase 2 (PC2) (64). However, there are data suggesting that aggregation alone is not sufficient for sorting. Modifications of the chromogranin B sequence (65) can prevent the correct sorting of these peptides to the RSP, while their *in vitro* aggregation properties appeared unaltered. The insulin-like growth factor-1 (66) does not aggregate in the TGN, but is still sorted in the RSP.

The second hypothesis, originally proposed in 1985 by Kelly (67), involves *cis*-acting sorting signals within a protein destined for the RSP that interact with membrane-bound sorting receptors. Sorting receptors, possibly located in the forming SG, direct segregation of the protein for further packaging into SGs. Protein aggregation within the ISG can occur in this model, but is more critical for product concentration than sorting *per se.* Evidence supporting this second hypothesis has come from experiments involving chimeric proteins (68–73), where the fusion of constitutively secreted protein to a protein destined for RSP caused a rerouting of

this protein to the RSP. Conversely, proteins that have their sorting signal domains modified may be misrouted from the RSP into the constitutive pathway, as demonstrated for POMC (74), chromogranin A and B (75), PC2 (69), and glycine α-amidating monooxygenase (PAM) (76). A related hypothesis is sorting by retention, in which all proteins are initially targeted to ISGs, after which proteins that do not belong in the RSP are removed to their final destination, *e.g.*, lysosomal enzymes (57, 77).

It has been proposed recently that the membrane form of CPE, localized to the TGN, is a sorting signal receptor (78). CPE is proposed to direct POMC, proinsulin, proenkephalin, but not chromogranin A, into the RSP (79). Thus, CPE is a common sorting receptor for some, but not all, prohormones, and there must be other sorting receptors to direct trafficking of other proteins to the RSP (79). However, Irminger *et al.* (80) have provided evidence refuting the claim that CPE is a sorting receptor for proinsulin. In those studies they used pancreatic islets isolated from CPE-deficient (Cpe^{fat}/Cpe^{fat}) and control ($Cpe^{fat}/+$) mice to examine whether the trafficking of proinsulin and insulin was affected. They found that CPE was not essential for the sorting of proinsulin to the RSP (80). However, similar experiments with procholecystokinin in Cpe^{fat}/Cpe^{fat} mice indicate that CPE does function as a sorting receptor (81).

With this as a background, we turn specifically to the processing of pro-TRH. The elucidation of the rat prepro-TRH sequence in 1986 was of key importance to understanding the processing of pro-TRH to TRH and non-TRH peptides. Rat prepro-TRH is a 29-kDa polypeptide composed of 255 amino acids. The rat precursor contains an N-terminal 25-amino acid leader sequence, 5 copies of the TRH progenitor sequence Gln-His-Pro-Gly flanked by paired basic amino acids (Lys-Arg or Arg-Arg), 4 non-TRH peptides lying between the TRH progenitors, an N-terminal flanking peptide, and a C-terminal flanking peptide (82, 83). The N-terminal flanking peptide (prepro-$TRH_{25–50}$-R-R-prepro-$TRH_{53–74}$) is further cleaved at the C-terminal side of the arginine pair site to render prepro-$TRH_{25–50}$ and prepro-$TRH_{53–74}$, thus yielding a total of 7 pro-TRH-derived peptides (Fig. 1).

The biosynthesis of TRH and other pro-TRH-derived peptides follows the same prohormone-processing mechanisms described above, beginning with mRNA-directed ribosomal translation, followed by posttranslational limited proteolysis of the larger precursor, proTRH. This process occurs while pro-TRH is transported from the TGN to newly formed ISGs (84). These granules then mature and are targeted to sites of secretion at the plasma membrane of the cell. Rat, mouse, and human pro-TRH, similar to other peptide hormone precursors such as pro-enkephalin, contains multiple copies of one of its peptide products, in this case, the progenitor for TRH, Gln-His-Pro-Gly. Most of the products derived from pro-TRH are targeted into the RSP. Cleavage of the precursor to generate biologically active TRH occurs at paired basic residues by the action of PC1 and PC2 (31, 35, 37) followed by the action of CPE to remove the basic residue(s) (85). Gln-His-Pro-Gly is then amidated by the action of PAM, which uses the C-terminal Gly as the amide donor, and the Gln residue undergoes cyclization to a pGlu residue to yield TRH.

In the last few years, this laboratory has elucidated the processing steps involved in the synthesis of TRH and pro-TRH-derived peptides. An understanding of the biosynthesis and processing of pro-TRH is critical to appreciating how, when, and where modulation of this central regulator of the HPT axis takes place during physiologically appropriate modulation of thyroid function. It is also critical to understanding the function of TRH and other pro-TRH-derived peptides in extrahypothalamic regions of the brain, or outside of the nervous system, as discussed in *Section VI* of this review. Initial studies of TRH biosynthesis were difficult because of the low levels of TRH and other pro-TRH-derived peptides produced in hypothalamic tissue *in vivo*. In the search for a better system to perform these studies, initial work was done by stably transfecting prepro-TRH cDNA into transformed cell lines (86, 87) to attain higher levels of pro-TRH expression. Among the cell lines investigated, only AtT_{20} (corticotroph, mouse) and RIN 5F (insulinoma, rat) cells were able to efficiently cleave the TRH precursor at paired basic amino acid residues to generate mature TRH, as well as pro-TRH-derived peptides, which were identical to those previously identified *in vivo* (88, 89). GH_4C1 (somatommamotroph, rat) and 3T3 (fibroblast, rat) cells were unable to process the pro-TRH precursor. When these studies were performed, little information was available about PCs, their role in prohormone processing, or their expression in different tissues and cell lines. Retrospectively, it was determined that the cell lines capable of processing proTRH, AtT_{20} and RIN 5F cells, also express PC1 and PC2 (90).

A model of pro-TRH posttranslational processing was developed in experiments with transfected AtT_{20} cells expressing rat prepro-TRH (30). By means of Western blot analysis, immunoprecipitation followed by SDS-PAGE, and RIA, it was determined that pro-TRH is present in transfected AtT_{20} cells and in primary cultures of hypothalamic neurons, an endogenous source of pro-TRH, as a 26-kDa protein (37). Pulse-chase studies indicated that the 26-kDa precursor is cleaved at two mutually exclusive sites to generate the first intermediate forms (Fig. 1). One cleavage generates a 15-kDa N-terminal peptide (prepro-$TRH_{25–151\ or\ 157}$) and a 10-kDa C-terminal peptide (prepro-$TRH_{154\ or\ 160–255}$). An alternate cleavage generates a 9.5-kDa N-terminal peptide (prepro-$TRH_{25–106\ or\ 112}$) and a 16.5-kDa C-terminal peptide (prepro-$TRH_{109\ or\ 115–255}$). These cleavage steps occur in the TGN (Fig. 2), before packaging into ISGs (29, 84), in agreement with similar studies of the cellular location of early processing for POMC and somatostatin (SRIF) (53).

In subsequent steps, the 15-kDa N-terminal intermediate moiety of pro-TRH is processed to a 6-kDa peptide, corresponding to prepro-$TRH_{25–74}$, and a 3.8- kDa peptide, corresponding to prepro-$TRH_{77–106}$. It is proposed that processing of the remaining 10-kDa C-terminal fragment produces the 5.4-kDa C-terminal flanking peptide prepro-$TRH_{208–255}$, and the 5.6-kDa peptide prepro-$TRH_{160–199}$ (30, 91). Antisera against prepro-$TRH_{178–199}$ (pFE_{22}) recognizes the 10-, 5.6-, and 2.6-kDa (prepro-$TRH_{178–199}$) C-terminal peptides both in transfected AtT_{20} cells and in extracts from rat PVN, lateral hypothalamus, and ME. Prepro-$TRH_{178–199}$ is further cleaved to two smaller moieties of 1.6 and 0.84 kDa, prepro-$TRH_{178–184}$ and prepro-$TRH_{186–199}$ (pFQ7 and pSE_{14}), respec-

tively (Fig. 1). Processing of the 9.5-kDa N-terminal fragment arising from the alternative cleavage of the 26-kDa prohormone at residues 107–108 is postulated to result in the production of the N-terminal peptides, prepro-TRH_{25-50}, prepro-TRH_{53-75}, and prepro-TRH_{83-106}, while the 16.5-kDa fragment is processed to produce the 5.6-kDa prepro-$TRH_{160-199}$ and the 5.4-kDa prepro-$TRH_{208-255}$ (Fig. 1).

Recent experiments confirm the proposed model that the C-terminal 10-kDa peptide derived from an initial cleavage at residues Lys^{152}-Arg^{153} is the precursor to two peptides of 5.6- (prepro-$TRH_{160-199}$) and 5.4-kDa (prepro-$TRH_{208-255}$) (Fig. 1). After the cleavage of basic residues at positions 199–200 or 207–208, the 5.6-kDa peptide is further processed to generate the prepro-$TRH_{160-169}$ and prepro-$TRH_{178-184}$. prepro-$TRH_{178-184}$ is further cleaved to two novel peptides, prepro-$TRH_{178-184}$ and prepro-$TRH_{186-199}$ (91), Fig. 1).

B. Intracellular sites of pro-TRH processing

Initial studies in 1993 showed that processing of the 26-kDa pro-TRH precursor to smaller intermediates occurred before packaging into ISGs (29). The experimental strategy used to more precisely define the intracellular sites of pro-TRH processing was to block peptide transport from one cellular compartment to another and to characterize processing that occurred before the point of blockade (84). To study processing that occurs in the RER, peptides were blocked in their exit from this compartment with brefeldin A (BFA) treatment of AtT_{20} cells expressing the prepro-TRH cDNA (30, 87). BFA is a fungal metabolite that blocks ER-to-Golgi transport of proteins by reversibly inhibiting the exchange of GDP for GTP in the GTP-binding protein that is a key component in the vesicular transport, the ARF protein. This prevents protein recruitment to intracellular membranes and inhibits subsequent vesicle formation. To study the processing steps that occur in the GC, cells were incubated at reduced temperatures. Incubation of cells at 20 C prevents packaging of proteins into ISGs at the TGN and has been used previously to study the processing of other prohormones (53, 92). Figure 2 summarizes the results of these blockade experiments, described more fully below.

When transfected AtT_{20} cells expressing prepro-TRH were treated with BFA to block ER-to-GC transport, the 26-kDa pro-TRH precursor accumulated 4-fold over control levels, indicating a significant degree of post-ER processing. How-

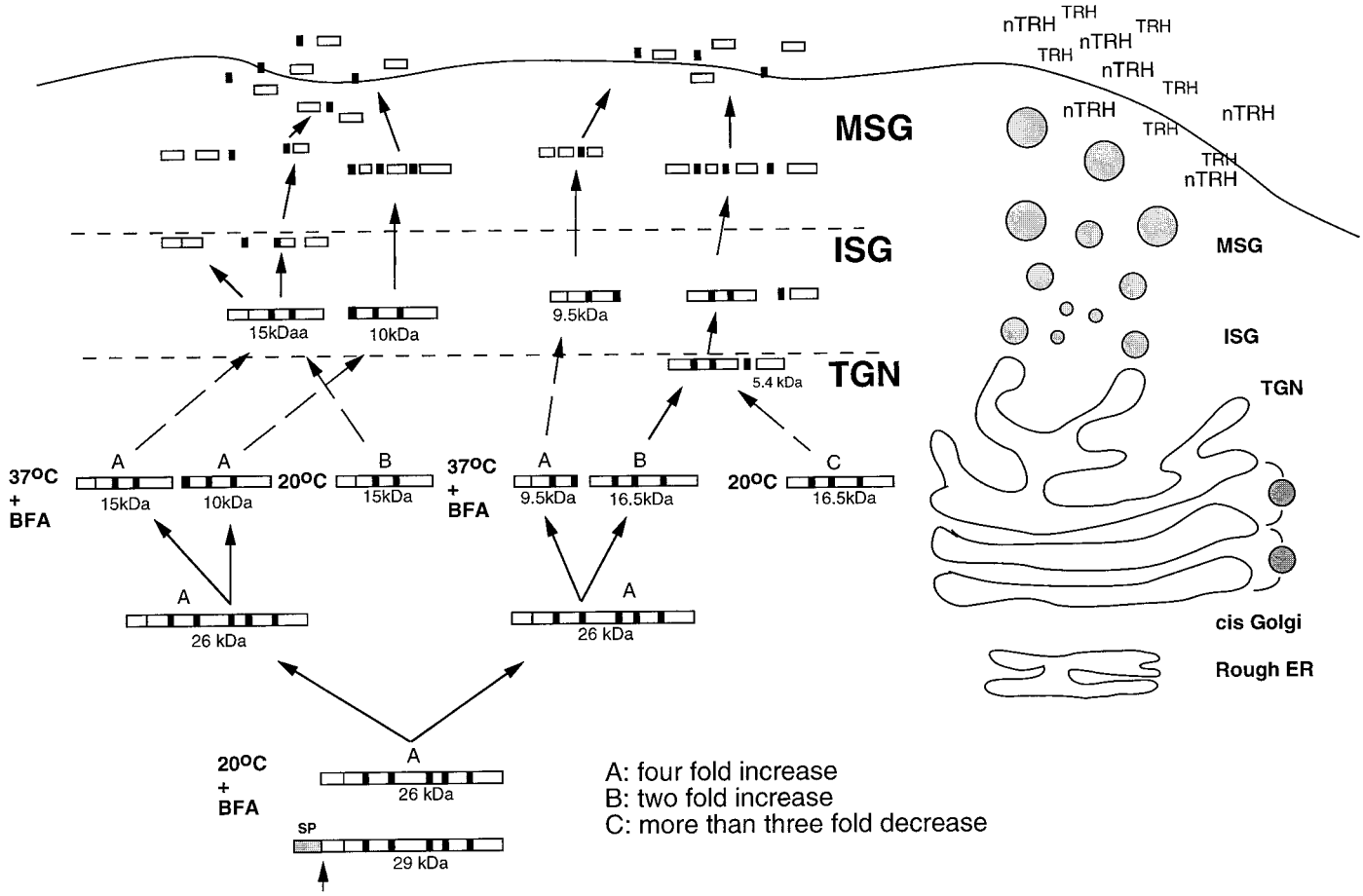


FIG. 2. Schematic representation of the proposed intracellular processing of pro-TRH. On the *left* is indicated the biochemical processing cascade that is initiated in the GC by PC1 (*Section II.B* and *D*). The effect of BFA and temperature blockade on the intracellular trafficking of peptides is described in *Section II.B*. The PC1 and PC2 enzymatic activities in this cascade are described in *Section II.D*. On the *right*, a *cartoon* showing the distribution of organelles within the RSP. TGN, *trans*-Golgi-network; MSG, mature secretory granules; ISG, immature secretory granules; nTRH, non-TRH peptides. [Reproduced with permission from I. P. Cruz and E. A. Nillni: *J Biol Chem* 271:22736–22745, 1996 (84).]

ever, some processing of 26-kDa pro-TRH to the 15-kDa and the 9.5-kDa N-terminal intermediates, and the 16.5-kDa and 10-kDa C-terminal intermediates, was seen. The accumulation of the 16.5-kDa intermediate was not as great as that of the 15-kDa intermediate when compared with control levels (4-fold for the 15 kDa and about 2-fold for the 16.5 kDa) (84). To further clarify where the initial site of pro-TRH cleavage occurred, a combination of temperature blockade and BFA treatment was performed. With this strategy it was demonstrated that the 26-kDa precursor protein (accumulated 4-fold) is processed in the GC (possibly in the TGN) to generate the 15-kDa/10-kDa and the 9.5-kDa/16.5-kDa intermediate pairs. When the fate of the 15- and 16-kDa intermediates was analyzed, while they were retained within the TGN at 20 C, the 16-kDa C-terminal intermediate was further processed at basic residues 206–207 to the 5.4-kDa C-terminal peptide. In contrast, the 15-kDa N-terminal intermediate appeared to undergo processing in a post-GC compartment, *i.e.,* the SGs. This observation strongly suggests that these two intermediates follow different paths of processing (84).

Evidence supporting differential distribution for the N- and C-terminal peptides comes from recent immunocytochemical (ICC) studies using transfected AtT_{20} cells, which indicate that pro-TRH, as well as the 15- and the 6-kDa N-terminal intermediates, are located in the GC and TGN (Fig. 3A). In contrast, end products, including prepro-$TRH_{25–50}$, prepro-$TRH_{160–169}$, and TRH, are only present in SGs along the plasma membrane and in cell processes (Fig. 3F). C-terminally directed antisera that recognize pro-TRH and the 16.5- and 5.4-kDa C-terminal peptides result in positive immunostaining in the GC, along the plasma membrane, and in cell processes (Fig. 3H). Thus, C-terminal intermediates appear to reach further along the RSP before processing than their N-terminal counterparts. This differential processing might serve as a mechanism to regulate the timing of production of peptides such as prepro-$TRH_{160–169}$, prepro-$TRH_{178–199}$, and prepro-$TRH_{53–75}$, and possibly TRH. For example, the 16.5-kDa intermediate, which is processed in the TGN, contains prepro-$TRH_{178–199}$ and preproTRH$_{160–169}$. A portion of such peptides, formed before their entry into SGs, might exit the cell via the constitutive pathway to maintain a basal level of release independent of TRH secretion.

Primary cultures of hypothalamic neurons, developed in this laboratory, provided a second model system in which to study pro-TRH processing. After 12–14 days *in vitro* (36), these hypothalamic neurons show growth of neurites similar in morphology to peptidergic neurons. Most neurons are bipolar with long axons containing varicosities, boutons, and growth cones. Many of the growth cones are in contact with neurites of other neurons. Dendrite-like structures are also observed. N-terminal antiserum in these cultured hypothalamic neurons (36) stains the GC, ISGs budding from the GC, and terminal boutons (Fig. 3, B and E). Immunoelectron microscopy (IEM) confirms positive staining in the GC (Fig. 3E). Some ISGs budding from the TGN-like were also stained. Since positive staining was detected in boutons, it was proposed that these intermediates are processed to mature pro-TRH-derived peptides near the axon terminal, before secretion. ICC using anti-TRH, anti-prepro-$TRH_{53–75}$, and anti-prepro-$TRH_{160–169}$ showed positive staining only in the neuronal processes and axon terminals while the soma remained unstained (Fig. 3G). The positive staining observed in neuronal processes and axon terminals with these antibodies was confirmed by IEM (Fig. 3G, *inset*).

Note that in transfected AtT_{20} cells using the same antiserum, immunostaining was seen only in GC and ISGs, but not in cellular processes (Fig. 3A). ICC on transfected AtT_{20} cells using anti-TGN_{38}, a TGN marker, indicated that the TGN can be projected away from the GC toward budding ISGs (Fig. 3C). Thus, in AtT_{20} cells processing of the 15-kDa N-terminal peptide takes place somewhere between the TGN and ISGs, suggesting that prepro-$TRH_{25–50}$, prepro-$TRH_{53–75}$, and prepro-$TRH_{83–106}$ are already formed by the time SGs mature.

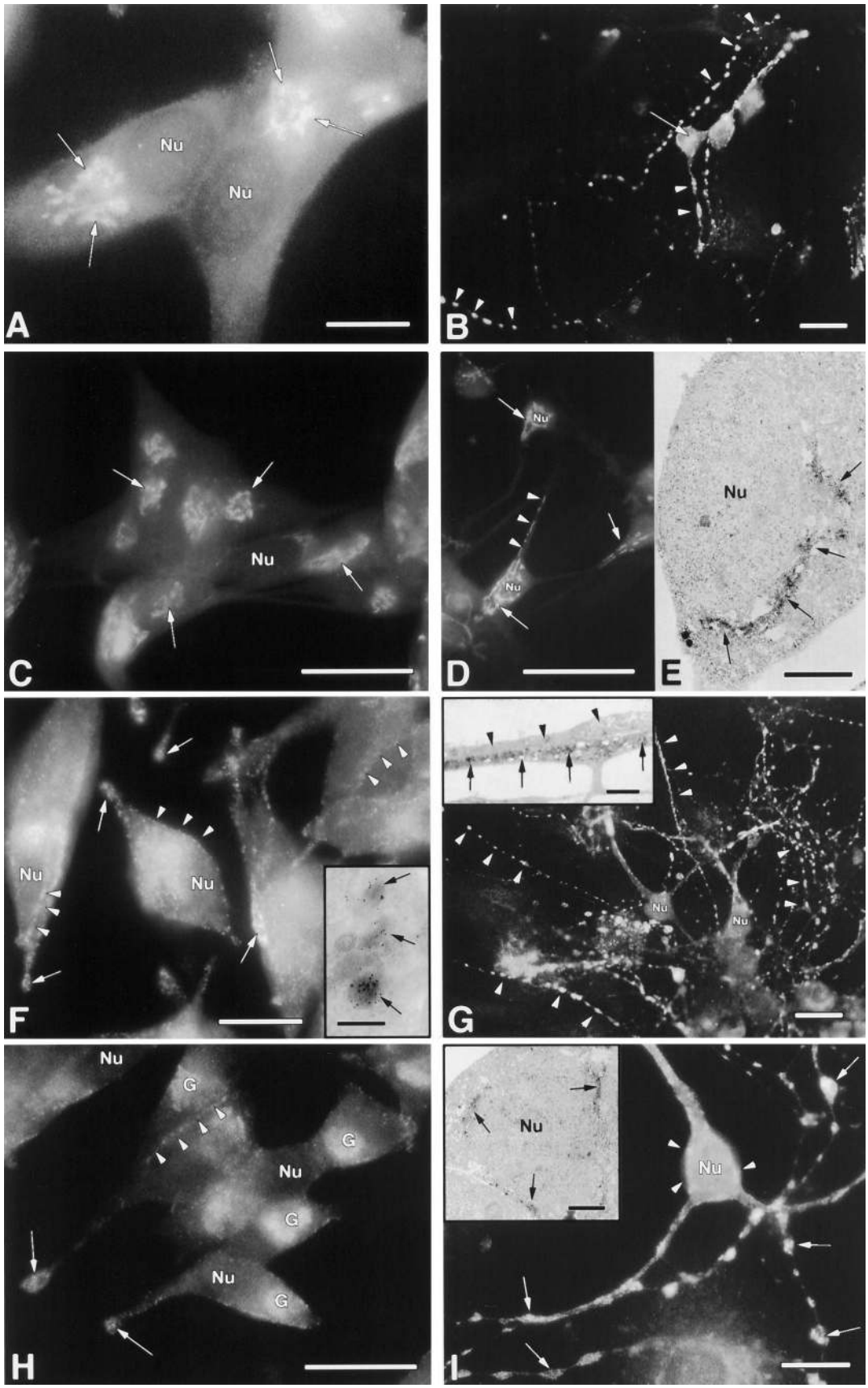
Using the C-terminal antiserum that recognizes pro-TRH and the 16.5-, 10-, and 5.4-kDa peptides, positive staining was visualized in a patchy cytoplasmic distribution (Fig. 3I), often closely associated with the nucleus. Immunoreactivity was also observed in neuronal boutons, axon terminals, and unbranched growth cones. IEM confirmed that the patchy cytoplasmic areas were within the ER and GC (36) (Fig. 3I, *inset*); all layers of the GC (*cis*, medial, and *trans*) were immunostained with this antibody (36). The contrasting staining patterns for the two antisera (N- and C-terminal) (Fig. 3, B and I) suggest the existence of a different peptide distribution for N-terminal *vs.* C-terminal peptides, possibly due to different intracellular routing of intermediates to SGs. However, no conclusive data are yet available to confirm this hypothesis.

C. Tissue-specific processing of pro-TRH

Increased knowledge of differential processing has led to a better understanding of how multiple biological peptides, with different functions, are generated from the same prohormone. This concept is further reinforced by the observation that certain regions in the brain can give rise to several different pro-TRH-derived peptides in addition to, or instead of, TRH. This will become clear in a review of the neuroanatomical distribution of pro-TRH and pro-TRH-derived peptides.

TRH-positive axons in the ME originate from neuronal perikarya in the PVN. The PVN is composed of two major components, the magnocellular and the parvocellular divisions. The parvocellular division contains most of the TRH neurons that project to the ME. A large population of immunoreactive neurons is located in medial and periventricular parvocellular subdivisions, organized in a triangular configuration, symmetric to the dorsal aspect of the third ventricle, whereas the anterior parvocellular subdivision neurons are more disperse (93). However, not all TRH-containing neurons in the PVN project to the ME (94). In addition to the PVN, TRH neurons are present in many other regions of the hypothalamus (93). As these populations of neurons have no known projections to the ME, and are not regulated in conjunction with the thyrotropic neurons of the PVN, it is presumed that they do not subserve a direct hypophysiotropic function.

The largest concentration of hypothalamic TRH neurons outside of the PVN are found in the dorsomedial nucleus,



lateral hypothalamus, and preoptic area, including medial, periventricular, suprachiasmatic, and the sexual dimorphic nucleus of the preoptic area (93). In addition to the PVN, TRH neurons are present in many other regions of the CNS including regions in the diencephalon, telencephalon, mesencephalon, myelencephalon, and spinal cord (Table 1). An extensive anatomic description of TRH neurons and TRH fibers in these tissues has been reported previously (93). While the presence of TRH in these regions is clear, relative levels are largely unknown because most mapping has depended on ICC detection in animals pretreated with colchicine. Detailed microdissection studies, combined with RIA, have been done for some areas, including the brainstem and hippocampus. These studies do not require colchicine pretreatment, are more quantitative than ICC detection, and reveal an even broader distribution for TRH than appreciated by ICC. However, their neuroanatomical resolution falls short of that of ICC, and these latter studies are the primary source of TRH data presented in Table 1.

In several areas of the brain where production of pro-TRH is found, TRH and pro-TRH-derived peptides are also detected (Table 1). In the case of pro-TRH, the reticular nucleus of the thalamus contains abundant prepro-TRH mRNA and several pro-TRH-derived peptides in their extended forms, but does not contain mature TRH (95). Moreover, the N-terminal extended forms of TRH, TRH-prepro-$TRH_{160–169}$ and TRH-prepro-$TRH_{178–199}$ are major end products of pro-TRH processing in the olfactory lobe (OB) (96, 97), but not in the hypothalamus where pro-TRH is completely processed to non-TRH peptides and TRH (96). In the ME, PVN, and preoptic area (POA), pro-TRH is fully processed to its mature forms, while in the OB less than 60% of N-terminal prepro-$TRH_{25–50}$ is formed. Similarly, while in the OB the 10-kDa C-terminal intermediate (see Fig. 1) is the main end product, in the ME and PVN this intermediate is fully processed to prepro-$TRH_{160–169}$ and prepro-$TRH_{178–199}$ (Fig. 1). Finally, in the POA partial processing of the 10-kDa peptide is observed, and the lateral hypothalamus contains lower levels of both N- and C-terminal pro-TRH-derived peptides as compared with the ME and POA (E. A. Nillni, unpublished data). Table 1 summarizes the current information regarding neuroanatomical distribution of intermediate forms of pro-TRH processing, mature pro-TRH-derived peptides, and TRH peptides in perikarya and fibers.

D. The role of PCs and CPs

The PCs are a family of seven subtilisin/kexin-like endoproteases including furin, PC1 (also known as PC3), PC2, PC4, PACE4, PC5-A (also known as PC6-A), its isoform PC5-B (also known as PC6-B), PC7 (also known as LPC), and PC8 (also known as SPC7) (32–34, 98–101)). The structure of these serine proteinases resembles both the bacterial subtilisins and yeast kexin (90, 102, 103). These enzymes cleave at the C-terminal side of single, paired, or tetra basic amino acid residue motifs (104), followed by removal of remaining basic residue(s) by CPs (50, 51). The selective expression of PC1 and PC2 in endocrine and neuroendocrine cells suggests they are significant in prohormone processing (32, 90, 98, 105). PC1 and PC2 have been shown to process pro-TRH (31, 35, 37, 106), proinsulin (104, 107, 108), proenkephalin (109), prosomatostatin (110, 111), and POMC (112, 113) to various intermediates and end products in coexpression experiments.

Like their substrates, PC1 and PC2 undergo maturation from larger precursor proteins. Maturation of pro-PC1 begins in the ER and continues in the TGN (90). In contrast, pro-PC2 maturation begins in the TGN and continues in the SGs; active PC2 and PC1 accumulate in SGs (114, 115). PC2 has been proposed to produce smaller peptides from intermediates resulting from cleavage by PC1 (109, 112, 113). Consistent with this, PC1 is implicated in the early cleavage of POMC (112, 113, 116) and proinsulin (117). Thus, the compartments where these enzymes are active also are determined by differential timing of their respective maturation.

The distribution of neurons containing mRNAs encoding prepro-TRH, PC1, and PC2 in the PVN and other areas of the

FIG. 3. Subcellular localization of pro-TRH, intermediate and end products of processing, and TRH in transfected AtT_{20} cells encoding prepro-TRH, and in primary cultures of hypothalamic neurons. The cells were fixed with 4% paraformaldehyde followed by immunostaining with different antibodies against the pro-TRH sequence. Fluorescein isothiocyanate conjugated to goat antirabbit globulin was used as a probe. Panel A, AtT_{20} cells: positive staining in the GC and TGN (*arrows*) using an antibody against pro-TRH and N-terminal intermediate forms (anti-pCC_{10}). Bar = 25 μm. Panel C, AtT_{20} cells: cells immunostained with anti-TGN38 (*arrows*), a TGN marker. Bar = 50 μm. Panel F, AtT_{20} cells: a typical positive staining along the plasma membrane (*arrow heads*), a common granule distribution of corticotropic cells, and processes (*arrows*) using anti-non-TRH peptides and anti-TRH. Bar = 25 μm. Panel F, AtT_{20} cells; *inset*: typical positive staining of SGs by IEM using anti-pST_{10} antibodies (5 nm gold particles). Bar = 200 nm. Panel H, AtT_{20} cells: positive staining in the GC and processes using an antibody that recognizes pro-TRH and C-terminal intermediate forms (anti-pYE_{17}). Bar = 50 μm. Panel B, Hypothalamic neurons: positive staining in the GC (*arrow*) and TGN using an antibody against pro-TRH and N-terminal intermediate forms, and in all boutons distributed along the neuronal processes (*arrowheads*) Bar = 50 μm. Panel D, hypothalamic neurons: cells immunostained with anti-TGN38, a TGN marker. Bar = 50 μm. Panel E, hypothalamic neurons, a higher magnification of panel B showing positive staining in stacked Golgi cisternae and in some forming granules (*arrows*) using the peroxidase-DAB reaction (*arrows*) Bar = 5 μm. Panel G, hypothalamic neurons: positive fluorescence is observed only in neurites (*arrowheads*) and axon terminals, while the cell body remain unstained. Bar = 50 μm. Panel G, hypothalamic neurons; *inset*: an IEM of neurites using peroxidase-DAB staining reaction. Of the two adjacent neurites shown, the lower one is positively stained (*large arrows*), whereas the upper one is negative (*small arrow*). Bar = 1 μm. Panel I, hypothalamic neurons: positive staining in several areas of the cell body (*arrowheads*) and in all boutons distributed along the neuronal processes (*arrowheads*) using an antibody against pro-TRH and C-terminal intermediate forms. Bar = 25 μm. Panel I, hypothalamic neurons, *inset*: a higher magnification of cytoplasmic areas from panel I showing positive staining in the endoplasmic reticulum and GC (*arrows*) as well as in SG near the plasma membrane (*arrows*). Bar = 2 μm. nu, Nucleus; G, Golgi complex. The polyclonal antibodies used in this ICC are as follow: Anti-pCC_{10} [made against a synthetic decapeptide (Cys-Lys-Arg-*Gln-His-Pro*-Gly-Lys-Arg-Cys)], which recognizes prepro-$TRH_{25–255}$ (26 kDa) prepro-$TRH_{25–151}$ (15 kDa) prepro-$TRH_{25–112}$ (9.5 kDa) prepro-$TRH_{25–74}$ (6 kDa). Anti-pYE_{17} (made against prepro-$TRH_{240–255}$), which recognizes prepro-$TRH_{25–255}$ (26 kDa) prepro-$TRH_{115–255}$ (16.5 kDa), prepro-$TRH_{160–255}$ (10 kDa), prepro-$TRH_{208–255}$ (5.4 kDa), anti-pST_{10} (made against prepro$TRH_{160–169}$), and anti-TRH. [Panels B, D, G, and I were reproduced with permission from E. A. Nillni *et al.*: *Endocrinology* 137:5651–5661, 1996 (36). © The Endocrine Society.]

TABLE 1. Current information available regarding pro-TRH biosynthesis, processing in different tissue location, its relation to PC1 and PC2, neuroendocrine inputs, and physiological significance

Tissue	pro-TRH-derived peptides	TRH		PCs			Coexpressing PCs and pro-TRH	Neuroendocrine inputs to TRH neurons	Physiological significance
		Perikarye	Fibers	PC1	PC2	PC5			
Telencephalon									
Olfactory bulb	Partial processing prepro-TRH154–169 prepro-TRH172–199								
Accessory			+						
External plexiform layer		+		+++	++	++	PC2		
Internal plexiform layer		+					PC2		
Mitral cells				+	++	++++			
Ependymal cells				0	0	0			
Granule cells				0/+	+	0			
Periglomular cells				+	++	++			
Glomerular layer		+	+				PC2		
Anterior olfactory nucleus		+		+++	++++	++++			
Tenia tecta				++++	+++	0	PC1		
Hippocampal formation									
CA1				+++	+++++	++			
CA2		+		++	++++	+			
CA3		+		++++	+++++	+++++			
Scattered hilar cells				++++	+++	++++			
Stratum oriens			+						
Dentate gyrus									
Molecular Layer		+		0	0	0	PC1,PC2		
Granular Layer		+		+++++	+++	+	PC1,PC2		
Nucleus accumbens			+	+	++	0			
Caudate-putamen		+	+	+++++	+++	0	PC1,PC2		
Amygdala									
Medial nucleus		+	+	+++	++	+++	PC1,PC2		
Central nucleus		+	+	0/+	0/+	+	PC1,PC2		
Anterior cortical nucleus				++	+	+++	PC1,PC2		
Basolateral nucleus				+++	++++	+++	PC1,PC2		
Septum			+	++	++++	++			
Bed nucleus, anterior commissure		+							
Bed nucleus, stria terminalis		+	+	+++	++	+	PC1,PC2		
Diagonal band of Broca		+	+				PC1,PC2		
Entorhinal cortex		+							
Medial preoptic area				+++	+	++	PC1,PC2		
Pyriform cortex			+						
Diencephalon									
Thalamus									
Anteroventral nucleus				+++	++	0			
Anteromedial nucleus				++	++++	0/+			
Anterodorsal nucleus				++++	++++	0/+			
Centrolateral nucleus				++	++++	0/+			
Centromedial nucleus				+++	++++	++++			
Mediodorsal nucleus				+++	++++	0			
Paraventricular nucleus			+	+++	++++	+++++			
Paratenial nucleus			+						
Reticular nucleus	Some intermediates, no TRH		+				0		
Reuniens nucleus				0	+++	0/+			
Rhomboid nucleus				+++	++++	+++			
Ventrolateral nucleus				0/+	++++	0			
Ventromedial nucleus			+	0/+	+++	0			
Dorsal lateral geniculate nucleus				0	++++	0			
Medial geniculate nucleus									
Dorsal				0/+	++++	0			
Ventral				0/+	+++	0			
Medial				0/+	++++	0			
Marginal Zone				+++	+	0			
Habenula									
Medial									
Lateral part				++	+	0			
Medial part				+	++++	0			
Lateral			+	++	+	0			
Hypothalamus									
Anterior hypothalamus		+	+						
Suprachiasmatic nucleus				+++	++	++	PC1,PC2		
Periventricular nucleus				++++	+	+++	PC1,PC2		
Supraoptic nucleus		+		+++++	+++	+++++	PC1,PC2		

TABLE 1. Continued

Tissue	pro-TRH-derived peptides	TRH		PCs			Coexpressing PCs and pro-TRH	Neuroendocrine inputs to TRH neurons	Physiological significance
		Perikarye	Fibers	PC1	PC2	PC5			
Paraventricular nucleus	pro-TRH is fully processed							NE+, E-?, DA+, 5HT+, NPY-, Som-, EOPs-, VIP+?, GABA-?, IL-1-	Theromoregulation, T_3/T_4 feedback, stress, TSH circadian rhythm, starvation, response to infection. pFE22 and pSE14 increased during suckling
Magnocellular part		+	+	+++++	++	+++++	PC1,PC2		
Parvocellular part		+	+	++	+++	+++	PC1,PC2		
Arcuate nucleus		+	+	+++	++	++	PC1,PC2		
Lateral hypothalamic area	Lesser processing as compared with PVN and ME	+	+	+++++	++++	++++	PC2		
Median eminence	TRH + non-TRH peptides		+					NE+, E?, DA-, Som-, EOPs-, IL-1?	Theromoregulation. T_3/T_4 feedback, stress, TSH circadian rhythm, starvation, response to infection
Ventromedial nucleus			+	+	++	+	PC2		
Dorsomedial nucleus		+	+	+++	+++	+++	PC1,PC2		
Medial mammillary nucleus				+	++++	+++			
Lateral mammillary nucleus				+	+++	+			
Perifornical region		+							
Premammillary nucleus			+						
Preoptic area	pro-TRH is fully processed	+	+						
Subfornical organ			+						
Mesencephalon and metencephalon									
Locus coeruleus			+	++	++++	+++			
Motor nucleus V			+						
Oculomotor nucleus			+	+++	++++	+++			
Parabrachial nucleus			+						
Periaqueductal gray	pro-TRH is fully processed	+	+	++	+++	++	PC1,PC2		Possible pain modulation
Raphe magnus nucleus				+++	+	0	PC1,PC2		
Red nucleus			+	++	++++	++++			
Substantia nigra, pars lateralis		+							
Substantia nigra, pars compacta				+++	+++	+++			
Substantia nigra, pars reticulata				+	+++	+++			
Trochlear nucleus			+						
Myelencephalon									
Cochlear nucleus		+		++++	+++	++			
Dorsal motor nucleus, vagus		+	+						
External cuneate nucleus		+							
Facial nucleus			+	+++	++++	++++			
Hypoglossal nucleus			+	+++	+	++++			
Inferior olive			+	++	++++	++++			
Lateral reticular nucleus		+							
Nucleus ambigus			+						
Nucleus tractus solitarius									
Medial			+	++++	++	++++			
Lateral				++	+++	++++			
Pontine nuclei		+		+++	++++	+++++	PC1,PC2		
Raphe nuclei									
Dorsal		+	+	+++	+++	+++++	PC1,PC2		
Median		+	+	+++	++	+++	PC1,PC2		
Reticular formation			+						
Spinal Cord	pST10 detected								
Anterior funiculus			+						
Central canal			+						
Dorsal horn (laminae II and III)		+	+						
Intermediolateral column			+						
Lateral funiculus			+						
Ventral horn (lamina IX)			+						
Cell lines									
Transfected AtT20 cells	pro-TRH is fully processed			PC1	low PC2		PC1,PC2		
Transfected RIN 5F cells	pro-TRH is fully processed			PC1	PC2				
Transfected GH4C1 cells	no pro-TRH processing								
Primary hypothalamic neurons	pro-TRH is fully processed			PC1	PC2		PC1,PC2		

The major source of contribution to this table comes from literature cited in this review and from our published and unpublished results.

brain has been determined using *in situ* hybridization (106) (Table 1). The glomerular layer of the OB displays coexpression of prepro-TRH with PC2 mRNA, but not PC1, whereas in the tenia tecta coexpression of mRNA for prepro-TRH with PC1, but not PC2, is evident. The PVN displays prepro-TRH mRNA coexpression with both enzymes, whereas the lateral hypothalamus shows coexpression of prepro-TRH mRNA with PC2 mRNA, but not PC1 mRNA. Double *in situ* hybridization indicates that in the PVN, PC2 mRNA is present in 60–70% of TRH neurons, and PC1 is present in 37–46% of TRH neurons (118). Even though these investigators found a trend for more coexpression of mRNA for prepro-TRH with PC2 than PC1, coexpression alone does not define which enzyme is more important in the processing of pro-TRH *in vivo*.

During the last few years, Nillni's laboratory has provided unequivocal evidence for the role of PC1 and PC2 in the processing of pro-TRH (31, 35–37). In coinfection experiments, where recombinant vaccinia viruses are used to coexpress PC1, PC2, PACE4, PC5-B, and furin, together with rat prepro-TRH in constitutively-secreting LoVo cells or in the regulated-secreting endocrine GH_4C1 cell line, RIA of LoVo-derived secreted products demonstrates that furin cleaves the precursor to generate both N- and C-terminal intermediates, while PC5-B does not produce any peptide. PC1, PC2, and PACE4 only produce N-terminal intermediates, and less efficiently than furin. Interestingly, in LoVo cells, furin cotransfection produces TRH-Gly at much greater levels than any of the other PC enzymes. Recent data indicate that furin, which is ubiquitously expressed in all tissues, may serve a role in processing of prosomatostatin within the constitutive pathway (110, 111). Since LoVo cells only contain the constitutive secretory pathway, these results suggest that pro-TRH can be processed to a certain extent without entry into the RSP. However, caution must be taken with this interpretation because under conditions of viral expression, the unusually high level of virus in coinfected cells can produce disruption of cellular compartments. The products resulting from coexpression of prepro-TRH with either furin or PC1 are similar, in agreement with their similar specificity observed in a number of cell coexpression experiments (109, 111) and *in vitro* data (119).

In GH_4C1 cells, PC1, PC2, furin, PC5-B, and PACE4 produce both N-terminal and C-terminal peptides derived from pro-TRH. Significantly, TRH-Gly and TRH are produced in highest amounts by PC1, PC2, and furin. Further analysis of the cleavage specificity of PC1 and PC2 reveals that PC1 is primarily responsible for cleavage of the entire TRH precursor to mature TRH, as it can generate all products at significantly higher levels than PC2 (Fig. 4). While 7B2 is known to be involved in the maturation of PC2 (Fig. 4) (90, 120) it does not augment the ability of PC2 to cleave pro-TRH to either N- or C- terminal forms. Subsequently, we have examined the role of PC1 and PC2 in the formation of prepro-$TRH_{178-199}$ by coexpressing rat prepro-TRH cDNA with PC1, PC2, and 7B2 in GH_4C_1 cells (91). PC1 effectively cleaved pro-TRH to immunoreactive forms recognized by anti-prepro-$TRH_{178-199}$, while PC2 played a minor role, even in the presence of 7B2 (Fig. 1).

Even though PC1 displays a greater ability to process pro-TRH than PC2, PC2 can process certain regions of the pro-TRH sequence (37). For example, in cells coexpressing pro-TRH and PC2, but not pro-TRH and PC1, greater quantities of 2.8-kDa peptide (pEH_{24}, $preproTRH_{83-106}$) relative to 3.8-kDa peptide (TRH-pEH_{24}, prepro-TRH_{77-106}) are found, while the converse is true for PC1 coexpressing cells. These data suggest that PC2 may be important in generating TRH from this intermediate. In support of a physiological role for both PC1 and PC2, pro-TRH is coexpressed and colocalized with PC1 and PC2 in primary cultures of hypothalamic neurons (Fig. 5) (37).

SDS-PAGE fractionation reveals that PC2 has a cleavage specificity that differs from that of PC1. In cells coinfected with PC1 and pro-TRH, two prominent moieties of 5.6 kDa and 2.6 kDa are formed. The latter is similar in size to prepro-$TRH_{178-199}$. In cells coinfected with PC2 and proTRH, the 5.6-kDa peptide is not present, but the 2.6-kDa peptide and a smaller form of about 1.6 kDa are observed. Microsequencing analysis of prepro-$TRH_{178-199}$ peptide incubated *in vitro* with purified PC2 demonstrates one specific cleavage at Arg_{185} (. . . Glu^{182}-Leu^{183}-Gln^{184}-Arg^{185}-↓-Ser^{186}-Trp^{187}-Glu^{188}-Glu^{188}-Lys^{189}. . .) generating two novel peptides, pFQ_7 and pSE_{14} (Fig. 1).

These two novel peptides, prepro-$TRH_{178-184}$ and prepro-$TRH_{186-199}$ are present in rat PVN, lateral hypothalamus, and ME (91). Thus, the antibody generated against the prepro-$TRH_{178-199}$ sequence recognizes the 10-kDa peptide, a 5.6-kDa form that probably is prepro-$TRH_{160-199}$ (30), a 2.6-kDa peptide that is prepro-$TRH_{178-199}$, and two smaller moieties of 1.6 and 0.84 kDa that are proposed to be prepro-$TRH_{186-199}$ and prepro-$TRH_{178-184}$, respectively (Fig. 1). Figure 6 shows a diagrammatic representation of rat pro-TRH and its cleavage by PC1 and PC2 as proposed from the most recent studies and compared with previous *in vitro* studies (31, 35, 91).

Table 1 combines current neuroanatomical distribution data for prepro-TRH mRNA, pro-TRH, and pro-TRH-derived products with the distribution of PC mRNAs and enzymes. Also included is a summary of pro-TRH processing in transfected cells and primary cultures of hypothalamic neurons and pituitary cells. Several important conclusions can be drawn. pro-TRH is widely distributed in the hypophysiotropic and extrahypophysiotropic areas of the brain. The widespread expression of pro-TRH, PC1, and PC2 mRNAs, with their overlapping distribution in many areas of the rat CNS, indicates the striking versatility provided by tissue-specific processing in generating quantitative and qualitative differences in non-TRH peptide products as well as TRH. Examples of these differences for several tissues are presented in the first column of the table and described above. A most striking example is the reticular nucleus of the thalamus, where PC1 and PC2 are not coexpressed with pro-TRH. TRH is not produced in this nucleus, indicating a central role for PC1 and PC2 in maturation to TRH. However, other pro-TRH intermediates are present in the reticular nucleus, suggesting PCs other than PC1 and PC2 might be involved in processing of pro-TRH for this particular region of the CNS (106).

Differential processing has been reported for other prohormones, and these differences relate to alterations in the expression of various PCs within different cell types. POMC

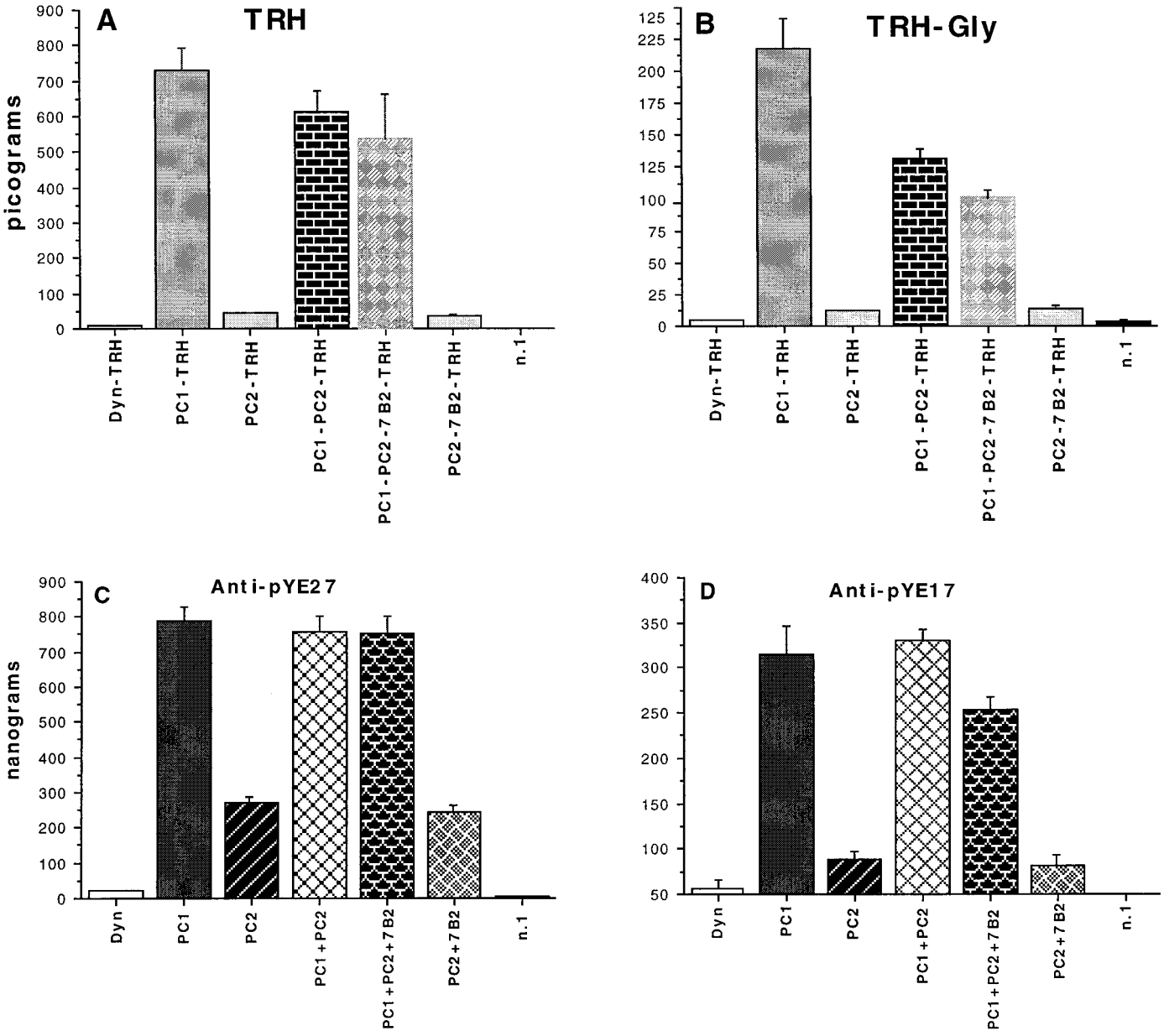


FIG. 4. Cleavage of pro-TRH as determined using anti-TRH (A) RIA, anti-TRH-Gly (B), using anti-pYE$_{27}$ (C) RIA, and anti-pYE$_{17}$ (D) RIA. Coinfections of pro-TRH were done with Dynorphin as a control, PC1, PC2, PC1-PC2, PC1-PC2–7B2, and PC2–7B2 in GH4C1 cells. n, Noninfected cells. RIAs were performed against resuspended serum free media. Cell means of recognized products in picograms are plotted against the indicated coinfected construct. Data are the mean values of six identical wells per condition, with $P < 0.05$ on Tukey-Kramer. [Reproduced with permission from P. Schaner *et al.*: *J Biol Chem* 272:19958–19968, 1997 (37).]

is processed primarily to ACTH, β-endorphin, and N-POMC$_{1–77}$ in the anterior pituitary (melanotrophs). In turn, these products are further processed to α-MSH, β-endorphin$_{1–31}$, N-POMC$_{1–49}$, and γ-MSH in the intermediate lobe and brain (38). Differential processing of a common polypeptide precursor is dependent upon the processing enzymes expressed in each specific cell type. Proenkephalin, which contains seven identical copies of met-enkephalin, is processed to large intermediate forms in the adrenal medulla, whereas this precursor is cleaved primarily to the pentapeptide met-enkephalin in the brain (39). The biological actions of substance P (SP) depend on the enzymatic processing of its precursor by the processing enzymes prolylendopepetidase to yield SP$_{5–11}$, and endopepetidase 3.4.24.11 to yield SP$_{1–7}$. While SP$_{1–7}$ acts as an analgesic, inhibits aggression, and enhances learning and memory, the SP$_{5–11}$ enhances pain transmission, stimulates aggression, and blocks learning and memory (40). In the brain, procholecystokinin (pro-CCK) is processed to produce only CCK8 amide, while in the gut the precursor is cleaved to larger molecules, such as CCK12, 22, 33, 38, 58, and 83 amide (121). Transfection experiments have shown that proneuropeptide Y (pro-NPY) can be cleaved by cell lines expressing either PC1 or PC2, but pro-NPY is primarily processed by PC2 in superior cervical

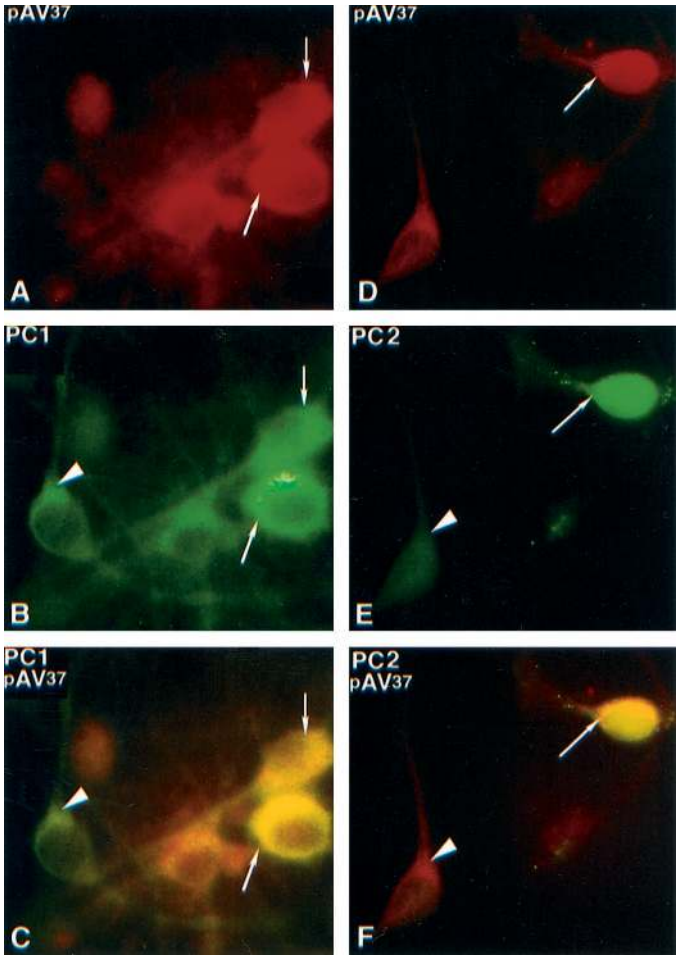


FIG. 5. Colocalization of pro-TRH with PC1 and PC2. Neuronal cells cultured for up to 14 days in four-chamber labTeck slides were fixed with 4% paraformaldehyde followed by immunoreaction with anti-PC1 or anti-PC2. Fluorescein isothiocyanate conjugated to goat antirabbit globulin was used as a probe. Texas Red-X-succimidylester directly conjugated to anti-pAV_{37} antibodies was used as a probe for pro-TRH. Panel A shows positive immunostaining for pro-TRH peptides (*red color, arrows*). Panel B shows positive immunostaining for PC1 (*green color, arrow* and *arrowhead*). Panel C shows the protein colocalization of pro-TRH and PC1 (*yellow-orange color* indicated by *arrows*). Some neuronal cells contain PC1 but not pro-TRH (panels B and C, *arrowhead*). Panel D shows positive immunostaining for pro-TRH peptides (*red color*, *arrow*). Panel E shows positive immunostaining for PC2 (*green color*, *arrow* and *arrowhead*). Panel F shows the protein colocalization of pro-TRH and PC2 (*yellow-orange color* indicated by *arrow* and *arrowhead*). Some neuronal cells contain PC1 but not pro-TRH (panels B and C, *arrowhead*). Thirty-millimeter slides were digitized with a video camera and appropiate macro lens using BioVisionframe grabbera software (Perceptics Corp., Knoxville, TN). Images of the *red* and *green* planes were combined using Adobe Systems, Mountain View, CA) to show areas of colocalization. The resulting images were printed with a Mitsubishi CP210 dye sublimation printer (Apunix Computer Services, San Diego, CA). [Reproduced with permission from P. Schaner *et al.*: *J Biol Chem* 272: 19958–19968, 1997 (37).]

ganglia (122). Thus, differential processing of neuropeptitdes including pro-TRH, pro-NPY, POMC, pro-CCK, SP, and proenkephalin provides a critical mechanism through which cells regulate the levels of specific peptides to fulfill different physiological requirements, a mechanism potentially more versatile than the alternative splicing of mRNA.

As mentioned in *Section IIA*, CPs remove remaining C-terminal basic residues from prohormone intermediates that are initially cleaved by PCs. Experiments with the fat/fat mouse model of CPE deficiency (123) support a role for CPE in the processing of pro-TRH (85). Mice homozygous for the *fat/fat* mutation are obese, diabetic, and infertile. These mice have a missense (Ser to Pro) mutation at CPE residue 202 that abolishes enzymatic activity (123). Hypothalamic TRH levels are depressed 65% in *fat/fat* mice relative to heterozygous controls. SDS-PAGE demonstrates hypothalami from both *wt/fat* and *fat/fat* mice contain moieties different from those of the *wt/wt* mice. Specifically, 6.5-, 3.0-, 2.6-, and 1.6-kDa forms of the pro-TRH sequence are detected, and their levels differ significantly between the two groups. Compared with *wt/fat* mice, *fat/fat* mice hypothalami contain 20-, 3-, and 2-fold elevations in the 6.5-, 3.0-, and 2.6-kDa species. These data indicate that the *fat/fat* mutation produces qualitative changes in pro-TRH processing, and that CPE is involved in the later stages of pro-TRH processing. However, cell transfection experiments would help to rule out secondary phenotypic changes caused by the CPE mutation in these mice. Further, since hypothalami from *fat/fat* mice contain immunoreactive TRH, additional CPs must also be able to process pro-TRH to TRH, assuming that the TRH detected is not a cross-reactive non-TRH species. CPs such as carboxypeptidase D, with similar enzymatic properties to CPE, are also present in compartments of the secretory pathway and are distributed in many tissues, including the brain (50).

Interestingly, in *fat/fat* mice, levels of TSH, T_3, and T_4 were normal, suggesting that 34% of normal TRH levels is sufficient to maintain the thyroid function. This last observation is important because it is hypothesized that the five identical progenitor sequences of TRH contained in the prohormone may not be processed to mature TRH at all times, and that only a few of them may be needed to maintain the thyroid function. In cultured hypothalamic cells at steady state, previous studies had shown that the ratio of mature TRH to prepro-$TRH_{25–50}$ and the 5.4-kDa C-terminal peptide (Fig. 1) is 3:1 instead of the theoretical 5:1 for complete processing (36). A similar ratio is seen in transfected AtT_{20} cells (30). In the rat brain, the ratio of TRH to other pro-TRH-derived peptides sequences is almost 1:1 in the hypothalamus, and 1.5:1 in the olfactory lobe (89). The above ratios are indicative of incomplete yields of TRH from pro-TRH, although only if degradation rates do not contribute significantly to these various ratios. Further, the relative immunoreactivites of various antisera used to their iodinated tracers has not been defined, leaving exact molar ratios difficult to calculate. Still the range of TRH to pro-TRH-derived peptides found in various tissues makes it likely that full TRH yields are not achieved in all, or even most, tissues.

If not all TRH progenitor sequences are cleaved from pro-TRH, the resulting TRH progenitor sequences, linked to amino- or carboxyl-terminal extensions, would not be detected in usual TRH RIAs. The TRH assay is specific for mature TRH, needing both the amino-terminal pyro-Glu and carboxy-terminal Pro-amide for detection. Nonimmunoreactive TRH progenitors might also retain untrimmed basic amino acid residues, carboxyl-terminal glycine residues, etc. Another explanation for the lower than 5:1 ratio of TRH to other

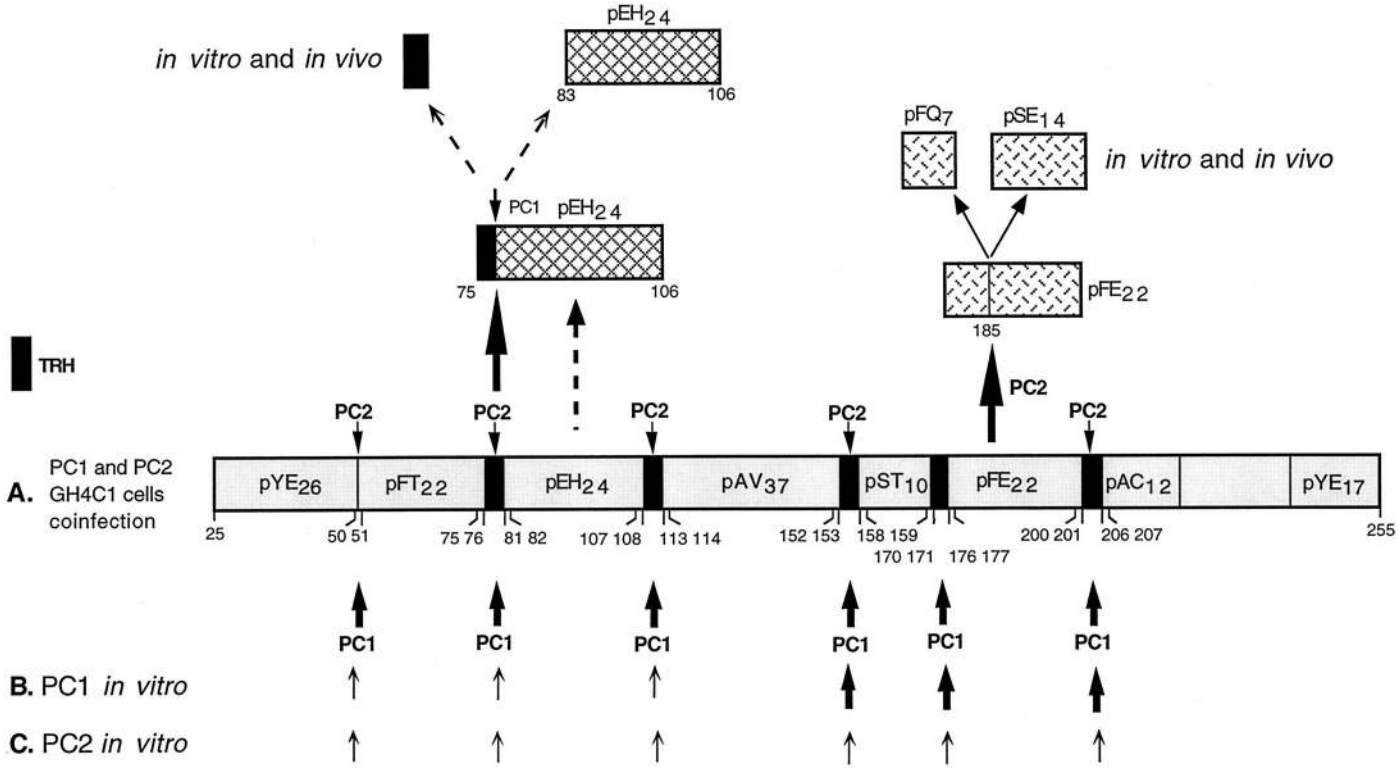


FIG. 6. Diagramatic representation of rat pro-TRH and its cleavage by PC1 and PC2 as showed in our previous studies done *in vitro* and coexpression conditions (see *Section II.D*). The *arrows* indicate the site of cleavages and whether they are major (*thick arrow*) or minor (*thin arrow*) sites for each enzyme. [A portion of this figure was reproduced with permission from P. Schaner *et al.*: *J Biol Chem* 272:19958–19968, 1997 (37).]

products could be that TRH is extracted less efficiently from cells than these other peptides, although this seems unlikely because TRH is the smallest of the peptides, and doping experiments indicate TRH is extracted with high efficiency under our conditions. As described above, it is proposed that the processing of peptides derived from the N-terminal portion of pro-TRH is substantially different from the processing of those of the C-terminal end. This suggests the possibility of differential maturation of TRH molecules depending on location within the pro-TRH sequence (84). If this is the case, either the three TRH molecules derived from the C-terminal side, or the two from the N-terminal side, may play a more primary role in hormonogenesis. It is also possible that certain TRH molecules become biologically active at different times than others, or only some of them reach maturity while the rest are degraded by the TRH-degrading enzymes, depending upon physiological needs (see *Section VII*). Finally, cells may simply produce excess TRH that may or may not be used.

In summary, we have presented an overview of the current knowledge of pro-TRH biosynthesis, its processing, its tissue distribution, and the role of known processing enzymes in pro-TRH maturation. Evidence is presented suggesting differential processing for pro-TRH at the intracellular level is physiologically relevant. The data indicate that PC1 is primarily responsible for most pro-TRH cleavage events. PC2 is involved in specific processing events that occur later in the secretory pathway, specifically in the formation of the second TRH molecule from the N-terminal side of prepro-$TRH_{83–106}$, and the proteolytic cleavage of prepro-$TRH_{178–199}$ to generate the novel prepro-$TRH_{178–184}$ and prepro-$TRH_{186–199}$ peptides.

E. Neuropeptide and catecholamine regulation of pro-TRH biosynthesis and processing

Immunoreactive TRH (iTRH) axon terminals are present in high density in the external layer of the rat ME, in close apposition to capillaries of the hypophysial-portal system (124). These axons originate from neuronal perikarya located in the PVN, the "thyrotrophic area" of the rat hypothalamus. Destruction of this region results in disappearance of up to 94% of TRH in the external layer of the ME and reduction of TSH secretion from the anterior pituitary gland (125). As described in *Section IIC* (Table 1), in addition to the PVN, iTRH neurons are present in other regions of the hypothalamus, including the POA, anterior hypothalamus, and supraoptic, arcuate, dorsomedial, and premmamilary nuclei, as well as basolateral and prefornical hypothalmus (93).

Although many neurons in the PVN contain more than one peptide, TRH neurons are unique in being almost always unassociated with other known peptides (126). This makes the regulation of pro-TRH-derived peptide biosynthesis very specific. As described in more detail below, TRH neurons in the PVN are located in a region where they can be regulated by a number of neuroendocrine inputs. TRH neurons are densely innervated by norepinephrine (NE)-containing axons that stimulate TRH secretion (124). TRH neurons are also

densely innervated by neuropeptide Y (NPY) neurons. In smaller numbers, SRIF and endogenous opioid peptide (EOP) terminals are also in contact with TRH neurons (124). *In vivo*, these various neuroendocrine inputs may affect the levels of prepro-TRH mRNA and the posttranslational processing of pro-TRH by influencing the biosynthesis and maturation of PC1 and PC2. At the present time, the effect of this input on pro-TRH processing is unknown. Evidence for coordinated regulation of mRNAs for processing enzymes and their substrates has been documented in several cases (127). In contrast, outside the hypothalamus TRH is colocalized with other substances. For example, in the descending bulbospinal pathway, TRH is colocalized with SP and serotonin (5-HT) (128). Regulation of these TRH neuronal systems is much less well characterized than the thyrotropic neurons of the hypohyseal-portal system.

TRH-synthesizing neurons in the rat PVN receive a large number of afferent neuroendocrine inputs. Axon collaterals of parvocellular neurons ramify within the medial parvocellular PVN and establish numerous synaptic contacts with perikarya and dendrites of other parvocellular as well as magnocellular neurons, *e.g.*, SRIF afferents are derived from the PVN itself (129). The majority of inputs to TRH neurons are derived from the diencephalon, telencephalon, and brainstem (130). The paraventricular and medial parvocellular divisions of the PVN are densely innervated by NE-containing and epinephrine (E)-containing inputs from the medulla and pons (131). Further, NE-containing neurons densely innervate the midregion of the external layer of the ME. These inputs activate tuberoinfundibular neurons. Intracerebroventricular (icv) injections of NE, E, and α2-adrenergic agonists stimulate basal TSH secretion (132, 133), and NE/E treatment of hypothalamic preparations stimulates TRH release (134). Inhibitors of catecholamine (CA) biosynthesis or α2-adrenergic antagonists lead to a fall in basal TSH secretion. Thus, NE and E exert a tonic, stimulatory regulation on TSH secretion principally through α2-adrenergic receptors. Stimulated release from the ME appears to be postsynaptically mediated via α1-adrenergic receptors (135). In contrast, locus coeruleus (LC) afferents are inhibitory, being activated during stress (136). NE/E excitation of PVN TRH neurons mediates the rise in TSH in response to acute cold exposure or hypovolemia (135, 137, 138). However, it has also been proposed that α1-adrenergic receptors mediate a phasic inhibitory regulation of TSH release. The data on NE/E modulation of TRH biosynthesis may be reconciled by an examination of how these inputs affect the posttranslational processing of pro-TRH, as well as examining their effects on PC biosynthesis. Peripheral levels of T_3, T_4, or TSH may also influence NE/E effects on TRH biosynthesis and/or release.

The PVN also receives prominent dopamine (DA) inputs from the posterior and dorsal areas of the hypothalamus, the zona incerta of the subthalamic region, and the A14 region of the anterior hypothalamus (124). Mesencephalic A9 and A10 dopaminergic neurons also project to the PVN. In turn, large terminal fields to the ME originate in the arcuate nucleus and periarcuate nucleus regions of the hypothalamus. In contrast to the NE/E system, DA inputs appear to inhibit TRH secretion, mainly at the level of the ME (139). Augmentation of DA neurotransmission inhibits basal and/or cold-stimulated TSH release, while DA antagonism has the opposite effect, although some studies have failed to replicate these findings (124). In addition, TRH release may be indirectly inhibited by DA-stimulated secretion of SRIF (140). Conversely, DA stimulates TRH release from isolated hypothalamic fragments (134, 140), again reinforcing the need to examine TRH biosynthesis in both *in vitro* and *in vivo* systems. Within the HPT axis, thyroid hormones appear to modulate DA levels in the ME, and TSH increases the ability of DA to inhibit TRH (141) (Table 1).

A wide array of neuropeptides, including NPY, TRH itself, SRIF, EOPs, neurotensin (NT), and vasoactive intestinal polypeptide (VIP), have inputs to the PVN and/or external layer of the ME (142–146). Other mediators, including γ-aminobutyric acid (GABA) and various cytokines, also appear to regulate TRH or TSH secretion, but there is as yet no anatomical evidence to support a direct action on TRH neurons in the PVN and/or ME (124). Anatomically, NPY appears most prominent in its inputs to the periventricular and medial parvocellular divisions of the PVN (147). NPY cell bodies principally reside in the medulla, often coexisting with NE and E (148), but other sources come from throughout the brain, including the arcuate nucleus of the hypothalamus itself. Indeed, the arcuate nucleus is the major source for NPY fibers innervating the TRH neurons in the PVN (149). Few NPY-containing axons project to the ME. The effects of NPY on tuberinfundibular TRH are not yet well understood. NPY neurons also innervate SRIF neurons in the PVN, which would allow indirect regulation of TRH biosynthesis or secretion (124). Central administration of NPY reduces NE utilization in the PVN, as well as TSH release, indicating an inhibitory influence. *In vivo*, NPY also increases hypothalamic DA content, as well as DA turnover in the ME, the net result of which would reduce TRH release as well (150). Physiologically, NPY is critical to integrating thyroid function, food intake, and thermoregulation (151) (Table 1).

Inputs containing EOPs represent a second rich innervation to the PVN. These originate in the arcuate nucleus, periarcuate area, and amygdala (144). The dorsal raphe projects 5-HT/enkephalin (ENK) axons, and the posterior hypothalamus-mammallary bodies send GABA/histamine/ENK projections, to the PVN. The ME contains numerous ENK, dynorphin, and endorphin (END) synapses originating from the PVN and arcuate nucleus. Both END and ENK inhibit TRH release from the hypothalamus, and ENK and morphine inhibit TRH secretion from the ME (152). There is additional evidence that ENK indirectly inhibits tuberoinfundibular TRH via DA release (153).

Recent data indicate that pro-TRH processing is regulated by opiate withdrawal (27). Opiate withdrawal increases prepro-TRH mRNA, and the N-terminal prepro-$TRH_{53–74}$ and prepro-$TRH_{83–106}$ peptides, in the rat PAG, whereas the level of TRH is unaltered (27, 154). New data also show suckling increases the production of prepro-$TRH_{178–199}$ and prepro-$TRH_{186–199}$ (see *Section IVC* and *E*). (91). These results demonstrate that levels of various products derived from pro-TRH can be posttranslationally regulated in an independent fashion under altered physiological conditions. Thus, it is

logical that neuroendocrine inputs into the PVN can affect pro-TRH processing as well.

Finally, we note that while the genomic organization of the rat prepro-TRH gene is well described (155, 156), the molecular mechanisms regulating the expression of this gene are incompletely understood. The 5′-region of the prepro-TRH gene contains TATA and GC box sequences, also present in the promoter region of other neuropeptide genes (155). In addition, sequences similar to a cAMP response element (CRE), and negative thyroid response elements (TREs), are present. The region between −47 and +6 of the rat prepro-TRH gene is active in CA77 TRH-secreting medullary thyroid carcinoma cells (155, 157), but not in transgenic mice (158). Inclusion of most of exon 1 (bp −47 to +84) increases promoter activity in CA77 cells and activates the promoter in transgenic mice, principally in prepro-TRH gene-producing tissues. Thus, *cis* element(s) located within exon 1 are necessary for the expression of the rat prepro-TRH gene *in vivo* (158). In CA77 cells, the human prepro-TRH gene is regulated by thyroid hormone through two distinct classes of negative TREs (157), similar to other neuropeptide genes such as prepro-SRIF (159).

F. Glucocorticoids modulate the biosynthesis and processing of pro-TRH

Glucocorticoids evoke a broad spectrum of responses in many eukaryotic cells by stimulating or repressing the transcription of glucocorticoid-regulated genes, including those of peptide hormones (160). The primary effect of glucocorticoids on gene transcription can occur by specific binding of the steroid receptor complex to DNA at the site of glucocorticoid response elements. Glucocorticoids can also interfere with the action of other transcription factors through protein-protein interactions and may elicit secondary effects at the posttranscriptional, translational, and posttranslational levels (161–163). For example, glucocorticoids stimulate processing of the precursors for atrial natriuretic factor and neurotensin (NT) (162, 163). Glucocorticoids also regulate the posttranslational maturation, the intracellular trafficking, and the extracellular release of the mouse mammary tumor virus (164).

Glucocorticoids enhance TRH gene expression in several *in vitro* cell systems, including hypothalamic neurons, anterior pituitary cells, and thyroid C cells, an effect that occurs, at least in part, through transcriptional activation (165). Dexamethasone substantially elevates biosynthesis of the 26-kDa TRH prohormone and its intermediate products in cultured anterior pituitary cells, consistent with an overall up-regulation of both the biosynthesis and processing of the TRH precursor (161). This explains why glucocorticoids act not only at the transcriptional level, but also at the translational/post-translational level. This question can be addressed in experiments with AtT_{20} cells transfected with prepro-TRH cDNA driven by a CMV-IE promoter not responsive to physiological signals. Dexamethasone causes a 75% increase in newly synthesized 26-kDa pro-TRH without altering prepro-TRH mRNA levels, suggesting that glucocorticoids raise translation rates and/or slow processing of pro-TRH. In fact, dexamethasone treatment accelerates TRH precursor processing.

Interestingly, processing of the N- *vs.* the C-terminal intermediates in the AtT_{20} cells is influenced differentially by glucocorticoids. Levels of the N-terminally derived peptide prepro-$TRH_{25–50}$ are enhanced while levels of the 5.4-kDa C-terminally derived peptide are reduced. TRH content is increased (Fig. 7) (161). How could dexamethasone differentially affect the processing of N- *vs.* the C-terminal intermediates? Glucocorticoids may alter pro-TRH processing through changes in the expression of processing enzymes, as well as morphological alterations in AtT_{20} cells. For example, GC volume is obviously enlarged in AtT_{20} cells treated with dexamethasone. Although speculative, these changes may

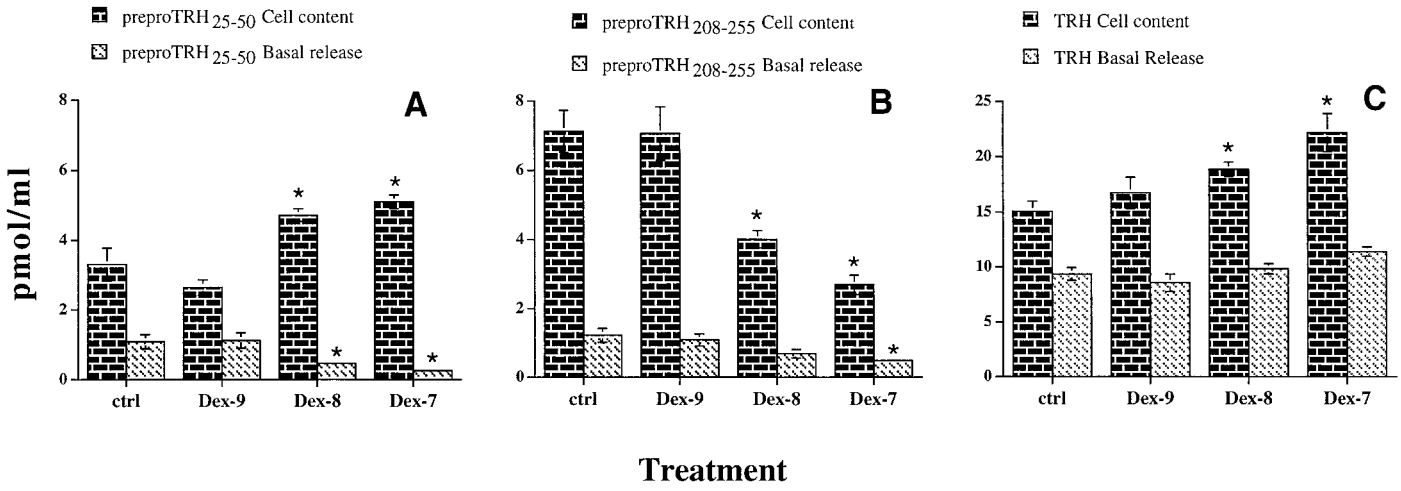


FIG. 7. Effect of dexamethasone on the accumulation and release of pro-TRH-derived peptides in AtT_{20} cells transfected with a prepro-TRH cDNA. AtT_{20} cells were cultured in six-well plates; cellular extracts and release media (2 h basal release) were processed further for the determination by RIA of the intracellular accumulation and basal release of the N-terminal peptide prepro-$TRH_{25–50}$ (A), the C-terminal peptide prepro-$TRH_{208–255}$ (B), and TRH itself (C) whose five copies are derived from both N- and C-terminal intermediates. A representative experiment (n = 6 wells for each group) is depicted. Data are presented as mean values ± SEM. * $P < 0.05$ compared with control. [Reproduced with permission from T. O. Bruhn *et al.*: *Endocrine* 9:143–152, 1998 (161).]

slow down the normal transport of intermediate products from the TGN to ISGs, thereby altering the accumulation or degradation of intermediate forms through changes in processing enzyme exposure (84, 161).

In vivo, more diverse effects of glucocorticoids on TRH gene expression have been reported. prepro-TRH mRNA-expressing neurons outside the PVN do not appear to be affected by changes in adrenal status, in contrast to the suppression caused by glucocorticoids in the PVN (166). The action of glucocorticoids on PVN prepro-TRH mRNA-expressing neurons may involve the hippocampus and amygdala, which convey negative feedback by glucocorticoids on CRF-expressing neurons in the PVN (167). Thus, direct positive regulation by glucocorticoids may be overridden by an indirect negative regulation causing a net reduction of prepro-TRH mRNA in the PVN while positive and negative regulation may sum to no change prepro-TRH mRNA-expressing neurons outside of the PVN (166).

In conclusion, glucocorticoids induce changes in the biosynthesis and processing of pro-TRH by affecting both transcription and translation rates, and by differentially influencing the processing of N- *vs.* C-terminal intermediates of pro-TRH. At the translational and posttranslational level, these effects result in an increase in TRH production, with more complicated differential effects on the accumulation of other N- and C-terminal pro-TRH-derived peptides. It is clear that control over the diverse range of pro-TRH-derived peptides within a specific cell is accomplished mostly from the regulation at the posttranslational level rather than the translational or transcriptional levels. Three examples supporting this hypothesis are presented in this review: 1) pro-TRH processing in the PAG is regulated during the opiate withdrawal, so that levels of TRH remain unchanged, but other pro-TRH-derived peptides are induced (*Section II.B.6*); 2) pro-TRH processing is regulated during suckling, where a selective, yet dramatic, increase in prepro-$TRH_{178–199}$ and prepro-$TRH_{186–199}$ peptides is observed (*Section IV.D* and *E*); and 3) in the absence of transcriptional effects, glucocorticoids induce differential processing of pro-TRH in both primary cultures of pituitary cells and transfected AtT_{20} cells encoding prepro-TRH cDNA (this section).

G. Leptin regulates pro-TRH biosynthesis

Food deprivation in animals and humans results in endocrine and metabolic changes including decreases in circulating thyroid hormones, TSH, insulin, GH, gonadal hormones, and gonadotropins. Previous work in starved rats has shown a decrease in hypothalamic, but not thalamic, reticular, prepro-TRH mRNA, as well as decreased circulating TRH. This supports the concept that hypothyroidism produced after starvation is of hypothalamic origin (168). Leptin is a recently discovered peptide hormone that is synthesized and released by adipose tissue. Leptin also is decreased in starvation. Absence of leptin is responsible for the obese phenotype of *ob/ob* mice, and administration of this hormone to these animals decreases plasma corticosterone, suggesting that leptin is capable of inhibiting the hypothalamic-pituitary adrenal axis. In normal rats and mice, leptin inhibits hypothalamic CRH release (169).

Leptin may have an important role in the neuroendocrine regulation of the HPT axis (170). During prolonged fasting in rats, low levels of T_3 and T_4 are observed, and TSH is in the low to normal range. As described above, this is due in part to suppression of prepro-TRH gene expression in PVN neurons. Since the decrease in thyroid hormone levels is blunted in mice and rats by systemic leptin, it has been proposed that the decrease in leptin detected during fasting alters the set point for feedback inhibition by thyroid hormones on the biosynthesis of prepro-TRH mRNA (170). The mechanism of such leptin regulation of prepro-TRH biosynthesis is unknown. It is hypothesized that leptin has direct actions on cell bodies in the arcuate nucleus, positively regulating POMC, and thus α-MSH, and negatively regulating NPY and the Agouti-related peptide (151). NPY afferents on TRH neurons are inhibitory (see *Section II.E*). In preliminary studies done in this laboratory, both leptin and α-MSH elevate prepro-TRH mRNA, pro-TRH, and TRH secretion in primary hypothalamic cultures (our unpublished results). Using the same primary cultures of hypothalamic neurons, leptin dose-dependently increases pro-TRH synthesis and TRH secretion. Immunocytochemical analysis reveals that approximately 40–50% of the hypothalamic cells are positive for the leptin receptor. Of these, approximately 10–15% colocalize with TRH (Fig. 8). These data suggest that the regulation of pro-TRH biosynthesis and TRH release in response to starvation includes direct regulatory actions of leptin and α-MSH on hypothalamic TRH neurons involved in HPT axis homeostasis (171).

In summary, leptin effects on pro-TRH biosynthesis include: 1) an inhibitory action of leptin on NPY release from the arcuate nucleus, which in turn may reduce the inhibitory action of this peptide on TRH release from the PVN; 2) a stimulatory action of leptin on α-MSH release from the arcuate nucleus, which may stimulate TRH release from the PVN; and 3) a direct action of leptin on TRH neurons located in the PVN.

III. Function of TRH

A. The HPT axis

The hypophysiotropic role of TRH in the control of thyroid function has been extensively reviewed elsewhere, and the reader is referred to the excellent review by Scanlon and Toft (172). Therefore, this section will only briefly describe the role of TRH in the HPT axis, including relevant new reports, to aid in the understanding of other sections of this review.

TSH is synthesized and secreted by the thyrotrophic cells in the anterior pituitary and is the major regulator of the thyroid gland. TSH secretion is primarily regulated by negative feedback from circulating thyroid hormone and by stimulatory input from the hypothalamus through TRH action on thyrotrophs. There are other factors known to regulate TSH secretion, including glucocorticoids in the systemic circulation and SRIF and DA from the hypothalamus. There is evidence supporting the view that glucocorticoids in man suppress endogenous hypothalamic TRH secretion (173). Further support for this hypothesis was demonstrated in adrenalectomized rats in which pro-TRH mRNA levels

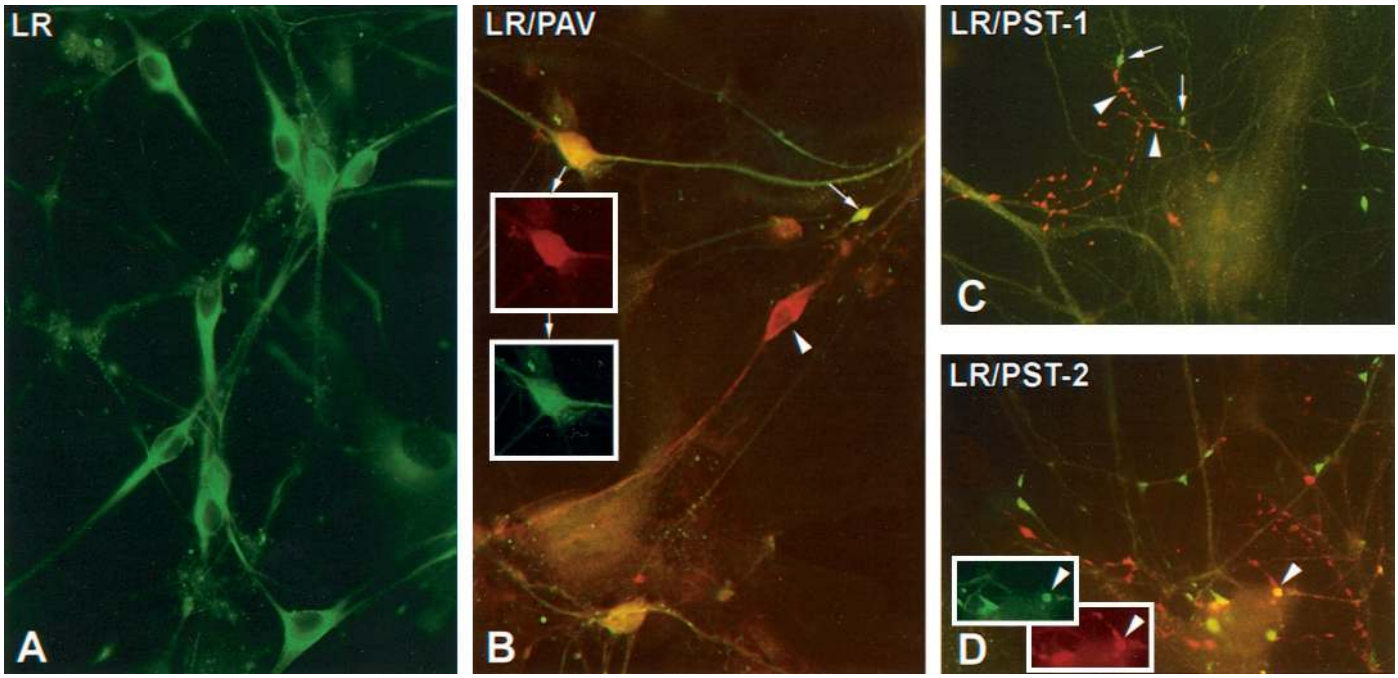


FIG. 8. Colocalization of pro-TRH with the leptin receptor (LR). Neuronal cells cultured for up to 14 days in four chamber labTeck slides were fixed with 4% paraformaldehyde followed by immunoreaction with anti-LR and anti-pro-TRH. Fluorescein isothiocyanate conjugated to goat antirabbit globulin was used as a probe. Texas Red-X-succimidylester directly conjugated to anti-pAV_{37} or anti-pST_{10} antibodies were used as a probe for pro-TRH. Panel A shows positive immunostaining for LR in neurons untreated with Triton X-100. Staining is mainly localized on the surface of cell body and dendrites. Panel B shows cells with positive immunostaining for pro-TRH using anti-pAV37 (*red color*, *arrowhead*) and LR (*green color*, *arrow*). Colocalization of pro-TRH with LR (*yellow-orange color* indicated). The *insets* show the same cell with each individual staining. A distinct population of neuronal cells contains pro-TRH but not LR and vice versa. Among the total cells with positive staining for pro-TRH, approximately 30% or less of those cells colocalize with LR. Panel C shows positive immunostaining for pro-TRH using anti-pST10 (*arrowheads*, *red*) and for LR (*arrows*, *green*), which are present in different subcellular locations. Panel D shows positive immunostaining for pro-TRH peptides (*red color*, *arrowheads*) that colocalize with LR (*green color*). Thirty millimeter slides were digitized with a video camera and appropiate macro lens using BioVisionframe grabbera software (Perceptics Corp., Knoxville, TN). Images of the *red* and *green* planes were combined using Adobe Systems to show areas of colocalization. The resulting images were printed with a Mitsubishi CP210 dye sublimation printer (Apunix Computer Services, San Diego, CA).

increase 1.6-fold, an increase reversible with dexamethasone (166). The role of glucocorticoids *in vivo* and *in vitro* has been described in the previous sections. Both SRIF and DA inhibit TSH release by direct effects on thyrotrophs. As discussed in *Section II.C.*, SRIF perikarya in the POA, periventricular parts of the anterior hypothalamus, and a few in the PVN, and TRH neurons in the PVN, project to the ME. TRH and SRIF are the two main TSH-regulating hypophysiotropic neurohormones released into the hypophysial portal vasculature. The maintenance of euthyroidism is dependent on a highly regulated balance of neuropeptides and neurotransmitters, where the dominant positive hypothalamic control for TSH is TRH, and the principal feedback control is through thyroid hormones. However, thyroid hormones also provide direct negative feedback on prepro-TRH gene expression as well. The relationship of thyroid hormone regulation to pro-TRH processing is undefined.

Even though TRH is the major regulator of the synthesis and secretion of TSH, and thus plays a pivotal role in the HPT axis, in a recent study, homozygous TRH gene knockout mice were shown to be viable, fertile, and exhibit normal development (174). Whereas the TRH−/− mice showed normal serum PRL and GH levels, thyroid hormone levels were significantly reduced as compared with the wild-type heterozygous mice. The targeted disruption of the prepro-TRH gene caused a characteristic tertiary hypothyroidism, and a substantial decrease in insulin secretion resulting in a profound hyperglycemia. These authors suggested that in addition to abnormalities of the thyroid function, TRH may be involved in the pathogenesis of diabetes mellitus (174).

B. Extrahypophysiotropic TRH

More than two-thirds of iTRH in the brain is found outside of the traditional "thyrotrophic zone" of the hypothalamus (175, 176). This extrahypophysiotropic TRH is believed to function as a neuromodulator of known neurotransmitters (177, 178). Indeed, it might act as a neurotransmitter itself; it is present in secretory granules whose exocytosis is responsive to membrane depolarization, it acts through specific receptors that are widely distributed throughout the CNS, and it is rapidly cleared through specific catabolic pathways (179).

While the following discussion focuses on TRH, many other neuropeptides and neurotransmitters play critical roles in the biological functions discussed below. In several areas of the brain, TRH is colocalized with other neurotransmitters and/or neuromodulators, including 5-HT and SP in the bulbospinal pathway, DA in the olfactory bulb, and histamine, ENK, and NPY in various loci of the hypothalamus (128).

Where TRH is directly affected by, or directly affects, other neuroactive agents, they have been discussed, but otherwise their roles are left for review elsewhere. Further, in the following we describe many effects of TRH. In fact, these have been demonstrated using TRH and/or TRH analogs. Due to space constraints, we will not distinguish effects by TRH *vs.* its analogs. Available TRH analogs have higher affinities for the TRH receptor, longer half-lives, etc. and are reviewed elsewhere (180, 181).

Finally, for several TRH effects, the metabolite histidylproline diketopiperazine, or cyclo (His-Pro) (CHP) also has agonist or antagonist effects. CHP is present in the CNS and peripheral tissues in levels that often exceed levels of TRH (182). While CHP is a known breakdown product of TRH, there are data that CHP is also derived from precursors other than TRH (183). Further, high-affinity binding sites for CHP have not been identified (184) (but see Ref. 185). Thus, the precise biological meaning of CHP effects is unknown. Figure 9 summarizes proposed physiological roles for extrahypophysiotropic TRH.

1. TRH and ergotrophic effects. In diverse ways TRH is excitatory when infused into animals (179). Activation is reflected in organism-wide effects on arousal, sleep, cognition, locomotion, and mood (186). Metcalf and Dettnar (187) first used Hess's term "ergotrophic" to describe the endogenous activating effects of TRH in the brain that are described below.

a. TRH in arousal and sleep: Systemic (188, 189) and central (190) TRH increase wake time and/or decrease sleep time in multiple species. More dramatic is the ability of TRH to arouse animals from drug narcosis induced by alcohol (191, 192), β-endorphin (193), tetrahydrocannabinol (THC) (194), benzodiazepines (195), and barbituates (196, 197). icv Anti-TRH antiserum doubles anesthetic-induced sleep time, supporting an endogenous role for TRH in arousal (198). Of unclear significance, CHP is more effective than TRH in decreasing sleep time and reversing ethanol-induced narcosis, but does not affect TRH modulation of barbiturate-induced sleep time in mice (199, 200).

TRH analepsis is most strongly induced by infusions into the medial septal area, the diagonal band of Broca, or the nucleus basalis of Meynert (186, 201). TRH levels rise in the medial septum as rats recover from ethanol-induced sedation (202). Further, TRH enhances cholinergic activity in the septo-hippocampal and nucleus basalis-cortical systems (203–205), pathways that play a central role in reversal of drug-induced narcosis (206). Atropine blocks both analepsis and cholinergic enhancement when TRH is infused into these areas. However, antagonism of the analeptic response to *systemic* TRH requires both ACh and NE blockade, supporting mediation by other neuroanatomical sites (192, 207). The posterior lateral hypothalamus, the midbrain reticular formation, and TRH-containing fibers passing through, or synapsing in, the dorsal septum are additional neuroanatomical substrates that might mediate arousal through noradrenergic mechanisms (203).

There are limited data that TRH may be useful to enhance arousal in pathological states. TRH prevents the postconcussive elevation of cortical ACh and reduction of cortical NE seen in mice (208) and decreases time of unconsciousness in head-injured mice (209). Canine models of narcolepsy show improvement after treatment with TRH (189).

b. TRH in cognition: The ergotrophic effects of TRH on consciousness and arousal are often detected along with improvements in measures of learning and memory, consistent with the important role of ACh in both processes (210). TRH improves performance in learning-impaired mice, an effect that is blocked by scopolamine (211). TRH restores learning and/or memory deficits in rats made cognitively deficient by anticholinergic treatment, electrochemical shock treatment (ECS), or surgical lesions (212–214). These effects are largely mediated by enhanced cortical ACh

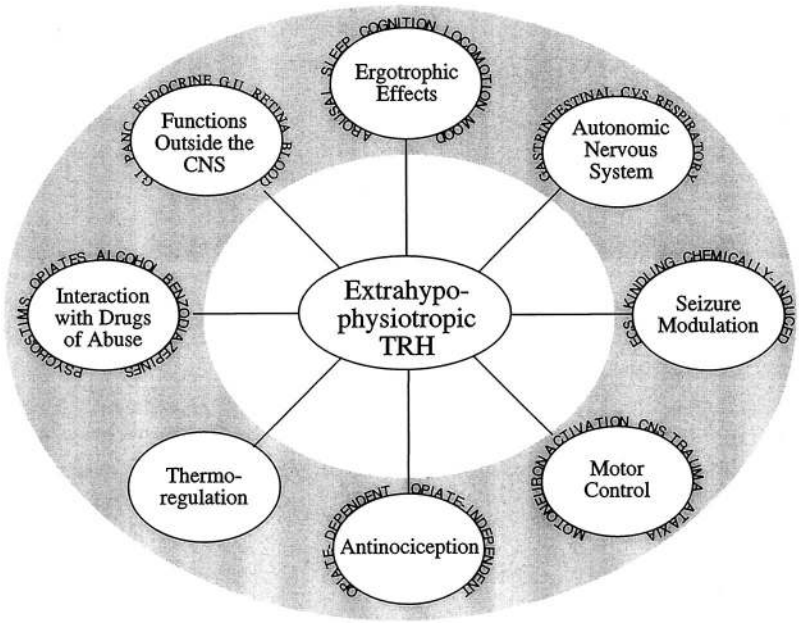


FIG. 9. Physiological roles of extrahypophysiotropic TRH. Extrahypophysiotropic TRH is implicated in a wide range of physiological processes, as well as processes affected by drugs of abuse. These functions are broken down into areas portrayed as ellipses within the *darker* annulus surrounding extrahypophysiotropic TRH in the *center*. Major subjects discussed in the text for each area are shown *outside the ellipses*.

release with TRH infusion into the nucleus basalis of Meynert and increased hippocampal ACh with TRH infusion into the medial septum-diagonal band (215). Facilitated release of NE (216) and *N*-methyl-D-aspartate (NMDA) receptor activation (217) also are implicated in cognitive enhancement by TRH. Human trials have demonstrated only modest cognitive improvements in patients with alcoholic dementia (218), in the ECS postictal state (219), and in Alzheimer's disease (220, 221).

c. TRH in locomotor activation: Systemic TRH elicits a number of motoric and behavioral activating effects in a wide range of species (178, 222). Further, TRH antagonizes locomotor depression induced by alcohol (223) and β-endorphin (193). TRH elicits locomotor activation when injected into the NAc (224, 225), the ventral tegmental area (VTA), the caudate (222), the septal nuclei (225), and the ventromedial hypothalamus (226).

Locomotor activation by TRH principally is mediated by DA. Repeated TRH treatment in rats elevates DA in the cerebral cortex and increases tyrosine hydroxylase activity, the rate-limiting enzyme in DA biosynthesis in the brain stem. These effects correlate with dose- and time-dependent increases in locomotor activity (227). DA antagonists and/or DA depletion block locomotor activation by systemic TRH (228). Consistent with TRH activity on the mesocorticolimbic system, systemic TRH increases DA release in the NAc (224, 229), and bath application of TRH induces DA release from NAc (230) and septal (231) brain slices.

Intraaccumbens TRH increases DA metabolism in the NAc, while icv TRH or intra-VTA TRH do not, although these latter treatments elicit locomotor excitation (222). Further, TRH can stimulate locomotion at doses that have no effect on NAc DA (232). These data support redundant but distinct TRH mechanisms, in addition to those in the mesocortical DA system, that induce locomotion (233). Other studies indicate that opiate antagonists and α-adrenergic antagonists can attenuate TRH-induced locomotion (234). A single study reports that CHP is able to stimulate locomotion (200).

d. TRH and antidepressant effects: In a number of behavioral assays used to screen compounds for antidepressant efficacy, TRH tests positively (235). This potentiation is independent of effects on TSH or thyroid hormones; however, PRL similarly can potentiate desipramine effects in the forced swim test (236). For the most part, DA receptor antagonists block the antidepressant effects of TRH (237), although it is difficult to tease out locomotor effects from antidepressant effects in some behavioral tests. TRH action in some antidepressant screens is blocked by opioid receptor antagonists or α2-adrenergic blockade (237). Further, antidepressant treatments alter TRH levels in rat brain, but not in a clear "antidepressant pattern" (238). In human trials, promising early results (239, 240) have given way to larger studies that indicate TRH is of limited benefit in depression (241, 242). Recent studies using intrathecal TRH reveals significant reductions in symptomatology of patients with refractory depression, although tachyphylaxis to the effects develops rapidly (243, 244). Recently, a compelling model has been put forward that clinical depression results from pathologically overdriven glutamatergic circuits in the limbic forebrain that have escaped inhibitory regulation by TRH (245).

2. TRH and autonomic nervous system function. The brainstem distribution of TRH supports a prominent role in autonomic nervous system (ANS) function. Fully 65% of medullary TRH is associated with dorsal vagal complex (DVC) neurons of the nucleus tractus solitaris (NTS), nucleus intercalatus and commisuralis, the dorsal motor nucleus (DMN) of the vagus, and, to a lesser extent, the nucleus ambiguus (246). Injection of TRH onto DMN neurons is uniformly excitatory, while applications onto NTS neurons are inhibitory (247). The majority of DVC iTRH derives from fibers arising from the medullary raphe nuclei that pass through the DVC (93, 248). However, cells within the DMN express prepro-TRH mRNA and pro-TRH (95, 249), consistent with some endogenous TRH production.

A descending bulbar-spinal pathway, in particular, from the nucleus interfascicularis hypoglossi and the nucleus paragigantocellularis lateralis, projects to the intermediolateral (IML) column of the spinal cord (250). Fibers and terminals of this tract are closely apposed to preganglionic sympathetic neurons. TRH fibers and preganglionic sympathetic neurons are also found around the central canal and in the intermediate gray matter of lamina VII (93, 251). While some studies indicate that more than 90% of TRH immunoreactive neurons also stain positively for 5-HT, and 75% express immunoreactive SP (252), only 43% of IML TRH is ablated by 5-HT neurotoxins (250). Indeed, more than 90% depletion of 5-HT in the spinal cord reduces spinal cord TRH by only 66% (253). Thus, a sizable pool of TRH-containing neurons are not serotonergic.

a. TRH and gastrointestinal function. TRH inhibits food and water intake: TRH inhibits food and water intake, consistent with its high levels in the ventromedial hypothalamus (176, 254), a center important to regulation of food intake (255), and its interaction with NPY and NE, both important to intake behavior (93). Systemic TRH reduces food intake less effectively than icv TRH, arguing for a central effect (256). Parenteral TRH can suppress eating without altering blood glucose levels (257) and without affecting TSH (256). icv TRH also reduces water intake (258), although others have reported icv TRH reduces food intake far more than water intake (259). icv TRH suppression of stress-induced eating is antagonized by D-ala-met-enkephalin (260), although DA transmission in the nigrostriatal pathway and lateral hypothalamus also affects stress-induced eating (261).

The hypothalamus serves as a principal brain substrate to coordinate hunger and satiety; it is generally held that the ventromedial hypothalamus serves to signal satiety, and the lateral hypothalamus, hunger (255). Injection of TRH into the ventromedial hypothalamus is most potent in producing adipsia and anorexia, and lateral hypothalamus injection is selectively potent for adipsia (262). Iontophoretic application of TRH onto ventromedial hypothalamic neurons results in facilitation of glucoreceptors, and hence, decreased feeding drive (263). However, others argue that the lateral, not ventromedial, hypothalamus is most critical for TRH-induced anorexia (226). TRH administration into the NAc is mildly anorexic (262). Systemic and icv CHP also suppress sponta-

neous food and water intake and stress-induced feeding, although it is less potent than TRH in feeding (264). In water-deprived rats, CHP is equipotent with TRH in reducing drinking (265). Thus, it cannot be ruled out that the TRH effects discussed above are actually the result of CHP as a TRH metabolite.

TRH enhances gastric acid secretion: Vagal preganglionic neurons arising from the rat DVC and nucleus ambiguus terminate in the gastrointestinal tract (266, 267), in close proximity to nerve efferents of the greater curvature and pylorus of the stomach (268). These comprise the major medullary projections to the stomach. In the cat, retrograde tracing does not support a descending tract from the nucleus ambiguus, but the remainder of the DVC participates in the bulbogastric projection (268). The high concentration of TRH receptors in the DVC (269, 270) in close proximity to NTS vagal afferents (266) is consistent with modulation by TRH afferents from the NTS and the medullary raphe nuclei. As well, peripheral signals of gastric distention and gastric secretion are carried by vagal afferents back to the medulla to activate DVC neurons. The codistribution of afferent and efferent pathways in the vagus provide a means for bulbogastric TRH neurons to modulate gastrointestinal responses to physiological signals, such as gastric distension, the cephalic phase of gastric acid secretion, etc. (271, 272).

Central TRH is far more potent than iv TRH in inducing gastric acid secretion (18, 273). icv Anti-TRH inhibits gastric acid secretion in pylorus-ligated (274) and cold-restrained (18) rats, supporting an endogenous role for the peptide. TRH stimulates gastric acid secretion independent of hypophysiotropic effects or effects on gastrin (275, 276). Further, TRH injection into the DVC is 10 times more potent than icv TRH in stimulating gastric acid secretion (18, 277, 278). Bilateral DVC injection of anti-TRH antiserum significantly reduces gastric acid secretion in response to icv TRH (279), or chemical or electrical activation of medulla raphe pallidus (RPa) neurons (280), supporting the central role of the DVC in TRH effects on gastric acid secretion.

Atropine injection into the DMN does not completely block TRH-stimulated gastric acid secretion (279), because other loci, including the nucleus ambiguus, lateral hypothalamus, and the ventromedial hypothalamus, can mediate TRH-induced gastric acid secretion (18, 281). TRH action also is partly mediated through α2-adrenergic receptors and enhanced sympathetic outflow that modulates the vagus (275, 277, 282). By unknown mechanisms a number of peptides in the DVN, including CRF, bombesin, calcitonin gene-related peptide (CGRP), calcitonin (CT), endogenous opiates, and, curiously, gastrin releasing peptide, inhibit TRH-induced gastric acid secretion (18). CHP and TRH-OH have no such activity (277, 283).

Kainic acid stimulation of afferent nucleus raphe obscurus (ROb) neurons mimics the induction of gastric acid secretion by TRH injection into the DVC (278). In addition, the caudal raphe nuclei-DVC pathway mediates cold-induced vagal stimulation of gastric acid secretion and erosion formation (284). Further, anti-TRH antisera injected into the DMN abolishes the ability of excitatory amino acid injection into the RPa to enhance indomethacin-induced gastric erosion formation (285). Thus, excitation of the raphe nuclei enhances DVC outflow, and one of the mediators of this effect is TRH. 5-HT (286) and SP (287) afferents from the raphe nuclei to the DVC modulate TRH effects.

icv TRH administration indirectly affects gastric acid secretion by increasing pepsin secretion and gastric mucosal blood flow and secretion (18). This effect is partly inhibited by DVC injection of anti-TRH antiserum, and surprisingly, is independent of increased gastric acid secretion (279). Thus, TRH may provide a means to regulate pepsinogen secretion without altering acid production. Intracerebral (ic) TRH-stimulated gastric mucosal blood flow is vagally mediated, via stimulation of an L-arginine-nitric oxide (NO) pathway independent of histamine H1 receptors or capsaicin-sensitive afferents (288). In addition, ic TRH enhances gastric secretion of 5-HT and 5-HT entry into the portal vasculature, an effect that again is vagally mediated (289).

TRH effects on gastrointestinal contractility and transit: ic, But not iv, TRH increases gastric contractions and gastric emptying in most species (290–292). Enhanced gastric motility is reproduced by direct infusion into the DMN but not the nucleus ambiguus (268), an effect completely blocked by vagotomy (293). Systemic morphine (294), or DVC injections of CRF (295), bombesin (296), and interleukin-1β (IL-1β) (297) inhibit the TRH effect. Since gastric contractility is inhibited by excitatory amino acid injection into the DVC (298), it is likely that TRH is inhibitory to DVC neurons controlling gastric contractility. Indeed, TRH injections onto NTS neurons reduce their spontaneous activity (299).

The ROb, RPa, and nucleus raphe magnus (RMg) provide afferents to the DVC (300, 301). In the cat, the DMN receives its strongest inputs from the caudal RPa and ROb, where TRH neurons are enriched (301). Glutamate or electrical excitation of the caudal RPa and ROb, but not rostral RPa or RMg, results in enhanced gastric contraction. This effect is abolished by vagotomy and anti-TRH antibody injection into the DVC (302). Surprisingly, TRH stimulates ROb and RPa TRH afferents to the DVC. This effect is completely abolished by vagotomy or atropine into the ROb, markedly attenuated by atropine into the RPa (303), and antagonized by SP or VIP into the ROb (304). ic Antisense oligonucleotides to the TRH receptor block the increase in gastric motility seen with TRH injection into either the ROb or the downstream DVC, while glutamate excitation is unaffected. Thus, TRH activates both TRH and cholinergic afferents to the DVC, which in turn increase gastric motility (305). Finally, gastric contractility also is increased by TRH injection into the hypothalamic paraventricular nucleus or the central nucleus of the amygdala, an effect abolished by subdiaphragmatic vagotomy. However, unlike medullary effects, the frequency of gastric contractions after these injections is attenuated (306, 307).

Motility in the proximal small intestine and ascending colon and cecum is also mediated by a central effect on vagal outflow (291, 308). However, central depletion of brain catecholamines blocks the contractile response in the duodenum, indicating a critical role for catecholamines as well as ACh in TRH central regulation of bowel motility, at least in some regions of the gut (309). Acceleration of small intestinal transit appears to occur through a separate pathway from

that described for the stomach (310). TRH (≥100 ng) increases small intestine transit only when injected into the medial septum, or lateral and anterior hypothalamus, in anesthetized rats. icv, But not iv TRH, also reverses net water absorption in the jejunum and ileum, an effect entirely abolished by vagotomy (311). Large colon transit in rabbits is increased by iv or icv TRH (312) and is associated with accumulations of fluid in the colon (289). TRH effects on colonic transit are mediated by vagal and sacral cord parasympathetic outflow, as well as serotonergic transmission (18). In humans, iv TRH retards glucose and xylose absorption by the gut (313).

TRH effects on pancreas and liver. In normoglycemic rats, acute systemic TRH will induce hypoglycemia, with little effect on peripheral pancreatic hormones (314). Central TRH potently blocks epinephrine-induced hyperglycemia, presumably via combined parasympathetic/sympathetic induction of insulin secretion (315). Central TRH antagonizes hyperglycemia induced by treatments other than epinephrine, including central injections of CRF, ENK, and glucagon, as well as systemic 2-deoxyglucose, foot shock, immobilization, or endotoxin (315).

Pancreatic effects of TRH are most likely paracrine. TRH is synthesized in the insulin-producing β-cells (316). In neonatal pancreas, TRH and insulin appear to be secreted via the same potassium-, cAMP-, and protein kinase C-responsive pathways (317); in adult pancreas, TRH secretion is inversely related to insulin secretion (318). While TRH does not affect insulin release (319), TRH and CHP inhibit 2-deoxyglucose-stimulated pancreatic secretion in a dose-dependent manner (320), and TRH enhances arginine-stimulated glucagon release (319). In isolated rat pancreas perfusates, anti-TRH antiserum reduces glucose- and arginine-stimulated glucagon secretion, and exogenous TRH enhances basal glucagon secretion if endogenous TRH is first cleared (318).

TRH also mediates central effects on pancreatic secretion. CSF injection of TRH, and microinjection into the DVC, stimulates exocrine pancreatic volume, protein, and bicarbonate secretion via vagal outflow (321, 322). VIP is also an important nonmuscarinic mediator of TRH-stimulated pancreatic secretion, while CGRP, via noradrenergic mechanisms, opposes TRH pancreatic stimulation (321). Curiously, in isolated rat pancreatic acinar cells, TRH inhibits carbachol- and ceruletide-stimulated, but not OAG- or CCK-stimulated amylase secretion (320, 323). Thus, certain pancreatic secretory pathways may show opposite peripheral and central effects by TRH. Finally, TRH has a trophic effect on the pancreas (324). Chronic administration of TRH for 10 days via gastric fistula significantly increases pancreatic weight, DNA content, and protein content, although enzyme concentrations are not proportionally elevated, so that their final concentrations are reduced.

ic RX77368, a TRH analog, stimulates hepatic DNA synthesis 24–72 h post injection in a dose-dependent manner (325). iv Administration is ineffective. The effect is abolished by hepatic vagotomy or atropine. Further, ic RX77368 enhances hepatic blood flow 15–90 min post injection. This regulation is abolished by hepatic vagotomy, atropine, indomethacin, or the NO synthesis inhibitor, NG-nitro-L-arginine methyl ester (326).

b. TRH and cardiovascular function: TRH reverses shock of varying etiologies in a number of animal species (327). However, the precise cardiovascular changes induced by TRH vary markedly with dosage, species, and experimental state, in particular, whether the animal is anesthetized or conscious, and normotensive *vs.* hypotensive. While TRH displays many cardiovascular effects, CHP and TRH-OH have minimal cardiovascular activity (328).

In rabbits, both anesthetized and conscious, iv TRH increases blood pressure and causes peripheral vasoconstriction (328, 329). Effects in anesthetized rats are the same, except peripheral vasodilatation is seen (330); this peripheral vasodilatation is reversed by cholinergic blockade (328). Overall, TRH modulates blood pressure through combined parasympathetic and sympathetic effects (328, 330, 331). DA plays a lessor role (332), and naloxone is ineffective in altering these effects (333). As well, iv TRH in rats completely reverses systemic NT-induced hypotension and attenuates the central pressor effect of NT (334).

In anesthetized rabbits, but not conscious rabbits, cerebral vasodilatation is induced by iv TRH, bringing cerebral blood flow back to the level observed in conscious animals. Once cerebral blood flow is normalized, TRH has little effect (329). Cerebral vasodilatation is not the result of alterations in peripheral blood pressure, blood gases, loss of autoregulation, change in cerebral metabolism, or change in oxygen saturation gradients (328). Cerebral vasodilatation in anesthetized rabbits is partially blocked by the α2-adrenergic antagonist yohimbine, although yohimbine has no effect on TRH-induced elevation of mean arterial pressure (335). Neither vagotomy nor cholinergic blockade reduces these effects (328). Transection at the mesencephalic pons abolishes TRH-induced cerebral vasodilatation without affecting its systemic pressor effect (336). Cordotomy at the C1 level abolishes the pressor effect of TRH, but not its effect on the cerebral vasculature. Thus, peripheral pressor and vasoconstriction effects are mediated more caudally than those increasing cerebral blood flow.

icv TRH significantly elevates blood pressure and heart rate in anesthetized (337, 338) and conscious (339) rats. In anesthetized rats, icv TRH increases blood flow to most organs, and this is abolished by bilateral vagotomy (328). In conscious rats, much more peripheral vasoconstriction is found (340), although this effect is reversed in hypovolemic states (341). icv TRH elevates plasma levels of NE and E independently of plasma renin activity or vasopressin. The vascular effects of icv TRH in rats are mimicked by TRH activation of sympathetic nuclei within the brain (342); intrathecal TRH induces its pressor response in rats and humans via increased sympathetic activity to the peripheral vasculature and adrenals (343), an effect mediated in rats by TRH receptors in the IML (344). Interestingly, sympathetic nerve responses in rats are partially attenuated by reduced thyroid activity, providing a second route for TRH regulation of cardiovascular function (345). Taken together, the data in rabbits and rats suggest that central TRH regulation of cardiac functions and organ blood flow distribution principally

are mediated through the sympathetic nerves and the adrenal medulla (339).

Selective electrolytic lesions have identified the dorsal raphe nuclei as mediating the pressor response induced by icv TRH. Reductions in blood pressure induce prepro-TRH mRNA in the RPa (346). These changes, in turn, effect the descending bulbospinal tracts and ascending tracts to the PVN (342). TRH infusion directly into the dorsal raphe nuclei reproduces the pressor, tachycardic, and sympatho-excitatory effects of icv TRH, and these effects are blocked by ganglioplegia with pentolinium. Further, chemical lesioning by the 5-HT-preferring toxin 5,7-dihydroxytryptamine (5,7-DHT) obliterates TRH-induced tachycardia, while the NE/DA-selective toxin 6-hydroxydopamine does not. These data strongly implicate the descending bulbospinal pathway to the IML column to boutons on preganglionic sympathetic neurons as the neuroanatomical pathway mediating the peripheral cardiovascular effects of TRH (347).

TRH in experimental CVS disease: In experimental models of anaphylactic shock, hemodynamic parameters in mice (348) and guinea pigs (349) are improved by iv TRH, largely through elevation of plasma E and NE (350). icv TRH mimics the protective effect of iv TRH (351). TRH action in endotoxic shock also is mediated through the sympathetic nervous system (350). In hemorrhagic shock, TRH elevates blood pressure either by improvement in cardiac output, or by increasing peripheral vascular resistance (352). However, after effects of the latter may be harmful and could explain why TRH efficacy for survival after hemorrhagic shock has varied among species (353, 354). Although principally mediated by catecholamines, icv TRH-induced hemodynamic improvements in shock are partially blocked by δ-opiate receptor antagonism (354) and vagotomy or atropine sulfate (355).

Few studies have examined the converse effect of shock on TRH. In rats, hemorrhagic shock decreases TRH in frontal cortex, septum, hippocampus, and hindbrain (356). TRH receptor binding is significantly decreased in septum and hindbrain. However, other experiments indicate that elevations of TRH in the medulla, midbrain, cortex, striatum, and cerebellum after hemorrhage are associated with better survival (357). Thus it appears that reductions in TRH neurotransmission in certain parts of the brain may contribute to the pathophysiology of shock. Further, TRH effects may be mediated, in part, by downstream effects on the thyroid axis (358) and plamsa vasopressin (204).

In spontaneously hypertensive rats (SHR), CSF TRH, prepro-TRH mRNA, TRH, and TRH receptor binding in the POA are all significantly elevated compared with Wistar-Kyoto (WKY) control rats (359). In SHR, elevated TRH receptor binding in striatum and hypothalamus correlates with the development of hypertension (360). Further, iv or icv TRH antiserum lowers arterial blood pressure in SHR rats, but not WKY rats. Chronic enalapril, a vasodilator, decreases blood pressure and reduces POA TRH levels, although another vasodilator, diltiazem, has no effect (359).

Animal models of stroke have provided a testbed for potential therapeutic application of TRH. In rats with middle cerebral artery (MCA) occlusion-induced infarcts, icv TRH given at 15 min and 24 h post surgery significantly improves survival, protects against ischemic damage, and reduces infarct size 10 days after surgery (361). Also in MCA infarcts, TRH increases blood flow to the infarct area (362). ip And oral TRH improve neurological deficits in MCA stroke (363) and improve recovery in cerebral damage induced by experimental hematoma (363). However, in gerbil and dog models of stroke, TRH fails to display efficacy (352).

c. TRH and respiration. icv TRH, in doses as low as 3 ng, significantly elevates blood pressure and heart rate in anesthetized rats, but a minimum 16 ng is required to raise the respiratory rate (364). Respiratory frequency is increased much more than tidal volume (16). Increases of respiratory frequency greater than tidal volume are also seen in conscious rats (365) and in rabbits (366). icv TRH raises respiratory rates in anesthetized rats partly through a DA D2 receptor mechanism (332). Further, icv TRH-induced respiratory stimulation is potentiated by pretreatment with naloxone, methylatropine, or low doses of GABA, but is unaffected by β-adrenergic blockade and is independent of TSH (367). TRH antagonism of opiate-induced respiratory depression (368) is described in more detail below in *Subsection 7*.

In isolated brain stem-spinal cord preparations from rat neonates, bath application of TRH induces respiratory rhythmic neural discharges (369). icv TRH produces rhythmic bursting activity in neurons of the NTS (370), and local injection of TRH into the NTS induces tachypnea, although with a slower onset than seen with icv TRH (367). The respiratory effect occurs in the absence of any change in blood pressure or heart rate. Shortening of inspiratory times, but not tachypnea, results from TRH injections into the ROb (371). Tachypnea, without cardiovascular or locomotor effects, is seen with microinjection into the interpeduncular nucleus of the reticular activating system (372).

There is significant anatomical support for a role of TRH in respiratory control. Botzinger neurons in the medulla, which inhibit respiratory motoneurons, and the more caudal ventral respiratory group, form close associations with TRH-immunoreactive boutons (373). These connections appear to be functional, since TRH injected into the pre-Botzinger complex in neonatal rat medullary slices increases respiratory discharge frequency (369). TRH-immunoreactive boutons are also prominent near nucleus ambiguus motoneurons that display rhythmic fluctuations with phrenic nerve discharges (374).

Developmental studies support a direct effect for TRH on hypoglossal motoneurons in the caudal medulla. These neurons innervate tongue muscles critical for airway inspiratory control and display respiratory-related activity. TRH increases hypoglossal neuron discharge frequency, duration, and amplitude in neonatal mouse slices. In adult rat brainstem slice preparations, high doses of TRH depolarize hypoglossal neurons and reduce their firing threshold (375). More rostral to the hypoglossal nucleus, TRH enhances the responsiveness of inspiratory neurons in the ventrolateral medulla (376).

iv Or ic TRH significantly stimulates diaphragmatic activity and antagonizes morphine depression of diaphragmatic activity (377). TRH potentiates the excitability of diaphragmatic motor nerve terminals (378). Further, injections

of TRH as low as 1 ng into the retrotrapezoid nucleus of anesthetized rats increase phrenic nerve firing frequency and amplitude. Only at 5 ng does TRH raise blood pressure. Both CHP and TRH-OH increase phrenic nerve firing frequency, but not amplitude, starting at 5 ng (379).

Preclinical literature describes the importance of thyroid hormones and steroid treatment on fetal lung development (380). However, in human trials TRH coadministration in steroid and surfactant therapy does not reduce newborn respiratory distress syndrome, chronic lung disease or associated neonatal complications, or death (381, 382).

3. TRH and seizure modulation. TRH was first reported to potentiate the anticonvulsant actions of phenobarbital in mice in 1975 (383) and has since been shown to be anticonvulsant in multiple animal models of seizures (384–386). Despite extensive preclinical data indicating that TRH is likely to serve as an anticonvulsant, or a potentiator of known anticonvulsants, few large trials with this agent have been conducted. For intractable epilepsy, modest results have been achieved (387, 388).

a. TRH and electroconvulsive seizures: One seizure paradigm commonly used to test the efficacy of anticonvulsant drugs is electroconvulsive seizure (ECS) (389). A single stage 5 seizure, induced after five ECS treatments given on alternate days (ECS $\times$ 5), elevates TRH in hippocampus, amygdala/pyriform cortex, whole cortex, and striatum, 48 h post seizure (17). Subconvulsive shocks given on alternate days for 5 days result in regulation only in the striatum. A separate study reported that ECS $\times$ 5 decreased NAc and lumbar spinal cord TRH 24 h after the last shock (390). In sum, ECS effects center on the hippocampus, amygdala and surrounding cortex, and the dorsal and ventral striatum.

The hippocampus most consistently demonstrates TRH induction after chronic ECS (391). A significant percentage of hippocampal TRH derives from extrinsic sources. ECS induction of prepro-TRH mRNA in the entorhinal cortex presumably leads, via the perforant pathway, to TRH increases in the dentate gyrus (17). In the hilar subregion of the hippocampus, which contains few or no perforant path terminals, fimbrae-fornix lesions do not block ECS induction of TRH, consistent with endogenous biosynthesis (392). There is no difference in basal TRH release from hippocampal slices dissected from ECS-treated vs. sham-treated animals. However, potassium-stimulated TRH release increases linearly 12, 24, and 48 h post seizure, and tissue content remains uniformly elevated throughout the postseizure period (393). Thus, in addition to elevations in steady state levels, there is a time-dependent shift of intracellular TRH into a potassium-responsive pool (394), which may enhance TRH release in response to afferent signaling.

Given the documented effect of TRH to increase cholinergic transmission in the hippocampus (395) and cortex (396, 397), it is logical to speculate that TRH could be used to reverse ECS-induced neurochemical and behavioral deficits (398, 399). Indeed, in rats post-ECS performance deficits are reversed by TRH.

b. TRH in kindled seizures: A second paradigm used to model epilepsy is kindling, where repeated electrical or chemical stimulation of limbic structures progressively lowers seizure threshold until an initial subthreshold stimulation becomes capable of reliably stimulating generalized (stage 5) seizures (400). In fully amygdala-kindled rats (five consecutive stage 5 seizures), prepro-TRH mRNA levels are significantly elevated 24 h following a stage 5 seizure in the dentate gyrus granular layer and the pyriform, entorhinal, and perirhinal cortices (401, 402). More detailed time course studies in fully kindled animals report significant elevations of prepro-TRH mRNA in the dentate gyrus granular layer, several nuclei of the amygdala, and layers II and III of the pyriform and entorhinal cortices. Increased levels are detected 3 h post seizure, peaks occur at 6–12 h post seizure, and levels return to baseline 24–48 h post-seizure (246, 392). The time course is similar in all regions, although slightly delayed in entorhinal cortex. The induction in prepro-TRH mRNA seen after full kindling may be observed unilaterally 24 h after partial kindled (stage 1–4) seizures, but is reliably and bilaterally observed only after fully kindled, generalized (stage 5) seizures (401).

Kindled seizures induce c-fos mRNA and Fos-related peptides, which in turn are postulated to induce prepro-TRH transcription via the AP-1 site in the prepro-TRH gene promoter (155, 403). Fos-like immunoreactivity and prepro-TRH mRNA are extensively colocalized, in some cases in up to 70% of cells, in the pyriform cortex, entorhinal cortex, and dentate gyrus granule cells (404). A second potential transcriptional regulator of prepro-TRH gene expression, corticosterone, also is rapidly induced during kindled seizures (166, 405).

Carbamazepine given contingently with kindling treatments attenuates prepro-TRH mRNA increases in the dentate gyrus, pyriform cortex, and ipsilateral entorhinal cortex. No attenuation is seen when carbemazepine is given noncontingently, *i.e.*, after the kindling treatment (406). These results are intriguing, indicating that the carbamazepine-TRH interaction might be altered by behavioral or other nonpharmacological interventions.

Regulation of TRH peptide levels, measured 48 h post seizure, correlates with the progression of amygdala kindling. Partial kindling induces TRH in pyriform cortex, with greater regulation in stage 3–4 seizures than stage 2 seizures (17, 407). In fully kindled rats, TRH is increased even more in pyriform cortex 48 h after stage 5 seizure. Increases are also seen in cingulate and frontal cortex, hippocampus, amygdala, and ventral striatum (408, 409). Similarly, chemically kindled rats show TRH induction in hippocampus, amygdala, pyriform cortex, and anterior cortex 48 h after stage 5 seizures (17). Both kindling and TRH regulation after kindling persist for 6 months after kindling.

In fully kindled animals, all subregions of the hippocampus show reduced levels of TRH 1 h after seizure, consistent with synaptic release and rapid degradation. Levels rise to control levels at 6 h, are elevated at 24 and 48 h, and again return to control levels at 144 h after seizure. Increases are largest in the dorsal hippocampus, including the dentate gyrus, hilus, and CA3 region. It is hypothesized (17, 409) that TRH elevations may mediate the postictal refractory period (410). In fully kindled rats, bilateral hippocampal TRH in-

fusions decrease seizure after-discharge duration and overt seizure duration in a dose-dependent manner, consistent with an anticonvulsant action in the hippocampus (394).

TRH receptor binding in the dentate gyrus and perirhinal cortex is decreased in amygdala-kindled rats compared with sham-kindled animals (402, 406). After a single stage 5 seizure in electrically kindled rats, hippocampal membrane TRH receptor binding is reduced 23–29%, and amygdaloid membrane binding is reduced by 21–22% (409, 411). Curiously, in amygdala-kindled rats, dorsal striatal receptor binding is increased 24 h after seizure and persists significantly elevated at 21 days, although no regulation in striatal TRH is reported. Thus, only in certain brain regions do receptor adaptations appear to compensate for elevated TRH levels.

c. TRH in chemically induced seizures: TRH regulation in other types of seizures differs somewhat from that seen in ECS and kindling. Limbic seizures induced by systemic kainic acid substantially increase TRH in the posterior cortex and in the underlying dorsal and ventral hippocampus (412). Smaller increases are detected in the anterior cortex, amygdala/pyriform cortex, and corpus striatum. The increases in TRH are longer lasting than described for ECS, peaking at 2–4 days and resolving by 14 days, except for the dorsal hippocampus, where TRH elevations persist beyond 2 weeks. TRH is rapidly elevated in the septum, hippocampus, and thalamus/midbrain after a single pentylenetetrazol-induced seizure. Pyriform cortex was not tested (384). Pentylenetetrazol-induced tonic-clonic seizures in dogs increase TRH in pyriform and frontal cortex, hippocampus, and amygdala 48 h after seizure (413). Soman-induced seizures mediated by excessive cholinergic activity result in particularly high induction of TRH in frontal cortex, hippocampus, pyriform cortex, and entorhinal cortex, and lower induction in the amygdala (414).

d. Mechanisms of TRH anticonvulsant action: Irrespective of the precise mechanism of seizure induction, repeated seizures ultimately induce prepro-TRH mRNA in pyriform cortex, amygdala, and hippocampus. These areas contain well characterized TRH receptor binding (415, 416). Further, the pyriform cortex region is a primary site for initiation of grand mal seizure activity, and the prepyriform region is exquisitely sensitive to direct application of chemical convulsants (417). One mechanism of TRH anticonvulsant effects may be through inhibition of L-glutamate excitation of neurons (418, 419), especially neurons of the perforant pathways synapsing with dentate gyrus granule cells (420). This inhibition would increase after treatments such as kindling that elevate TRH, providing a feedback control for further sensitization in phenomena such as kindling and long-term potentiation. Knoblach and Kubek (407) suggest that TRH may be coreleased with excitatory neurotransmitters at these sites as a means to modulate neuronal response. If TRH is inhibitory to calcium influx secondary to reducing excitatory amino acid neurotransmission, it may also serve a neuroprotective role. Also it is speculated that hippocampal TRH may interact with coexpressed endogenous opioid peptides in seizure-involved pathways to modulate seizure activity (93).

4. TRH and motor control.

a. TRH stimulates ventral horn motoneurons: Bulbospinal neurons that express prepro-TRH mRNA and iTRH descend from the medullary raphe nuclei, and the parapyramidal and paraolivar regions, to end in close apposition to motor neurons in lamina IX, and sparsely in lamina VIII, of the ventral horn of the spinal cord (421, 422). The raphe projections provide dense innervation of spinal motoneurons and are likely to enhance motor excitability, principally of proximal muscle groups (423, 424). In rat (425), rabbit (426), and human (427) spinal cord, the highest concentrations of TRH are found in the ventral horn. TRH is present in large granules within terminal boutons that synapse with dendrites, supporting a synaptic role (422). Some 60–90% of these bulbospinal neurons coexpress 5-HT with TRH (428, 429). Immunocytochemical and ablation studies also support coexpression of TRH with SP (422), although SP appears to have a more prominent role in autonomic nervous system function than in voluntary motor control (430). Surprisingly, in most species the ventral horn of the spinal cord is not enriched in TRH receptor binding (431). However, the human spinal cord displays elevated TRH receptor densities in laminae IX, which contains α-motoneurons (432).

An extensive literature describes TRH excitation of spinal cord ventral horn motoneurons (423, 433, 434) and hypoglossal motor neurons (435) by suppression of a distinct K^+ current and development of an associated Ca^{++}-sensitive inward current (436). In addition, TRH enhances motor neuron firing in response to excitatory amino acids (423), increases motoneuron recruitment by antidromic stimulation (437), and depolarizes ventral roots (438). The net effect of this excitation is augmentation of muscle tone, contractility, and spinal reflexivity (438). It should be noted that while TRH, SP, and 5-HT each can enhance excitatory amino acid activation of motoneurons, TRH excitation of ventral horn motoneurons is slower and less reliable than that observed with application of 5-HT or SP (423, 439). Neither TRH-OH nor CHP have any demonstrated effect on spinal motoneurons, so motoneuron effects are presumed to be direct actions of TRH (434, 436).

Denervation of the plantar foot muscles by botulinum toxin injection reveals reinnervation deficiencies in adult rats that have undergone ablation of the descending bulbospinal 5-HT/TRH neurons (440). However, gross motor performance, muscle cell count, electrophysiological properties, or α-motoneuron counts are not made abnormal by this ablation, arguing for an insignificant role for TRH in adult animals. White *et al.* (439) argue that TRH function is more significant in developing animals or on damaged motoneurons. TRH trophic effects (441) and enhancement of contractility (442) are demonstrated best in embryonic/neonatal preparations. More importantly, TRH-induced depolarization shows significantly less tachyphylaxis in isolated neonatal rat spinal cord preparations than in adult preparations (443). NE inputs in the ventral horn enhance both microiontophoretically applied TRH-induced excitation of motoneurons (444) and behavioral excitation elicited by intrathecal

TRH (426). Thus, TRH may function only under certain physiological states, such as stress or healing, that were not well tested in previous paradigms.

In preclinical studies, TRH displays significant beneficial effects in the Rolling mouse Nagoya (RMN), a mouse model of ataxia (445, 446). TRH is also effective in ameliorating ataxia induced by 3-acetylpyridine degeneration of the inferior olive in rats (446) and by cytosine arabinoside treatment in mice (447). Immobility and fall index scores for other ataxic mice models, including *staggerer, reeler, weaver*, and mice with Purkinje cell degeneration, are reduced by TRH (448, 449). The NMDA antagonist MK-801 completely blocks TRH improvement of ataxia induced by 3-acetylpyridine, providing one of the few clues as to how TRH might mediate its antiataxic effects (450).

Several clinical studies support potential utility for TRH in the treatment of inherited ataxias such as spinocerebellar degeneration. Several studies demonstrate improvements by TRH of motor, occulomotor, and electrophysiological abnormalities in inhereted ataxias such as spinocerebellar degeneration (451, 452). For the motor neuron disease amyotrophic lateral sclerosis (ALS), studies using higher doses of TRH demonstrate some transient imporvement in symptoms, particularly speech, swallowing, and respiratory function. Unfortunately, longer term results with systemic or intrathecal TRH (453, 454) indicate that TRH fails to slow progressive motor neuron loss and provides only temporary symptomatic relief.

b. TRH promotes recovery in experimental spinal cord and brain injury: TRH accumulates superior to the site of traumatic or ischemic spinal cord injuries (455, 456). In both cervical and lumbar injuries, TRH elevations are accompanied by modest but detectable down-regulation of TRH receptor binding proximal to the injury, in lamina X and the ventral horn gray matter, but not in the dorsal gray (457). The decrease in TRH receptor binding is detectable 48 h after injury and recovers by 3 weeks. Recovery of TRH receptor binding parallels the functional neurological recovery that occurs late after CNS injury (458). In mouse models of spinal cord motoneuron degeneration, TRH metabolism and levels are increased at sites of damage (459, 460).

Whether increased TRH is a neuroprotective adaptation or a damaging mediator of the disease process is unknown. However, it appears likely that TRH is beneficial. TRH is superior to naloxone or high-dose steroid treatment in promoting recovery in experimental CNS trauma models (461). In most studies, sc or iv TRH improves electrophysiological recovery of damaged spinal cord tissue (462, 463). Improvement may result from trophic effects of TRH on spinal motoneurons (204, 464, 465), from the ability of TRH to increase spinal cord blood flow (466, 467), or from the ability to reduce edema (468) at the site of spinal cord injury. TRH efficacy in spinal trauma recovery requires continuous intrathecal infusion of native TRH, presumably due to its short half-life. Further, while TRH and all its analogs activate spinal motoneurons (434), only analogs that preserve the C terminus of native TRH are beneficial in spinal trauma, indicating the healing and activating effects of TRH are separately mediated (469).

TRH and its analogs are also efficacious in promoting recovery in animal models of head injury. Cats with brain stem compression injury show improved neurological and EEG parameters (470), and mice with head impact injuries demonstrate less behavioral disturbance (471), after TRH treatment. In rats with fluid percussion-induced brain injury, the TRH analog NS-3 given 30 min after injury improves survival, neurological parameters, and motor function at 24 h post injury, and improvements persist for at least 4 weeks (472). There is significant interest in TRH as a treatment for human traumatic brain injury and spinal cord injury, either to prevent damage progression or to speed neuronal recovery (473). However, TRH effects on head trauma, or recovery in spinal cord trauma, have been modest at best (474).

5. TRH and antinociception. The presence of TRH and TRH receptors in the midbrain PAG, the raphe nuclei, and, to a limited extent, in the dorsal horn of the spinal cord is highly suggestive of a role in pain modulation (93). Antinociception to chemical stimuli has been demonstrated in mice with iv and sc TRH (475) and for icv TRH against visceral chemical and mechanical pain (476). In the rat, icv TRH increases reaction latencies to visceral acetic acid (477). TRH displays potencies comparable to morphine in some of these studies. Thermal analgesia with icv TRH has been demonstrated in the hot-plate test (476) and less frequently in the tail-flick test (477). icv TRH also potentiates stress-induced analgesia, including foot shock-induced analgesia and swim-induced analgesia (478). In general, the antinociceptive effects of TRH are short lived, typically lasting for less than 15 min (479).

icv CHP in mice is variably reported to induce antinociception to mechanical, thermal, and chemical stimuli (480), or to mechanical stimuli but not chemical stimuli (481). This effect is significantly less potent, but longer lasting, than that of TRH and is antagonized to a greater extent by sc naloxone than is TRH. TRH-OH also is reported to be antinociceptive to mechanical and chemical stimuli in mice (481). Thus, it is likely that both TRH and its metabolites are active in TRH-induced antinoception in its various forms.

The PAG is critical for integration of pain perception and the behavioral response to pain (482). The PAG expresses moderately high levels of prepro-TRH mRNA (28, 249), and significant amounts of iTRH are detected in cell perikarya and neuronal fibers of the PAG (93, 421, 483), and TRH receptor binding is detected at moderate levels (484) throughout the PAG. The neuroanatomical distribution of TRH and TRH receptor binding in all regions of the PAG implicates it in both opiate-dependent and opiate-independent pain mechanisms. The dorsolateral PAG mediates antinociception that is not modified by naloxone and is presumed to be opiate independent, while the ventrolateral PAG is more commonly associated with opioid-dependent antinociception (482). This may relate to the finding that TRH infusions into the ventral PAG decrease cold-water swim-induced antinociception in the rat in a dose-dependent manner, while dorsal PAG infusion has the opposite effect (485). Curiously, TRH in both placements antagonizes morphine antinociception and reduces swim-induced hypothermia and morphine-induced hyperthermia, while the converse, analgesia produced by TRH infusion into the ventral PAG,

is blocked by opiate antagonists (486, 487). TRH has no demonstrable effect on μ-, δ-, or κ-opiate receptor binding, or receptor occupancy, arguing against direct TRH regulation of opioid peptide release. It is postulated that TRH activates one or more types of inhibitory interneuron, which in turn reduce excitation of pain-excited opiate-responsive neurons in the PAG.

Complete blockade of TRH-induced antinociception by opiate antagonists occurs in some PAG infusion paradigms, but not with systemic TRH, which presumably activates nonopiate pain pathways in which TRH functions. Separate studies indicate that TRH injections into the NAc (488) and amygdala (477) produce antinociception. TRH antagonizes NT-induced antinociception (489) and both TRH and CHP antagonize THC-induced antinociception (194).

Clearly then, the relationship of TRH antinociception to endogenous and exogenous opiate systems is complex. As opposed to opiate-induced hypothermia, locomotor depression, catalepsy, and respiratory depression, systemic TRH does not antagonize acute opiate-induced analgesia (490), although hyperalgesia induced by high-dose naloxone is antagonized by TRH (491). In contrast to the case with drug-naive mice, TRH does not display antinociceptive effects to chemical stimuli in morphine-tolerant mice (487). In particular, Bhargava and colleagues (490) argue that TRH interacts preferentially with the κ-opiate system in mice. Other studies (487) indicate that tolerance either to morphine (μ- and δ-specific) or ethylketocyclazocine methanesulfonate (κ-preferring) equally antagonize TRH antinociception. Most studies indicate that TRH-opiate interactions do not occur via TRH modulation of opiate receptor binding, or through TRH-stimulated release of endogenous opioids. Rather, TRH acts through intermediary systems that, in turn, modulate opiate-mediated pain transmission, and perhaps vice versa. In the spinal cord, likely intermediary systems involve 5-HT and SP (492).

PAG-mediated antinociception is mediated via outputs to the ROb, RPa, and RMg (482), nuclei that express high levels of prepro-TRH mRNA (249). Further, antinociception by excitation of the nucleus reticularis paragiganticellularis (RPGi) is mediated, in large part, through reciprocal connectivity to the RMg (493). TRH into either the RMg or RPGi is antinociceptive (477). icv TRH also inhibits pain-excited neurons in the mesencephalic reticular formation (MAF) (494). The MAF is believed to form a second integration circuit for autonomic response to painful stimuli (495).

In addition to the descending bulbospinal pathway, TRH is also found in an intrinsic system of cell bodies in laminae II and the lamina II/III border of the dorsal horn (251, 496). Dorsal horn iTRH is not depleted by 5-HT neurotoxins, unlike ventral horn TRH (250). Laminae II also exhibit a high level of TRH binding in many species (250, 432, 484). Intrathecal TRH antagonizes morphine analgesia in the tail-flick test at most doses, indicating that the net action of TRH within the dorsal horn is to enhance transmission of nociceptive somatosensory information (492). iv TRH is reported to facilitate nociceptive transmission through the dorsal horn via positive modulation of NMDA receptor-mediated transmission (497).

6. TRH in thermoregulation. TRH plays a prominent role in integrating a number of thermogenic responses to cold (509). CNS injection of TRH elevates body temperature (498), and TRH antagonizes the hypothermic effects of a number of agents, including barbiturates, ethanol, chlorpromazine, bombesin, NT, and β-endorphin (204). Systemic TRH antagonizes morphine-induced hypothermia, while having little effect on analgesia (499). icv Anti-TRH antibodies in rats produce hypothermia, supporting an endogenous role for TRH in body temperature elevation (500). A principal site of TRH thermoregulation is the anterior hypothalamic POA (498). TRH into the POA inhibits heat-sensitive neurons and activates cold-sensitive neurons (501), which results in increased body temperature through peripheral vasoconstriction, increased metabolic heat production, and shivering (502). These effects require intact catecholamine neurotransmission (502).

Ablation of the POA does not eliminate TRH antagonism of pentobarbital-induced hypothermia, indicating that sites other than the POA can mediate TRH thermoregulation (503). Cold exposure elevates prepro-TRH mRNA levels (504) and TRH secretion (505) in the PVN. These changes elevate thyroid hormones and increase heat generation in brown adipose tissue (506). Systemic TRH has similar effects, *e.g.*, systemic TRH improves thermoregulation in neonatal lambs through increased fat oxidation (507). We note that cold exposure elevates TSH levels before TRH levels, probably because SRIF, which tonically inhibits TSH secretion, is rapidly down-regulated in the PVN by cold (508).

Cold-induced increases in prepro-TRH mRNA also are seen in the DMN (509) and caudal raphe nuclei (510). The raphe nuclei, which receive sensory information from the skin, project to spinal cord preganglionic sympathetic neurons. Further, the raphe nuclei provide TRH afferents to the NTS (511), which, in turn, projects to the DMN, and then the spinal cord preganglionic neurons. 5-HT projections from the NTS to the PVN provide feedback regulation to this stimulation (512). Excitation of spinal cord preganglionic sympathetic neurons results in postganglionic NE release and increased facultative thermogenesis via β- and α1-adrenoreceptors on brown adipocytes (513). Additional stimulation comes from direct projections of TRH neurons from the dorsal cap of the PVN to preganglionic sympathetic neurons in the thoracic and sacral spinal cord (514).

In mice, icv TRH and CHP antagonize ip THC-induced hypothermia (517). However, CHP elicits hypothermia when injected into the cerebral ventricles, an effect antagonized by TRH (515). The POA is believed to be the sole site mediating this action of CHP (516). Thus, the hypothermic response to icv TRH seen under certain conditions such as warm environments may result from TRH catabolism to CHP (501).

7. TRH and drugs of abuse. The psychomotor theory of addiction states that a common biological mechanism mediates both positive reinforcement and motor activation by drugs of abuse (518). Given the ergotrophic effect of TRH as a locomotor activator, interaction between TRH and drugs of abuse is likely. While the main focus of drug abuse research has centered on two principal loci in the mesolimbic DA pathway, the NAc and the VTA (519), a more extensive network,

the "limbic-motor circuit," with inputs to the NAc coming from many limbic areas and outputs going to both limbic areas and motor areas, is now appreciated (520).

In rats, systemic TRH and intra-NAc TRH mimic cocaine by inducing locomotor activation via release of DA and 5-HT in the NAc and striatum (222, 521, 522). Conversely, DA D2 agonists increase TRH release from striatal and NAc slices (523). Downstream components of the HPT axis act to reinforce psychostimulant effects (524). TRH neurons of the PVN receive DA and NE inputs that are regulated by cocaine (124). Further, DA and cocaine both activate the HPA axis; stress (or CRF) and cocaine elevate NAc and medial prefrontal cortex DA and cause similar neuronal adaptions (525, 526).

Few studies have directly examined the link between TRH and psychostimulants. Acute amphetamine lowers TRH in the caudate, NAc, and lateral septum (527, 528). Over time, TRH levels show some adaptation to chronic amphetamine, and TRH receptor binding increases (528). Acute cocaine significantly decreases prepro-TRH mRNA levels in the amygdala and hippocampus, 45 min after injection (529). Chronic cocaine regulates prepro-TRH mRNA in the NAc, amygdala, hippocampus, and hypothalamus. Prepro-TRH mRNA regulation is strongly dependent on the length of time after cocaine cessation and persists beyond 72 h post injection in the amygdala.

The role of TRH in morphine actions also is not well understood. TRH antagonizes a number of morphine's depressant effects, including sedation, hypothermia, and catalepsy (530, 531). Chronic TRH inhibits the development of tolerance to opiate-induced hypothermia and catalepsy (499, 532). δ- And κ-opiate receptor activation reduces TRH receptor binding, but TRH does not effect opiate binding (533). More directly, morphine reduces cortical and diencephalic TRH concentrations (534). Within the HPT axis, a clearer relationship between TRH and opioids exists. Morphine and opioid peptides reduce plasma TSH (535) and blunt cold-induced TSH release (536). Further, exogenous morphine at pharmacological doses inhibits TRH release via opiate receptors on TRH-secreting hypothalamic nerve terminals (509).

TRH is more strongly implicated in opiate withdrawal. While cessation of chronic cocaine use induces relatively little physical withdrawal (537), chronic morphine results in the development of physical dependence and the aversive state of withdrawal upon cessation of morphine use (537). The expression of the physical symptoms of withdrawal is mediated principally by the LC and PAG (538). A large body of data indicates that the intrinsic NE neurons of the LC undergo an up-regulation of their cAMP second messenger system in response to chronic morphine. When unopposed during morphine withdrawal, the up-regulated cAMP system drives increased firing by LC neurons (539). Extrinsic excitatory amino acid inputs from the nucleus paragigantocellularis lateralis contribute an additional 50% to LC neuronal excitability during withdrawal (540, 541). The medial hypothalamus, medial thalamus, amygdala, frontal cortex, hippocampus, and RMg also are implicated in withdrawal (542, 543).

The PAG expresses high levels of prepro-TRH mRNA (28). Mature TRH (483) and TRH receptor binding (484) are present in moderate levels throughout the PAG. Prepro-TRH mRNA is strongly induced in the PAG during opiate withdrawal (28). Fos-like immunoreactivity is greatly increased in the ventrolateral PAG during withdrawal (544) and may mediate induction of prepro-TRH mRNA (401).

While TRH levels in the PAG remain unchanged during opiate withdrawal (154), it has been found that ic TRH prevents withdrawal-induced hypothermia and decreases jumping during withdrawal in morphine-dependent mice (545). In contrast, ic TRH induces wet-dog shakes in normal animals, arguing that it augments withdrawal-like symptomatology (546). Opiate withdrawal increases TRH in the lateral hypothalamus, suggesting this region may also play a physiological role in opiate withdrawal (154). Thus, much remains to be learned about TRH effects on opiate withdrawal at sites other than the PAG.

The TRH analog TA0910 reduces alcohol-intake in alcohol-preferring rats (547) and in primates (548) in a dose-dependent manner. This appears mediated by DA D2 receptors (549). Behavioral reward to alcohol, as measured by punished responding rates, is enhanced by iv TRH (550). Alcohol alters TRH receptor binding (551). In long-sleep (LS) and short-sleep (SS) mice that display differential CNS sensitivity to ethanol, SS mice have greater sensitivity to TRH than LS mice during postnatal days 8–14 (552). It is hypothesized that a TRH receptor-mediated alteration results in enhanced development of the thyroid gland in SS mice. Alcohol-preferring rats compared with nonpreferring rats have significantly lower TRH levels in the medial and lateral septum. Upon exposure to alcohol, preferring rats are able to right themselves earlier than nonpreferring rats, and this correlates with elevations of medial septal TRH. However, these findings may be nonspecifically related to the analeptic action of TRH in this region (202). Of unclear relationship to alcohol preference, chronic ethanol in rats partially "uncouples" PVN TRH expression from peripheral thyroid response (553, 554) and, like opiates, ethanol blocks the TSH response to cold (553, 554).

A wide range of other addictive substances alter TRH receptor binding, including THC and chlordiazepoxide (551, 555). Furthermore, as described above, behavioral reward to pentobarbital and chlordiazepoxide, as well as alcohol, as measured by punished responding rates, is enhanced up to 3.5-fold by iv TRH (550). Goeders *et al.* (556) recently presented evidence that levels of benzodiazepine receptor binding are affected by the development of either behavioral tolerance or sensitization to cocaine. Conversely, benzodiazepines specifically attenuate cocaine self-administration (557). Since a number of benzodiazepines displace ^{3}H-methyl-TRH from TRH receptors (558, 559), this provides another suggestive link between TRH and drugs of abuse.

In summary, there are multiple pathways through which TRH affects virtually all classes of abused drugs. Understanding these interactions is likely to advance our understanding of addiction in general. More importantly, this understanding may provide new pharmacological approaches for the clinical treatment of substance abuse.

8. TRH outside of the CNS. TRH is phylogenetically old, present in invertebrates such as the lamprey that lack TSH or

the snail that lacks a pituitary (560, 561). It appears that mammalian endocrine functions for TRH have been "co-opted" (562) for a peptide already functioning in more basic ways. TRH is detected in many nonneural vertebrate tissues, although its functions in these tissues are not well understood. In most cases, peripheral TRH is not regulated coordinately with the HPT axis (563).

Prominent among the TRH-containing tissues are the gastrointestinal organs, including the stomach, duodenum, small intestine, colon, and rectum (562), where TRH may have peripheral effects to modulate gastrointestinal contractility. The pancreas is a rich source of TRH. Indeed, in neonates a significant portion of circulating TRH is derived from pancreas (561). Prepro-TRH mRNA is expressed in β-cells of the pancreatic islet (564), and TRH and somatostatin have opposing paracrine effects on glucagon secretion (565).

TRH is present at high levels in the genitourinary system including the ventral prostate, Leydig cells of the testes, the epididymis, and seminal vesicles (566, 567). Interestingly, propylthiouracil-induced hypothyroidism increases TRH in prostate and testis but reduces TRH in epididymis (566, 568). TRH receptor mRNA is expressed in the ovary and uterus (569), and TRH is present in placenta, amniotic fluid, and breast milk (177, 570). Again, reproductive TRH may act as a paracrine regulator (571).

TRH is also present in retina (572, 573), where its levels are light entrained (572). icv TRH raises intraocular pressure and induces marked mydriasis via combined sympathetic and parasympathetic effects (574). TRH receptor mRNA is found in human peripheral blood monocytes (PBMCs) and rat splenocytes (575). It affects secretion of TSH and immunoglobulins from blood elements (576, 577) and may be a trophic factor for certain blood elements (578). In the heart, TRH is expressed and has direct ionotopic effects (579, 580).

IV. Function of non-TRH pro-TRH-Derived Peptides

The rat TRH precursor yields, in addition to TRH, seven pro-TRH-derived peptides that display region-specific distributions (19, 82, 87, 96, 581). In addition, TRH progenitors, *i.e.*, extended forms of TRH that have not been fully processed, are found. Of these, the following discussion will focus on the immediate TRH progenitor, TRH-Gly. From the first immunohistochemical studies describing the distribution of pro-TRH, it was clear that precursor immunoreactivity was present in areas of the brain where mature TRH had not been localized (82, 95, 582). These regions included nuclei in the olfactory bulb, sexual dimorphic area of the POA, reticular nucleus of the thalamus, amygdala, hippocampus, cerebral cortex, and PAG (95). These conclusions were based on immunohistochemical localization of TRH in colchicine-treated animals. However, TRH localization based on micropunch isolation and RIA, where animals need not be colchicine treated, has detected TRH in many of these regions, including the amygdala and surrounding cortical regions, the hippocampus, and PAG. Still, certain regions, such as the reticular nucleus of the thalamus, appear to contain little or no mature TRH, while studies using antisera raised against non-TRH pro-TRH-derived in brain extracts demonstrate significant amounts of pro-TRH-derived products in this nucleus (Table 1). Thus, while *in situ* immunohistochemical studies remain of value, one must be aware that they are beset with difficulties in variable immunoreactivity in different loci of the brain, and they lack precise definition of the immunoreactive peptide moieties that are being detected.

In comparison to the known roles of TRH reviewed in *Section III.B*, there is precious little known about the biological activities of the other pro-TRH-derived peptides. In the hypothalamus and testis, both these non-TRH peptides and TRH are regulated within HPT parameters, while in other tissues, both TRH and non-TRH peptides are not (563, 583). Both TRH-Gly and prepro-$TRH_{178-199}$ are regulated by dexamethasone in the hypothalamus, but not in cerebellum, brain stem, retina, and stomach (583). Thus, clues to the roles of pro-TRH-derived peptides other than TRH must come from an examination of their regional distribution or evidence of regulation under specific physiological or pathological conditions.

A. prepro-$TRH_{160-169}$ (pST_{10})

Prepro-$TRH_{160-169}$ is the best characterized of the non-TRH pro-TRH-derived peptides. It is released from rat hypothalamic slices and the ME, thus making a hypophysiotropic role likely (20). Prepro-$TRH_{160-169}$ (also known as Ps4 and TRH-potentiating peptide) enhances TRH-stimulated TSH release from the anterior pituitary and stimulates TSHβ gene promoter activity (21). Thus, it acts in an opposite manner to feedback by T_3, which decreases TSH secretion (172) and inhibits TSH subunit gene expression (584). The peptide has been isolated from bovine hypothalamus and its amino acid sequence confirmed by Edman degradation (585). Prepro-$TRH_{160-169}$ is also unique in that a receptor for this peptide has been characterized (see *Section VI*) (586). Prepro-$TRH_{160-169}$ receptor binding is developmentally regulated, with an increase from birth to weaning, and then a gradual decline to adult levels at postnatal day 60 (587).

Within the CNS, prepro-$TRH_{160-169}$ is most enriched in hypothalamus, with lesser amounts in the spinal cord and olfactory bulb. The pituitary and striatum contain moderate levels. Its receptor binding is highest in the pituitary, hypothalamus, spinal cord, olfactory bulb, and hippocampus (588). Prepro-$TRH_{160-169}$ is rich in rat testis, but trace levels are detected in urinary bladder, vas deferens, and heart. Receptor binding is high in urinary bladder and vas deferens, heart, and testis (588). In adrenal extracts, RIA detects lesser amounts of prepro-$TRH_{160-169}$, which has been confirmed further in this tissue by chromatographic fractionation. The pancreas contains prepro-$TRH_{160-169}$ within β-cell secretory granules (318). Finally, prepro-$TRH_{160-169}$ function is not restricted to a hypophysiotropic one. In parallel fashion to its role in the pituitary, prepro-$TRH_{160-169}$ does not influence basal gastric acid secretion when injected into the DMN, but does potentiate the ability of TRH to do so. Prepro-$TRH_{178-199}$ has no effect when coinjected with TRH into the DMN, and neither does prepro-$TRH_{160-169}$ when coinjected with TRH into the nucleus ambiguus (22).

ECS elevates prepro-$TRH_{160–169}$ in hippocampus, amygdala, pyriform cortex, and anterior cortex, but not corpus striatum, motor cortex, LC, or ventrolateral medulla (589). In these studies, elevations of prepro-$TRH_{160–169}$ correlated with elevations in TRH-Gly and TRH in the hippocampus, amygdala, and pyriform cortex. Thus, prepro-$TRH_{160–169}$ may share a role with TRH in seizure modulation (17). More recently it has been reported that prepro-$TRH_{160–169}$ levels in the hippocampus and amygdala correlate with immobility times in the Porsolt forced-swim test, leading to speculation that the peptide may act independently or in concert with TRH to affect mood, learning, or memory (589). A review of the distribution and postulated functions of this peptide recently has been presented (590).

B. prepro-$TRH_{178–199}$ (pFE_{22})

The second most studied non-TRH pro-TRH-derived peptide, prepro-$TRH_{178–199}$, is also released from rat hypothalamic slices and the ME (20, 591) and is localized in dense-core granules of PVN neurons that project down to the ME. prepro-$TRH_{178–199}$ has been reported to be a corticotropin-inhibiting factor, acting to reduce POMC mRNA and inhibit ACTH release (592, 593). These data fit the clinical phenomenon of increased TSH in isolated ACTH deficiency (594). Further, α2-adrenergic inputs stimulate release of both pro-TRH products and ACTH (595), and thus, prepro-$TRH_{178–199}$ and ACTH may be coreleased into the ME, allowing the pro-TRH product to modulate ACTH release. Despite this logic, the relationship between the HPA and HPT axes remains incompletely understood, and other investigators have been unable to reproduce corticotropin inhibition by prepro-$TRH_{178–199}$ (25). The pancreas produces prepro-$TRH_{178–199}$, within the β-cell secretory granules (316), suggesting a potential involvement for this peptide in the regulation of glucose metabolism. Finally, prepro-$TRH_{178–199}$ acts as a PRL secretagogue in primary pituitary cultures. However, as described in the next section, it may be broken down to peptide products that also induce PRL secretion, so a direct effect remains to be proven. Prepro-$TRH_{178–199}$ levels rise during the early phases of suckling in rat pups (91). Again, a precise role for the peptide in lactation, suckling, etc. remains to be determined.

C. prepro-$TRH_{178–185}$ and prepro-$TRH_{186–199}$ (pFQ_7 and pSE_{14})

Suckling increases prepro-TRH mRNA in PVN and markedly increases TRH release during the first period of lactation (596). In experiments where we coexpressed rat prepro-TRH cDNA with PC1, PC2, and 7B2 in GH_4C_1 cells (*Section II.D*), we detected two novel peptides, prepro-$TRH_{178–185}$ (pFQ_7) and prepro-$TRH_{186–199}$ (pSE_{14}). These peptides are generated by cleavage of prepro-$TRH_{178–199}$ (pFE_{22}) by PC2 (see *Section II.D* and Figs. 1 and 6). We subsequently determined that these peptides are present in the rat PVN. In examining whether pro-TRH processing is altered by suckling, we found that in addition to prepro-$TRH_{178–199}$, the products prepro-$TRH_{178–185}$ and prepro-$TRH_{186–199}$ also increase release of PRL from primary pituitary cultures. Prepro-$TRH_{178–185}$ was the most active PRL secretagogue. In suckling experiments, where pups were separated from their mothers for 6 h and then reunited for 45 min to suckle, a 5-fold increase in PVN prepro-$TRH_{178–199}$ and prepro-$TRH_{186–199}$ and a 6-fold increase in serum PRL were observed over nonsuckling controls. While these data implicate these novel peptides in suckling, or a response to PRL, further experiments are required to rule out nonspecific effects of stress (91).

D. prepro-$TRH_{53–74}$ (pFT_{22})

Prepro-$TRH_{53–74}$ displays a unique localization in the rostral two-thirds of the ventrolateral PAG (95, 597). Electrical stimulation of, or injection of excitatory amino acids into, this region of the PAG produces analgesia (598, 599), and this region of the PAG is most sensitive to production of morphine-induced antinociception (600). Although the lateral PAG is more commonly associated with non-opioid-mediated antinociception (482), there is limited evidence that non-opioid antinociception is also mediated within the ventrolateral PAG (601). Thus, prepro-$TRH_{53–74}$ may be a candidate molecule to mediate nonopiate pain perception in this region or play a modulatory role in opiate-mediated pain mechanisms.

As described in *Section III.B.5*, the expression of prepro-TRH mRNA in the reticular nucleus of the thalamus, the PAG, the raphe nuclei, and, to a limited extent, in the dorsal horn of the spinal cord is highly suggestive of a role in pain modulation (93). Prepro-$TRH_{53–74}$ similarly is prominent in the ventrolateral PAG, RMg, and thalamic reticular nucleus, suggesting a role in opiate-dependent pain perception (95, 597). The reticular nucleus serves in gating of peripheral somatic sensory information from the dorsal thalamus to the cortex (602). Opiate withdrawal induces prepro-$TRH_{53–74}$ in the rat PAG, while TRH levels are unaltered (27), suggesting that the peptide may interact with opiate pain mechanisms in these pathways (406, 597).

E. prepro-$TRH_{83–106}$ (pEH_{24}) and prepro-$TRH_{208–255}$

As described above (*Section III.B.10*), during opiate withdrawal prepro-TRH mRNA is increased in the PAG (27, 28). Peptide analysis in the PAG demonstrates an accumulation of the N-terminal peptides prepro-$TRH_{53–74}$ (see subsection 7 above) and prepro-$TRH_{83–106}$, a reduction in the C-terminal peptide prepro-$TRH_{208–255}$, and no change in prepro-$TRH_{178–199}$ or TRH, in opiate-withdrawal *vs.* control animals (154). Opiate withdrawal also increases prepro-$TRH_{83–106}$ in the lateral hypothalamus. We speculate that during opiate withdrawal, pro-TRH processing may be altered in several brain regions, resulting in increased levels of N-terminally derived peptides (prepro-$TRH_{53–74}$ and prepro-$TRH_{83–106}$) and decreased levels of some C-terminally derived peptides (prepro-$TRH_{208–255}$).

F. TRH-Gly

Immunoreactivity of the immediate precursor of TRH, TRH-Gly, is widespread and detectable throughout the CNS in a similar distribution to TRH and is also present in the

prostate, serum, spleen, adrenals, kidney, and gastrointestinal organs. Levels of TRH-Gly are up-regulated by hypothyroidism and by thermal stress, following the pattern for TRH itself (603). The pancreas contains TRH-Gly within β-cell secretory granules (603). The ratio of TRH/TRH-Gly is highest in pituitary and hypothalamus and much lower elsewhere in neural tissue. TRH-Gly is much better characterized than other pro-TRH-derived peptides discussed above. TRH-Gly is increased in several limbic regions after chronic ECS, including hippocampus and pyriform cortex (391). These authors hypothesize that the increase correlates with effectiveness in increasing swim times in the forced-swim test, and thus it may serve an antidepressant and/or anticonvulsant function. TRH-Gly, independent of conversion to TRH, stimulates gastric acid secretion in a dose-dependent manner, although with a potency 100-fold less than TRH (18). Interestingly, TRH-Gly can directly activate TRH receptors in high concentrations (100 and 1000 nM). The IC_{50} of TRH-Gly for displacement of MeTRH (12 μM) is significantly higher than the TRH receptor dissociation constant (K_d) of 1.7 nM for MeTRH (604).

TRH in pathological conditions may induce secretion of GH (124). In normal controls TRH-Gly does not increase secretion of TSH, PRL, or GH (605). However, iv TRH-Gly can induce secretion of GH in patients with acromegaly and stimulate PRL and TSH release in women with anorexia nervosa. Preclinical studies indicate estrogen/progesterone treatment, as well as starvation, can enhance the ability of TRH-Gly to stimulate TSH and PRL release, although at a potency far below that for TRH (606). In most cases, TRH-Gly effects are difficult to separate from effects resulting from subsequent conversion to TRH. However, TRH-Gly may prove useful as a pharmacological challenge agent, even if an endogenous role in pituitary release is not confirmed.

V. Non-TRH pro-TRH-Derived Peptides Outside of the CNS

In vertebrates, non-TRH pro-TRH-derived peptides are only reported in tissues where TRH is also found. Their functions are a matter of conjecture, based on their regional and developmental patterns of expression. Anti-pCC_{10}, which detects immediate TRH precursors flanked by paired basic residues as well as larger intermediates, detects immunoreactivity in the pancreas (607), specifically the developing islet β-cells in rat neonatal pancreas (608). Levels of TRH peak at postnatal days 2–4 in parallel with levels of prepro-$TRH_{160–169}$ and prepro-$TRH_{178–199}$. Both TRH and the pro-TRH-derived peptides are nearly completely abolished by streptozocin treatment, indicating that they are derived principally from β-cells (564). Likewise, prepro-$TRH_{53–74}$, extended forms of this peptide, and the octa-TRH progenitor are localized to developing β-cells (608, 609). Immunoreactive pCC_{10} is also found in the retina (607). In adrenal extracts, not only TRH and immunoreactive pCC_{10} (607), but also prepro-$TRH_{160–169}$, is detected by RIA and confirmed chromatographically.

In CA77 thyroid parafollicular cells, prepro-TRH mRNA, 7-kDa and 3-kDa species recognized by anti-prepro-$TRH_{53–74}$, and TRH are detected (86). In thyroid tissue, prepro-TRH mRNA, the 7-kDa and 3-kDa species of immunoreactive prepro-$TRH_{53–74}$, and immunoreactive prepro-$TRH_{115–151}$ are found. Part of this immunoreactivity comigrates with synthetic prepro-$TRH_{115–151}$ standard on gel filtration and reversed-phase HPLC. In addition, immunohistochemical studies localize prepro-$TRH_{53–74}$ to parafollicular cells in thyroid tissue (610). Thus, within the thyroid, significant biological functions for non-TRH peptides remain to be deciphered.

TRH-Gly is present in high levels in the ventral prostate of the rat, as well as in the testis, epididymis, and seminal vesicles (566). Both TRH and TRH-Gly in the epididymis and prostate are regulated along with the HPT axis under certain conditions (566). Prepro-$TRH_{160–169}$ is also detected in high levels in the testis. Further, prepro-$TRH_{160–169}$ receptor binding is high in urogenital organs, second only to CNS tissues (586). Human placenta contains appreciable quantities of the human TRH progenitor octa-TRH and a larger non-TRH peptide, human prepro-$TRH_{192–222}$ (611).

VI. TRH and Other pro-TRH-Derived Peptide Receptors

A. The TRH receptor

Although a complete discussion of TRH receptors is beyond the scope of this review, some review is important as regards the fate of pro-TRH products that are released. Full-length cDNA clones for the first TRH receptor (TRH-R1) have been identified in mouse (612), rat (613), and human (614). Although there are splice variants for rat and mouse that differ at their C terminus, functional differences between these isoforms, or characterization of their relative occurrence in tissues and under various physiological conditions, has not been determined (615). A second TRH receptor, TRH-R2, has recently been cloned (616, 617). This G protein-coupled receptor is approximately 50% related to the TRH-R1 at the amino acid level and modulates calcium influx upon binding TRH. The expression of TRH-R2 differs from that of TRH-R1, being restricted to the CNS and being enriched in the spinothalamic tract, dorsal horn of the spinal cord, pontine nuclei, and cerebellum. This distribution in the first two sites is suggestive of a role in sensory perception and antinoception, while the later two areas of expression suggest a role in motor control. The identification of TRH receptor species, be they subtypes or members of a new family with distinct pharmacological and/or neuroanatomical profiles, is critical if we are to utilize our growing knowledge of TRH functions in the development of clinically useful therapeutics.

TRH-R1 is highly conserved between species, *e.g.*, at the nucleotide level the human receptor is 90.3% and 89.2% homologous to the mouse and rat receptors, respectively; the three receptors are approximately 95% conserved at the amino acid level. The receptors are members of the G protein-coupled receptor superfamily. Intracellular signal transduction is principally mediated by coupling to G_q and G_{11}. Ligand binding results in activation of phosphoinositide-specific phospholipase C (PPI-PLC) (618), resulting in PIP_2

hydrolysis, and subsequent production of inositol 1,4,5-triphosphosphate (IP3) and 1,2-diacylglycerol. This stimulates increased intracellular calcium, although the exact contributions of increased calcium influx *vs.* mobilization of intracellular stores is controversial (619). Downstream activation of protein kinase C (620), calcium/calmodulin-dependent protein kinase (621, 622), and mitogen-activated protein kinase (623, 624) also occurs. Under specific conditions in certain cell types, the TRH receptor also couples G_{i2} and G_{i3} and to a G_s-like protein that does not activate adenylate cyclase (625). These complexes are less well studied and represent the minority of TRH signal transduction.

Of particular interest to the potential clinical usefulness of TRH or TRH analogs is the phenomeneon of TRH receptor desensitization. Pituitary TRH receptors after several hours exposure to TRH display markedly reduced TSH, but not PRL release. The IP3 response to TRH displays homologous desensitization in as little as 10 sec of TRH exposure in transfected HEK 293 cells (626). This occurs by rapid uncoupling of the receptor and a decrease in PPI-PLC activity. In the same model system, intracellular calcium mobilization displays heterologous acute desensitization, with effects on other receptors whose signal transduction also depends on calcium elevation (627). While most G protein-coupled receptors undergo acute desensitization by phosphorylation (628), this has not been demonstrated for TRH receptors. Neither a specific protein kinase nor calcium concentration have been clearly implicated in TRH receptor desensitization (625). Because G_{11}, G_q, and PPI-PLC have not been shown to be targets for desensitization, it still is believed some form of modification of the TRH receptor may explain acute desensitization. Not to be overlooked, if TRH exhibits slowed dissociation from the TRH-receptor complex, reactivation of the receptor cannot occur (629). Acute desensitization of the TRH receptor is also dependent on cell type, with pituitary cells displaying the most desensitization (630).

A second principal mechanism for TRH receptor desensitization is agonist-induced internalization (631). Up to 80% of TRH receptors are internalized by pituitary cells, with a half-time of 2–3 min (631). Thus, this mechanism is used by TRH receptors to a greater extent than for many other G protein-coupled receptors and may represent a significant mechanism for clearance of secreted TRH. However, recent studies indicate that desensitization does not depend upon internalization, *i.e.*, if internalization is blocked, receptor uncoupling can still mediate desensitization (629). This is similar to the angiotensin II and muscarinin M3 receptors that are also coupled to G_q and G_{11}. While the TRH receptor undergoes internalization and recyclization without ligand binding, this "housekeeping" function is slow relative to TRH-induced endocytosis (632). THR receptor-ligand complexes are internalized in clathrin-coated vesicles (632). A portion of the receptor is targeted to lysosomes, while the remainder is recycled to the cell surface. Similarly, the ligand may remain associated with the receptor to return to the cell surface, or if it dissociates intracellularly, will be degraded in lysosomes or, possibly, reach the cell surface as well (633). Internalization of the TRH receptor is dependent upon sequence motifs within its C terminus (634), as well as sequences within the second transmembrane region or third intracellular loop that are necessary for G protein coupling (633). Further, optimal rates of internalization appear to require coupling to $G_{q/11}$ and PPI-PLC (635).

Over longer periods of time, TRH receptor binding is also reduced by down-regulation of receptor number. TRH receptor down-regulation occurs in response to TRH, thyroid hormones, and agents that raise cAMP levels (625, 636). Like acute desensitization, receptor down-regulation is dependent upon the cell of expression, as determined in transfected cell experiments (637). The best characterized mechanism of reduced TRH receptor number is reduction in TRH-R1 mRNA levels in GH3 and GH4C1 cells treated with TRH (638, 639). TRH-R1 mRNA regulation is tightly coupled to activation of the TRH signal transduction elements, protein kinase C, IP3, and intracellular calcium (638–640). Desensitization of TRH-R1 by cAMP is also mediated by protein kinase A. Conversely, a number of conditions, including hypothyroidism (641), and treatment with dexamethasone (639), estradiol (642), and cycloheximide elevate TRH-R1 mRNA. Both for TRH-R1 mRNA down- and up-regulation, direct effects on gene transcription rates can be demonstrated.

There is also considerable evidence for regulation of TRH receptor mRNA stability. In GH3 cells transfected with the TRH-R1 coding sequence under control of a cytomegalovirus promoter, TRH increases degradation of TRH-R1 mRNA (643). TRH-R1 mRNA degradation is induced in pituitary cell types by phorbol esters (644). Narayanan and co-workers (645) present evidence in GH_3 pituitary cells that TRH-R1 mRNA degradation is controlled both by *cis*-acting elements within the mRNA 3′-untranslated region and by induction of RNases. Cell transfection studies indicate that regulation of TRH-R1 mRNA degradation does not occur in nonpituitary cells (644, 646). Estradiol-induced increases in TRH receptor mRNA also appear to be mediated through reduction in TRH receptor mRNA degradation (642).

B. The prepro-TRH$_{160–169}$ (pST$_{10}$) receptor

Of the other pro-TRH-derived peptides, only prepro-$TRH_{160–169}$ has characterized receptor binding (647). Receptors for prepro-$TRH_{160–169}$ seem to be of a single class, with a higher affinity for [Tyr^0] pST_{10} (IC_{50} = 8.3 ± 1.2 nM) than the native pST_{10} (IC_{50} = 9.3 ± 1.2 μM). Recent studies indicate that pST_{10} receptors cosegregate with S-100 protein-positive cells in pituitary cultures, supporting their expression in the folliculo-stellate cells of the anterior pituitary. Binding sites for pST_{10} are developmentally regulated, with an increase from birth to weaning, and then a gradual decline to adult levels at postnatal day 60 (587). Signal transduction by these receptors is not yet characterized. Within the CNS, pST_{10} receptor binding is highest in pituitary, the hypothalamus, spinal cord, and olfactory bulb, as well as the hippocampus (648). Its receptor binding is very high, two-thirds of that in the pituitary, in urinary bladder, and vas deferens and in the heart and testis, at a level equivalent to the hypothalamus (588).

VII. TRH Degradation

The rapid degradation of TRH after release from cells represents a significant drawback in its potential use as a therapeutic agent. To overcome these difficulties, various analogs have been synthesized and evaluated by many group of investigators (181). Specific enzymes that act on TRH are found in many tissues including the brain, spinal cord, pituitary, liver, kidney, pancreas, adrenal glands, and blood. These enzymes not only inactivate TRH, but act in concert with biosynthetic processes to determine the steady state levels of TRH, and its metabolites, CHP and TRH-OH. The distribution and biological effects of TRH metabolites are discussed more fully in *Section III.B*.

Four key enzymes breakdown TRH: PAP I, PAP II, and thyroliberinase give rise to the stable cyclized metabolite CHP (also known as histidyl-proline-diketopiperazine or His-Pro-DKP), and prolyl endopeptidase gives rise to the deamidated free acid, TRH-OH (182). These enzyme pathways are shown schematically in Fig. 10. In the CNS, the soluble PAP I and prolyl endopeptidase, and the membrane-bound PAP II, are the principal enzymes acting to metabolize TRH (649). TRH degradation in serum and many peripheral tissues is through the serum enzyme thyroliberinase (172). Each enzyme is described below in more detail.

Pyroglutamyl aminopeptidase I (PAP I) (EC 3.4.19.3) is a soluble cysteine protease that removes the N-terminal pyroGlu residue from TRH (649). It also cleaves peptides such as LHRH, NT, and bombesin. Prolyl endopeptidase (EC 3.4.21.26) is a soluble serine protease that cleaves on the carboxyl side of the TRH proline residue to generate TRH-OH (649). It also acts on other neuropeptides, including LHRH, NT, and SP. These two enzymes are present in many of the same tissues, so that both CHP and TRH-OH can be generated from TRH.

Displaying greater substrate specificity is the ectoenzyme pyroglutamyl aminopeptidase II (PAP II) (EC 3.4.19.6) (650). Like PAP I, this 260-kDa metalloenzyme removes pyro-Glu from TRH. The distinguishing features of PAP II are its greater substrate specificity and being membrane bound. PAP II is present in CNS synaptosomal fractions, in adenohypophyseal plasma membrane, and liver and serum particulate fractions. Highest activity is observed in the hippocampus and cerebral cortex. PAP II has been identified in many species, being highest in rabbit CNS, and in most cases retains similar features. However, its substrate specificity in bovine synaptosomes, where it has been extensively characterized, is not as narrow as in other species (651). The localization of PAP II in the CNS is consistent with its proposed role in the degradation of synaptic TRH (652).

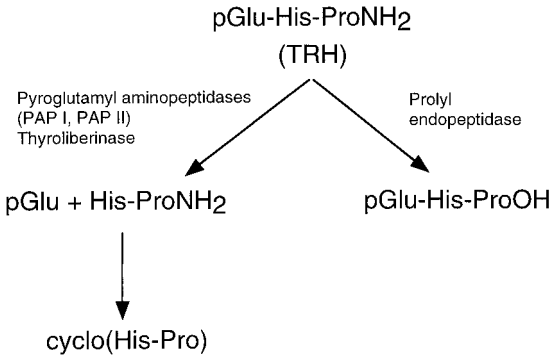


FIG. 10. Metabolic degradation of TRH by different TRH-degrading enzymes.

In the hypothalamus, PAP II activity is maximal at day 8 after birth, decreasing to adult values at day 45, while in the adenohypophysis it appears at day 8, peaks at day 30, and then decreases to adult values. In addition, thyroid hormone regulates the PAP II in the anterior pituitary but not in the brain (653, 654). In the PVN, TRH levels and PAP II activity do not correlate during pregnancy and lactation, indicating that PAP II is not the principal determinant of TRH levels (655). PAP II activity does vary with the estrous cycle (655). Furthermore, in brain areas other than the hypothalamus, PAP II activity decreases from days 9–20 coincident with increases in TRH and decreases in CHP (656), indicating PAP II activity can be a critical determinant of TRH steady state levels in some tissues. In sum, it appears that PAP II in areas under prominent endocrine control, such as the pituitary and PVN, subserves a different role than that in nonendocrine tissues.

Thyroliberinase, a fourth TRH-degrading enzyme, present in serum, is similar to PAP II but does not have the transmembrane anchor of PAP II. Like PAP II, thyroliberinase displays greater substrate specificity than PAP I or prolyl endopeptidase (657, 658). Thyroliberinase may be regulated by thyroid hormone; TRH half-life ranges from about 2 min in the plasma of thyrotoxic animals to 6 min in hypothyroid animals. In humans, the half-life of TRH is similar (172).

In studies examining the ontogeny of TRH catabolizing enzymes in pancreas, PAP I and prolyl endopeptidase are detected at early stages of rat pancreatic development, while PAP II remains undetectable. PAP I-specific activity increases until day 3 and decreases after day 5, and prolyl endopeptidase levels peak at 20 days. Because this development does not parallel that seen for TRH levels, it appears that TRH levels in neonatal rat pancreas are principally determined by biosynthetic rates (659).

The physiological significance of the soluble enzymes PAP I and prolyl endopeptidase within the brain and spinal cord are unclear, since in the case of neurotransmitter inactivation, TRH is probably degraded outside the neuron by ectoenzymes located on the cell surface, or within lysosomes after endocytosis. Membrane-bound ectoenzymes that are specific for TRH, such as PAP II, are more logically located for hydrolysis of synaptically released peptides. Soluble enzymes are better situated to control degradation of TRH during its transport in the hypophyseal portal vessels and systemic TRH. The exact mechanisms that control the amount of TRH that ultimately reaches the pituitary remain to be elucidated.

VIII. Concluding Remarks

This review describes the latest knowledge on pro-TRH research by providing new insights into its biosynthesis and processing, the neuroendocrine regulation thereof, the biological actions of its products, and the signal transduction and catabolic pathways used by those products. We empha-

size the wide diversity of non-TRH pro-TRH-derived peptides that are now known, and the intracellular pathways they take to reach maturity. The recent information available on tissue-specific processing of pro-TRH in the brain adds a further dimension to our understanding of differential processing and its importance in generating biological diversity.

Understanding of the role of non-TRH pro-TRH-derived peptides represents an exciting new frontier in pro-TRH research. During biosynthesis, these sequences within the precursor may function as structural or targeting elements that guide the folding and sorting of pro-TRH and its larger intermediates so that subsequent processing and secretion are properly regulated. The unique anatomical distribution of the pro-TRH end products, as well as regulation of their levels by neuroendocrine or pharmacological manipulations, described in this review, argues that these peptides will have unique biological roles. Some of these roles, such as for prepro-$TRH_{160-169}$, will be within the HPT axis, while many others will be unrelated to traditional thyroid function. This review also gathers together an extensive array of data indicating that TRH can function far beyond the HPT axis and should command significant future effort as a focus to develop new therapeutics. These therapeutics, in the form of TRH analogs or nonprotein peptidomimetics, and perhaps using novel delivery systems, will advance our ability to develop TRH and non-TRH pro-TRH-derived peptide agonists and antagonists that can target pro-TRH-derived peptides functioning in specific tissues or brain loci. It is hoped that these new drugs might provide novel treatment approaches for some of today's most difficult health and societal issues, including drug abuse, depression, chronic pain disorders, and sequelae of CNS injury.

Acknowledgments

The authors wish to thank April R. Bartnick and Shernett Griffiths for their administrative assistance, and James I. Koenig, Maryland Psychiatric Research Center, University of Maryland School of Medicine, for his excellent suggestions and discussions.

References

1. **Scharrer E, Scharrer B** 1940 Secretory cells within the hypothalamus. In: The Hypothalamus. Association for Research in Nervous and Mental Disease. Hafner Publishing, New York, pp 170–194
2. **Harris GW** 1948 Neural control of the pituitary gland. Physiol Rev 28:139
3. **Fink G** 1976 The development of the releasing factor concept. Clin Endocrinol (Oxf) 5:245S–260S
4. **Burgus R, Dunn TF, Desiderio D, Vale W, Guillemin R** 1969 Derives polypeptidiques de syntheses doues d'activite hypophysiotrope TRF. Nouvelles observations. CR Acad Sci Hebd Seances Acad Sci D 269:226–228
5. **Boler J, Enzmann F, Folkers K, Bowers CY, Shally AV** 1969 The identity of chemical and hormonal properties of the thyrotropin releasing hormone and pyro-glutamyl-histidil-proline amide. Biochem Biophys Res Commun 37:705–710
6. **Guillemin R** 1978 Peptides in the brain: the new endocrinology of the neuron. Science 202:390–402
7. **Schally AV** 1978 Aspects of hypothalamic regulation of the pituitary gland: its implications for the control of reproductive processes. Science 202:18–28
8. **Harris AR, Christianson D, Smith MS, Fang SL, Braverman LE, Vagenakis AG** 1978 The physiological role of thyrotropin-releasing hormone in the regulation of thyroid-stimulating hormone and prolactin secretion in the rat. J Clin Invest 61:441–448
9. **Hall R, Amos J, Garry R, Buxton RL** 1970 Thyroid-stimulating hormone response to synthetic thyrotropin releasing hormone in man. Br Med J 2:274–277
10. **Morley JE** 1981 Neuroendocrine control of thyrotropin secretion. Endocr Rev 2:396–436
11. **Bowers CY, Friesen HG, Hwang P, Guyda HJ, Folkers K** 1971 Prolactin and thyrotropin release in man by synthetic pyroglutamyl-histidyl prolinamide. Biochem Biophys Res Commun 45: 1033–1041
12. **Wilber JF, Utiger RD** 1968 *In vitro* studies on mechanism of action of thyrotropin releasing factor. Proc Soc Exp Biol Med 127:488–490
13. **Takahara J, Arimura A, Schally AV** 1974 Stimulation of prolactin and growth hormone release by TRH infused into a hypophysial portal vessel. Proc Soc Exp Biol Med 146:831–835
14. **Griffiths EC** 1985 Thyrotrophin releasing hormone: endocrine and central effects. Psychoneuroendocrinology 10:225–235
15. **Metcalf G** 1974 TRH: a possible mediator of thermoregulation. Nature 252:310–311
16. **Hedner J, Hedner T, Jonason J, Lundberg D** 1981 Central respiratory stimulant effect by thyrotropin releasing hormone in the rat. Neurosci Lett 24:317–320
17. **Kubek MJ, Low WC, Sattin A, Morzorati SL, Meyerhoff JL, Larsen SH** 1989 Role of TRH in seizure modulation. Ann NY Acad Sci 553:286–303
18. **Tache Y, Stephens Jr RL, Ishikawa T** 1989 Central nervous system action of TRH to influence gastrointestinal function and ulceration. Ann NY Acad Sci 553:269–285
19. **Lechan RM** 1993 Update on thyrotropin-releasing hormone. Thyroid Today 16:1–11
20. **Valentijn K, Bunel DT, Liao N, Pelletier G, Vaudry H** 1991 Release of pro-thyrotropin-releasing hormone connecting peptides PS4 and PS5 from perifused rat hypothalamic slices. Neuroscience 44:223–233
21. **Roussel J-P, Hollande F, Bulant M, Astier H** 1991 A prepro-TRH connecting peptide (prepro-$TRH_{160-169}$) potentiates TRH-induced TSH release from rat perfused pituitaries by stimulating dihydropyridine- and omega-conotoxin-sensitive Ca^{2+} channels. Neuroendocrinology 54:559–565
22. **Yang H, Tache Y** 1994 PreproTRH-(160–169) potentiates gastric acid secretion stimulation by TRH microinjected into the dorsal motor nucleus of the vagus. Neurosci Lett 174:43–46
23. **Redei E, Hilderbrand H, Aird F** 1995 Corticotropin release-inhibiting factor is preprothyrotropin-releasing hormone (178–199). Endocrinology 136:3557–3563
24. **McGivern RF, Rittenhouse P, Aird F, Van de Kar LD, Redei E** 1997 Inhibition of stress-induced neuroendocrine and behavioral responses in the rat by prepro-thyrotropin releasing hormone 178–199. J Neurosci 17:4886–4894
25. **Nicholson WE, Orth DN** 1996 Preprothyrotropin-releasing hormone-(178–199) does not inhibit corticotropin release. Endocrinology 137:2171–2174
26. **Bruhn TO, Taplin JH, Jackson IMD** 1991 Hypothyroidism reduces content and increases *in vitro* release of pro-TRH peptides from the median eminence. Neuroendocrinology 53:511–515
27. **Legradi G, Rand WM, Hitz S, Nillni EA, Jackson IMD, Lechan RM** 1996 Opiate withdrawal increases proTRH gene expression in the ventrolateral column of the midbrain periaqueductal gray. Brain Res 729:10–19
28. **Gahn LG, Sevarino KA** 1996 Preprothyrotropin-releasing hormone mRNA in the rat central gray is strongly and persistently induced during morphine withdrawal. Neuropeptides 30:207–212
29. **Nillni EA, Sevarino KA, Jackson IMD** 1993 Processing of proTRH to its intermediate products occurs before the packing into secretory granules of transfected AtT20 cells. Endocrinology 132:1271–1277
30. **Nillni EA, Sevarino KA, Jackson IMD** 1993 Identification of the thyrotropin-releasing hormone prohormone and its post-translational processing in a transfected AtT20 tumoral cell line. Endocrinology 132:1260–1270
31. **Nillni EA, Friedman TC, Todd RB, Birch NP, Loh YP, Jackson**

IMD 1995 Pro-thyrotropin-releasing hormone processing by recombinant PC1. J Neurochem 65:2462–2472
32. **Seidah N, Marcinkiewicz M, Benjannet S, Gaspar L, Beaubien G, Mattei MG, Lazure C, Mbikay M, Chretien M** 1991 Cloning and primary sequence of a mouse candidate prohormone convertase PC1 homologous to PC2, furin, and Kex2: distinct chromosomal localization and messenger RNA distribution in brain and pituitary compared to PC2. Mol Endocrinol 5:111–122
33. **Smeekens SP, Steiner DF** 1990 Identification of a human insulinoma cDNA encoding a novel mammalian protein structurally related to the yeast dibasic processing protease Kex2. J Biol Chem 265:2997–3000
34. **Smeekens SP, Avruch AS, LaMendola J, Chan SJ, Steiner DF** 1991 Identification of a cDNA encoding a second putative prohormone convertase related to PC2 in AtT20 cells and islets of Langerhans. Proc Natl Acad Sci USA 88:340–344
35. **Friedman TC, Loh YP, Cawley NX, Birch NP, Huang SS, Jackson IMD, Nillni EA** 1995 Processing of pro-thyrotropin-releasing hormone (pro-TRH) by bovine intermediate lobe secretory vesicle membrane PC1 and PC2 enzymes. Endocrinology 136:4462–4472
36. **Nillni EA, Luo LG, Jackson IMD, McMillan P** 1996 Identification of the thyrotropin-releasing hormone precursor, its processing products and its coexpression with convertase 1 in primary cultures of hypothalamic neurons. Anatomic distribution of PC1 and PC2. Endocrinology 137:5651–5661
37. **Schaner P, Todd RB, Seidah NG, Nillni EA** 1997 Processing of prothyrotropin releasing hormone by the family of prohormone convertases. J Biol Chem 272:19958–19968
38. **Eipper BA, Mains RE** 1980 Structure and biosynthesis of proadrenocorticotropin/endorphin and related peptides. Endocr Rev 1:1–27
39. **Liston D, Patey G, Rossier J, Verbanck P, Vanderhaeghen JJ** 1984 Processing of proenkephalin is tissue-specific. Science 225:734–737
40. **Hall ME, Stewart JM** 1983 Substance P and behavior: opposite effects of N-terminal and C-terminal fragments. Peptides 4:763–766
41. **Sanger F** 1959 Chemistry of insulin. Science 129:1340–1345
42. **Sachs H, Takabatake Y** 1964 Evidence for a precursor in vasopressin biosynthesis. Endocrinology 75:943–952
43. **Steiner DF, Oyer PE** 1967 The biosynthesis of insulin and a probable precursor of insulin by a human islet cell adenoma. Proc Natl Acad Sci USA 57:473–480
44. **Steiner DF, Clark JL, Nolan C, Rubenstein AH, Margoliash E, Aten B, Oyer PE** 1969 Proinsulin and the biosynthesis of insulin. Recent Prog Horm Res 25:207–282
45. **Howell SL, Taylor KW** 1967 The secretion of newly synthesized insulin *in vitro*. Biochem J 102:922–930
46. **Steiner DF, Cunningham D, Spiegelman L, Aten B** 1967 Insulin biosynthesis: evidence for a precursor. Science 157:697–700
47. **Chretien M, Li CH** 1967 Isolation, purification, and characterization of γ-lipotropic hormone from sheep pituitary glands. Can J Biochem 45:1163–1174
48. **Seidah NG, Chretien M** 1992 Proprotein and prohormone convertases of the subtilisin family recent developments and future perspectives. Trends Endocrinol Metab 3:133–140
49. **Steiner DF** 1998 The proprotein convertases. Curr Opin Chem Biol 2:31–39
50. **Xin X, Varlamov O, Day R, Dong W, Bridgett MM, Leiter EH, Fricker LD** 1997 Cloning and sequencing analysis of cDNA encoding rat carboxypeptidase D. DNA Cell Biol 16:897–909
51. **Fricker LD, Berman YL, Leiter EH, Devi LA** 1996 Carboxypeptidase E activity is deficient in mice with the fat mutation. Effect on peptide processing. J Biol Chem 271:30619–30624
52. **Eipper B, May V, Cullen EI, Sato SM, Murthy ASN, Mains RE** 1987 Cotranslational and posttranslational processing in the production of bioactive peptides. In: Meltzer HY (ed) Psychopharmacology: The Third Generation of Progress. Raven Press, New York, pp 385–400
53. **Xu H, Shields D** 1993 Prohormone processing in the trans-Golgi network: endoproteolytic cleavage of prosomatostatin and formation of nascent secretory vesicles in permeabilized cells. J Cell Biol 122:1169–1184
54. **Tooze SA, Chanat E, Tooze J, Huttner WB** 1993 Secretory granule formation. In: Loh YP (ed) Mechanisms of Intracellular Trafficking and Processing of Proproteins. CRC Press, Inc., Boca Raton, FL, pp 158–177
55. **Burgess TL, Kelly RB** 1987 Constitutive and regulated secretion of proteins. Annu Rev Cell Biol 3:243–293
56. **Matsuuchi L, Kelly RB** 1991 Constitutive and basal secretion from the endocrine cell line, AtT-20. J Cell Biol 112:843–852
57. **Arvan P, Castle D** 1998 Sorting and storage during secretory granule biogenesis: looking backward and looking forward. Biochem J 332:593–610
58. **Dannies PS** 1999 Protein hormone storage in secretory granules: mechanisms for concentration and sorting. Endocr Rev 20:3–21
59. **Kim JH, Lingwood CA, Williams DB, Furuya W, Manolson MF, Grinstein S** 1996 Dynamic measurement of the pH of the Golgi complex in living cells using retrograde transport of the verotoxin receptor. J Cell Biol 134:1387–1399
60. **Chandra S, Kable EP, Morrison GH, Webb WW** 1991 Calcium sequestration in the Golgi apparatus of cultured mammalian cells revealed by laser scanning confocal microscopy and ion microscopy. J Cell Sci 100:747–752
61. **Chanat E, Huttner WB** 1991 Milieu-induced, selective aggregation of regulated secretory proteins in the *trans*-Golgi network. J Cell Biol 115:1505–1519
62. **Yoo SH** 1996 pH- and Ca++ dependent aggregation property of secretory vesicle matrix proteins and the potential role of chromogranins A and B in secretory biogenesis. J Biol Chem 271:1558–1565
63. **Song L, Fricker LD** 1995 Calcium- and pH-dependent aggregation of carboxypeptidase E. J Biol Chem 270:7063–7967
64. **Shennan KI, Taylor NA, Docherty K** 1994 Calcium- and pH-dependent aggregation and membrane association of the precursor of the prohormone convertase PC2. J Biol Chem 269:18646–18650
65. **Chanat E, Weiss U, Huttner WB** 1994 The disulfide bond in chromogranin B, which is essential for its sorting to secretory granules, is not required for its aggregation in the trans Golgi network. FEBS Lett 351:225–230
66. **Schmidt WK, Moore H-P** 1994 Synthesis and targeting of insulin-like growth factor-1 to the hormone storage granules in an endocrine cell line. J Biol Chem 269:27115–27124
67. **Kelly RB** 1985 Pathways of protein secretion in eukaryotes. Science 230:25–32
68. **Tam WW, Andreasson KI, Loh YP** 1993 The amino-terminal sequence of pro-opiomelanocortin directs intracellular targeting to the regulated secretory pathway. Eur J Cell Biol 62:294–306
69. **Creemers JW, Usac EF, Bright NA, Van de Loo J-W, Jansen E, Van de Ven WJM, Hutton JC** 1996 Identification of a transferable sorting domain for the regulated pathway in the prohormone convertase PC2. J Biol Chem 271:25284–25291
70. **Moore HH, Kelly RB** 1986 Re-routing of a secretory protein by fusion with human growth hormone sequences. Nature 321:443–446
71. **Stoller TJ, Shields D** 1989 The propeptide of preprosomatostatin mediates intracellular transport and secretion of α-globin from mammalian cells. J Cell Biol 108:1647–1655
72. **Sevarino KA, Stork P, Ventimiglia R, Mandel G, Goodman RH** 1989 Amino-terminal sequences of prosomatostatin direct intracellular targeting but not processing specificity. Cell 57:11–19
73. **Parmer RJ, Xi X-P, Wu H-J, Helman LJ, Petz LN** 1993 Secretory protein traffic: chromogranin A contains a dominant targeting signal for the regulated pathway. J Clin Invest 92:1042–1054
74. **Cool DR, Fenger M, Snell CR, Loh PY** 1995 Identification of the sorting signal motif within pro-opiomelanocortin for the regulated secretory pathway. J Biol Chem 270:8723–8729
75. **Chanat E, Weiss U, Huttner WB, Tooze SA** 1993 Reduction of the disulfide bond of chromogranin B (secretogranin I) in the trans-Golgi network causes its missorting to the constitutive secretory pathway. EMBO J 12:2159–2168
76. **Milgram SL, Mains RE, Eipper BA** 1996 Identification of routing determinants in the cytosolic domain of a secretory granule-associated integral membrane protein. J Biol Chem 271:17526–17535
77. **Klumperman J, Kuliawat R, Griffith JM, Geuze HJ, Arvan P** 1998 Mannose 6-phosphate receptors are sorted from immature secretory granules via adaptor protein AP-1, clathrin, and syntaxin 6-positive vesicles. J Cell Biol 141:359–371
78. **Cool DR, Normant E, Shen F, Chen H-C, Pannell L, Zhang Y, Loh**

YP 1997 Carboxypeptidase E is a regulated secretory pathway sorting receptor: genetic obliteration leads to endocrine disorders in CPE^{fat} mice. Cell 88:73–83

79. **Normant E, Loh YP** 1998 Depletion of carboxypeptidase E, a regulated secretory pathway sorting receptor, causes misrouting and constitutive secretion of proinsulin and proenkephalin, but not chromogranin A. Endocrinology 139:2137–2145
80. **Irminger J-C, Verchere CB, Meyer K, Halban PA** 1997 Proinsulin targeting to the regulated pathway is not impaired in carboxypeptidase E-deficient Cpe^{fat}/Cpe^{fat} mice. J Biol Chem 272:27532–27534
81. **Lacourse KA, Friis-Hansen L, Samuelson LC, Rehfeld JF** 1998 Altered processing of procholecystokinin in carboxypeptidase E-deficient fat mice: differential synthesis in neurons and endocrine cells. FEBS Lett 436:61–66
82. **Lechan RM, Wu P, Jackson IMD, Wolfe H, Cooperman S, Mandel G, Goodman RH** 1986 Thyrotropin-releasing hormone precursor: characterization in rat brain. Science 231:159–161
83. **Lechan RM, Wu P, Jackson IMD** 1986 Immunolocalization of the thyrotropin-releasing hormone prohormone in the rat central nervous system. Endocrinology 119:1210–1216
84. **Cruz IP, Nillni EA** 1996 Intracellular sites of prothyrotropin-releasing hormone processing. J Biol Chem 271:22736–22745
85. **Nillni EA, Leiter EH, Wetsel WC**, The mutation in carboxypeptidase e (CPE) in the fat/fat mouse is associated with a defect in processing the prohormone for thyrotropin-releasing hormone (TRH). In: Program of the 79th Annual Meeting of The Endocrine Society, Minneapolis, MN, 1997, p 203
86. **Sevarino KA, Wu P, Jackson IM, Roos BA, Mandel G, Goodman RH** 1988 Biosynthesis of thyrotropin-releasing hormone by a rat medullary thyroid carcinoma cell line. J Biol Chem 263:620–623
87. **Sevarino KA, Goodman RH, Spiess J, Jackson IMD, Wu P** 1989 Thyrotropin-releasing hormone (TRH) precursor processing: characterization of mature TRH and nonTRH peptides synthesized by transfected mammalian cells. J Biol Chem 264:21529–21535
88. **Wu P, Lechan RM, Jackson IMD** 1987 Identification and characterization of thyrotropin-releasing hormone precursor peptides in rat brain. Endocrinology 121:108–115
89. **Wu P, Jackson IM** 1988 Post-translational processing of thyrotropin-releasing hormone precursor in rat brain: identification of 3 novel peptides derived from proTRH. Brain Res 456:22–28
90. **Seidah NG, Chretien M, Day R** 1994 The family of substilisin/kexin like pro-protein and pro-hormone convertases: divergent or shared functions. Biochimie 76:197–209
91. **Nillni EA, Koenig JI, Aird F, Seidah NG, Bartnick A**, Prepro-TRH178–199 and two novel peptides derived from its processing are regulated during suckling. In: Program of the 81st Annual Meeting of The Endocrine Society, San Diego, CA, 1999 (Abstract p1–319), p 202
92. **Milgram SL, Mains RE** 1994 Differential effects of temperature blockade on the proteolytic processing of three secretory granule-associated proteins. J Cell Sci 107:737–745
93. **Lechan RM, Toni R** 1992 Thyrotropin-releasing hormone neuronal systems in the central nervous system. In: Nemeroff CB (ed) Neuroendocrinology. CRC Press, Boca Raton, FL, pp 279–329
94. **Ishikawa K, Taniguchi Y, Inoue K, Kurosumi K, Suzuki M** 1988 Immunocytochemical delineation of thyrotropic area: origin of thyrotropin-releasing hormone in the median eminence. Neuroendocrinology 47:384–388
95. **Lechan RM, Wu P, Jackson IMD** 1987 Immunocytochemical distribution in rat brain of putative peptides derived from thyrotropin-releasing hormone prohormone. Endocrinology 121:1879–1891
96. **Bulant M, Delfour A, Vaudry H, Nicolas P** 1988 Processing of thyrotropin-releasing hormone prohormone (pro-TRH) generates pro-TRH connecting peptides. Identification and characterization of prepro-TRH-(160–169) and prepro-TRH-(178–199) in the rat nervous system. J Biol Chem 263:17189–17196
97. **Bulant M, Roussel J-P, Astier H, Nicolas P, Vaudry H** 1990 Processing of thyrotropin-releasing hormone prohormone (pro-TRH) generates a biologically-active peptide, prepro-$TRH_{160–169}$, which regulates TRH-induced thyrotropin secretion. Proc Natl Acad Sci USA 87:4439–4443
98. **Seidah NG, Gaspar L, Mion P, Marcinkiewicz M, Mbikay M, Chretien M** 1990 cDNA sequence of two distinct pituitary proteins homologous to Kex2 and furin gene products: tissue-specific mRNAs encoding candidates for pro-hormone processing proteinases. DNA 9:789
99. **Seidah NG, Day R, Hamelin J, Gaspar A, Collard MW, Chretien M** 1992 Testicular expression of PC4 in the rat: molecular diversity of a novel germ cell-specific Kex2/subtilisin-like proprotein convertase. Mol Endocrinol 6:1559–1570
100. **Seidah NG, Hamelin J, Mamarbachi M, Dong W, Tadros H, Mbikay M, Chrétien M, Day R** 1996 cDNA structure, tissue distribution, and chromosomal localization of rat PC7, a novel mammalian proprotein convertase closest to yeast kexin-like proteinases. Proc Natl Acad Sci USA 93:3388–3393
101. **Constam DB, Calfon M, Robertson EJ** 1996 SPC4, SPC6 and the novel protease SPC7 are coexpressed with bone morphogenic proteins at distinct sites during embryogenesis. J Cell Biol 134:181–191
102. **Hook VY, Azaryan AV, Hwang SR, Tezapsidis N** 1994 Proteases and the emerging role of protease inhibitors in prohormone processing. FASEB J 8:1269–1278
103. **Seidah NG** 1995 The mammalian family of subtilisin/kexin-like proprotein convertases. In: Shinde U, Inouye M (eds) Intramolecular Chaperones and Protein Folding. R.G. Landes Cie, Austin, TX, pp 181–203
104. **Rouille Y, Duguay SJ, Lund K, Furuta M, Gong Q, Lipkind G, Oliva Jr AA, Chan SJ, Steiner DF** 1995 Proteolytic processing mechanisms in the biosynthesis of neuroendocrine peptides: the subtilisin-like proprotein convertases. Front Neuroendocrinol 16: 332–361
105. **Schafer MK, Day R, Cullinan WE, Chretien M, Seidah NG, Watson SJ** 1993 Gene expression of prohormone and proprotein convertases in the rat CNS: a comparative *in situ* hybridization analysis. J Neurosci 13:1258–1279
106. **Pu LP, Ma W, Barker JL, Loh YP** 1996 Differential coexpression of genes encoding prothyrotropin-releasing hormone (proTRH) and prohormone convertases (PC1 and PC2) in rat brain neurons: implications for differential processing of pro-TRH. Endocrinology 137:1233–1241
107. **Steiner DF, Smeekens SP, Ohagi S, Chan SJ** 1992 The new enzymology of precursor processing endoproteases. J Biol Chem 267: 23435–23438
108. **Smeekens SP, Montag AG, Thomas G, Albiges-Rizo C, Carroll R, Benig M, Phillips LA, Martin S, Ohagi S, Gardner P, Swift HH, Steiner DF** 1992 Proinsulin processing by the subtilisin-related proprotein convertases furin, PC2, and PC3. Proc Natl Acad Sci USA 89:8822–8826
109. **Breslin MB, Lindberg I, Benjannet S, Mathis JP, Lazure C, Seidah NG** 1993 Differential processing of proenkephalin by prohormone convertases 1(3) and 2 and furin. J Biol Chem 268:27084–27093
110. **Galanopoulou AS, Kent G, Rabbani SN, Seidah NG, Patel YC** 1993 Heterologous processing of prosomatostatin in constitutive and regulated secretory pathways. Putative role of the endoproteases furin, PC1, and PC2. J Biol Chem 268:6041–6049
111. **Brakch N, Galanopoulou AS, Patel YC, Boileau G, Seidah NG** 1995 Comparative proteolytic processing of rat prosomatostatin by the convertases PC1, PC2, furin, PACE4, and PC5 in constitutive and regulated secretory pathways. FEBS Lett 362:143–146
112. **Benjannet S, Rondeau N, Day R, Chretien M, Seidah NG** 1991 PC1 and PC2 are proprotein convertases capable of cleaving proopiomelanocortin at distinct pairs of basic residues. Proc Natl Acad Sci USA 88:3564–3568
113. **Thomas L, Leduc R, Thorne BA, Smeekens SP, Steiner DF, Thomas G** 1991 Kex2-like endoproteases PC2 and PC3 accurately cleave a model prohormone in mammalian cells: evidence for a common core of neuroendocrine processing enzymes. Proc Natl Acad Sci USA 88:5297–5301
114. **Guest PC, Arden SD, Bennett DL, Clark A, Rutherford NG, Hutton JC** 1992 The post-translational processing and intracellular sorting of PC2 in the islets of Langerhans. J Biol Chem 267:22401–22406
115. **Zhou A, Mains RE** 1994 Endoproteolytic processing of proopiomelanocortin and prohormone convertases 1 and 2 in neuroendocrine cells overexpressing prohormone convertases 1 or 2. J Biol Chem 269:17440–17447

116. **Bloomquist BT, Eipper BA, Mains RE** 1991 Prohormone-converting enzymes: regulation and evaluation of function using antisense RNA. Mol Endocrinol 5:2014–2024
117. **Rhodes CJ, Lincoln B, Shoelson SE** 1992 Preferential cleavage of des-31,32-proinsulin over intact proinsulin by the insulin secretory granule type II endopeptidase. Implication of a favored route for prohormone processing. J Biol Chem 267:22719–22727
118. **Sanchez E, Charli JL, Morales C, Corkidi G, Seidah NG, Joseph-Bravo P, Uribe RM** 1997 Expression of the proprotein convertase PC1 and PC2 mRNAs in thyrotropin-releasing hormone neurons of the rat paraventricular nucleus of hypothalamus. Brain Res 761: 77–86
119. **Decroly E, Vandenbranden M, Ruysschaert JM, Cogniaux J, Jacob GS, Howard SC, Marshall G, Kompellli A, Basak A, Jean F, Lazure C, Benjannet S, Chretien M, Day R, Seidah NG** 1994 The convertases furin and PC1 can both cleave the human immunodeficiency virus (HIV)-1 envelope glycoprotein gp160 into gp120 (HIV-1 SU) and gp41 (HIV-1 TM). J Biol Chem 269:12240–12247
120. **Zhu X, Muller L, Mains RE, Lindberg I** 1998 Structural elements of PC2 required for interaction with its helper protein 7B2. J Biol Chem 273:1158–1164
121. **Beinfeld MC** 1997 CCK biosynthesis and processing: recent progress and future challenges. Life Sci 61:2359–2366
122. **Paquet L, Massie B, Mains RE** 1996 Proneuropeptide Y processing in large dense-core vesicles: manipulation of prohormone convertase expression in sympathetic neurons using adenoviruses. J Neurosci 16:964–973
123. **Naggert JK, Fricker LD, Varlamov D, Nishina PM, Rouillie Y, Steiner DF, Carroll RJ, Paigen BJ, Leiter EH** 1995 Hyperinsulinemia in obese fat/fat mice associated with a carboxypeptidase E mutation which reduces enzyme activity. Nat Genet 10:135–142
124. **Toni R, Lechan RM** 1993 Neuroendocrine regulation of thyrotropin-releasing hormone (TRH) in the tuberoinfundibular system. J Endocrinol Invest 16:715–753
125. **Brownstein MJ, Eskay RL, Palkovits M** 1982 Thyrotropin-releasing hormone in the median eminence is in processes of paraventricular nucleus neurons. Neuropeptides 2:197
126. **Ceccatelli S, Eriksson M, Hokfelt T** 1989 Distribution and coexistence of corticotropin-releasing factor, neurotensin-, enkephalin-, cholecystokinin-, galanin- and vasoactive intestinal polypeptide/peptide histidine isoleucine-like peptides in the parvocellular part of the paraventricular nucleus. Neuroendocrinology 49:309–323
127. **Dong W, Seidel B, Marcinkiewicz M, Chretien M, Seidah NG, Day R** 1997 Cellular location of the prohormone convertases in the hypothalamic paraventricular and supraoptic nuclei: selective regulation of PC1 in corticotrophin-releasing hormone parvocellular neurons is mediated by glucocorticoids. J Neurosci 17:563–575
128. **Hokfelt T, Tsuruo Y, Ulfhake B, Culheim S, Arvidsson U, Foster GA, Schultzberg M, Schalling M, Arborelius M, Freedman J, Post C, Visser T** 1989 Distribution of TRH-like immunoreactivity with special reference to coexistence with other neuroactive compounds. Ann NY Acad Sci 553:76–105
129. **van den Pol AN** 1982 The magnocellular and parvocellular paraventricular nucleus of rat: intrinsic organization. J Comp Neurol 206:317–345
130. **Sawchenko PE, Swanson LW** 1983 The organization of forebrian afferents to the paraventricular and supraoptic nuclei of the rat. J Comp Neurol 218:121–144
131. **Sawchenko PE, Swanson LW** 1982 The organization of noradrenergic pathways from the brainstem to the paraventricular and supraoptic nuclei in the rat. Brain Res Rev 257:275–325
132. **Krulich L** 1982 Neurotransmitter control of thyrotropin secretion. Neuroendocrinology 35:139–147
133. **Terry LC** 1986 Regulation of thyrotropin secretion by central epinephrine system. Studies in the chronically cannulated rat. Neuroendocrinology 42:102–108
134. **Grimm Y, Reichlin S** 1973 Thyrotropin-releasing hormone (TRH): neurotransmitter regulation of secretion by mouse hypothalamic tissue *in vitro*. Endocrinology 93:626–631
135. **Tapia-Arancibia L, Arancibia S, Astier H** 1985 Evidence for alpha1-adrenergic stimulatory control of *in vitro* release of immunoreactive thyrotropin-releasing hormone from rat median eminence: *in vivo* corroboration. Endocrinology 116:2314–2319
136. **Bjorklund A, Lindvall O** 1986 Catecholaminergic brain stem regulatory systems. In: Mountcastle VB, Bloom FE, Geiger SR (eds) Handbook of Physiology, The Nervous System, Section I: Neurophysiology. American Physiological Society, Bethesda, MD, vol 4:155
137. **Schettini G, Quattrone A, Di Renzo G, Lombardi G, Preziosi P** 1979 Effect of 6-hydroxydopamine treatment on TSH secretion in basal and cold-stimulated conditions in the rat. Eur J Pharmacol 56:153–157
138. **Arancibia S, Tapia-Arancibia L, Astier H, Assenmacher I** 1989 Physiological evidence for alpha1-adrenergic facilitatory control of the cold-induced TRH release in the rat, obtained by push-pull cannulation of the median eminence. Neurosci Lett 100:169–174
139. **Price J, Grossman A, Besser GM, Rees LH** 1983 Dopaminergic control of the rat thyrotroph. Neuroendocrinology 36:125–129
140. **Maeda K, Frohman LA** 1980 Release of somatostatin and thyrotropin-releasing hormone from rat hypothalamic fragments *in vitro*. Endocrinology 106:1837–1842
141. **Andersson K, Eneroth P, Roos P** 1985 Effects of TRH and a rat TSH preparation on discrete hypothalamic and forebrain catecholamine nerve terminal networks in the hypophysectomized male rat. Eur J Pharmacol 111:295–307
142. **Lechan RM, Jackson IMD** 1982 Immunohistochemical localization of thyrotropin-releasing hormone in the rat hypothalamus and pituitary. Endocrinology 111:55–65
143. **Palkovits M, Tapia-Arancibia L, Kordon C, Epelbaum J** 1982 Somatostatin connections between the hypothalamus and the limbic system in the rat brain. Brain Res 250:223–228
144. **Kharchaturian H, Lewis ME, Tsou K, Watson SJ** 1985 Beta-endorphin, alpha-MSH, ACTH, and related peptides. In: Bjorklund A, Hokfelt T (eds) Handbook of Chemical Neuroanatomy. GABA and neuropeptides. Elsevier, Amsterdam, vol 4:273
145. **Ibata Y, Kawakami F, Fukui K, Obata-Tsuto HL, Tanaka M, Kuba T, Okamura H, Morimoto N, Yanaihara C, Yanaihara N** 1984 Light and electron microscopic immunocytochemistry of neurotensin-like immunoreactive neurons in the rat hypothalamus. Brain Res 302:221
146. **Ceccatelli S, Fahrenkrug J, Villar MJ, Hokfelt T** 1991 Vasoactive intestinal polypeptide/peptide histidine isoleucine immunoreactive neuron systems in the basal hypothalamus of the rat with special reference to the portal vasculature: an immmunohistochemical and *in situ* hybridization study. Neuroscience 43:483–502
147. **Sawchenko PE, Pfeiffer SW** 1988 Ultrastructural localization of neuropeptide Y and galanin immunoreactivity in the paraventricular nucleus of the hypothalamus in the rat. Brain Res 474:231–245
148. **Everitt BJ, Hokfelt T, Terenius L, Tatemoto T, Mutt V, Goldstein M** 1984 Differential co-existence of neuropeptide Y (NPY)-like immunoreactivity with catecholamuines in the central nervous system of the rat. Neuroscience 11:443–462
149. **Legradi G, Lechan RM** 1998 The arcuate nucleus is the major source for neuropeptide Y innervation of thyrotropin-releasing hormone neurons in the hypothalamic paraventricular nucleus. Endocrinology 139:3262–3270
150. **Heilig M, Vecsei L, Wahlestedt C, Alling C, Widerlov E** 1990 Effects of centrally administered neuropeptide Y (NPY) and NPY 13–36 on the brain monoaminergic system of the rat. J Neural Transm Gen Sect 79:193–208
151. **Flier JS, Maratos-Flier E** 1998 Obesity and the hypothalmus: novel peptides for new pathways. Cell 92:437–440
152. **Arancibia S, Tapia-Arancibia L, Roussel JP, Assenmacher I, Astier H** 1986 Effect of morphine on cold-induced TRH release from the median eminence of unanaesthetized rats. Life Sci 38: 59–66
153. **Sharp B, Morley JE, Carlson HE, Gordon J, Briggs J, Melmed S, Hershman JM** 1981 The role of opiates and endogenous opioid peptides in the regulation of rat TSH secretion. Brain Res 219:335–344
154. **Nillni EA, Legradi G, Lechan RM**, Opiate withdrawal (OW) regulates proTRH post-translational processing in the ventrolateral column of the midbrain periaqueductal gray (PAG). Program of the 81st Annual Meeting of The Endocrine Society, San Diego, CA, 1999 (Abstract P1–323), p 203
155. **Lee SL, Stewart K, Goodman RH** 1988 Structure of the gene en-

coding rat thyrotropin releasing hormone. J Biol Chem 263:16604–16609
156. **Yamada M, Radovick S, Wondisford FE, Nakayama Y, Weintraub BD, Wilber JF** 1990 Cloning and structure of human genomic DNA and hypothalamic cDNA encoding human preprothyrotropin-releasing hormone. Mol Endocrinol 4:551–556
157. **Hollenberg AN, Monden T, Flynn TR, Boers ME, Cohen O, Wondisford FE** 1995 The human thyrotropin-releasing hormone gene is regulated by thyroid hormone through two distinct classes of negative thyroid hormone response elements. Mol Endocrinol 9:540–550
158. **Balkan W, Tavianini MA, Gkonos PJ, Roos BA** 1998 Expression of rat thyrotropin-releasing hormone (TRH) gene in TRH-producing tissues of transgenic mice requires sequences located in exon 1. Endocrinology 139:252–259
159. **Andrisani OM, Dixon JE** 1990 Somatostatin gene regulation. Annu Rev Physiol 52:793–806
160. **Beato M** 1989 Gene regulation by steroid hormones. Cell 56:335–344
161. **Bruhn TO, Huang SS, Vaslet C, Nillni EA** 1998 Glucocorticoids modulate the biosynthesis and processing of prothyrotropin releasing-hormone (proTRH). Endocrine 9:143–152
162. **de Nadai F, Rovere C, Bidard JN, Laur J, Martinez J, Cuber JC, Kitabgi P** 1993 Biosynthesis and posttranslational processing of the neurotensin/neuromedin N precursor in the rat medullary thyroid carcinoma 6–23 cell line. Effect of dexamethasone. Endocrinology 132:1614–20
163. **Shields PP, Dixon JE, Glembotski CD** 1988 The secretion of atrial natriuretic factor (99–126) by culture cardiac myocytes is regulated by glucocorticoids. J Biol Chem 263:12619–12628
164. **Kain SR, Jen TI, Firestone GL** 1993 Glucocorticoid-regulated trafficking of mouse mammary tumor virus proteins in permeabilized hepatoma cells. Requirements of intracellular membrane transport for maturation of the cytoplasmic phosphorylated polyprotein. J Biol Chem 268:19640–19649
165. **Luo LG, Bruhn T, Jackson IMD** 1995 Glucocorticoids stimulate thyrotropin-releasing hormone gene expression in cultured hypothalamic neurons. Endocrinology 136:4945–4950
166. **Kakucska I, Qi Y, Lechan RM** 1995 Changes in adrenal status affect hypothalamic thyrotropin-releasing hormone gene expression in parallel with corticotropin-releasing hormone. Endocrinology 136: 2795–2802
167. **Kovacs KJ, Mezey E** 1987 Dexamethasone inhibits corticotropin-releasing factor gene expression in rat paraventricular nucleus. Neuroendocrinology 46:365–368
168. **Blake NG, Eckland DJ, Foster OJ, Lightman SL** 1991 Inhibition of hypothalamic thyrotropin-releasing hormone messenger ribonucleic acid during food deprivation. Endocrinology 129:2714–2718
169. **Heiman ML, Ahima RS, Craft LS, Schoner B, Stephens TW, Flier JS** 1997 Leptin inhibition of the hypothalamic-pituitary-adrenal axis in response to stress. Endocrinology 138:3859–3863
170. **Legradi G, Emerson CH, Ahima RS, Flier JS, Lechan RM** 1997 Leptin prevents fasting-induced suppression of prothyrotropin-releasing hormone messenger ribonucleic acid in neurons of the hypothalamic paraventricular nucleus. Endocrinology 138:2569–2576
171. **Nillni EA, Bartnick A, Harris M, Hollenberg A, Bjorbaek C, Flier JS**, Leptin regulates proTRH biosynthesis. Program of the 81st Annual Meeting of The Endocrine Society, San Diego, CA, 1999 (Abstract OR-361), p 112
172. **Scanlon MF, Toft AD** 1996 Regulation of thyrotropin secretion. In: Braverman LE, Utiger RD (eds) Werner and Ingbar's The Thyroid: A Fundamental and Clinical Text, ed 7. Lippincott-Raven Publishers, Philadelphia, pp 220–240
173. **Brabant G, Brabant A, Ranft U, Ocran K, Kohrle J, Hesch RD, von zur Muhlen A** 1987 Circadian and pulsatile thyrotropin secretion in euthyroid man under the influence of thyroid hormone and glucocorticoid administration. J Clin Endocrinol Metab 65:83–88
174. **Yamada M, Saga Y, Shibusawa N, Hirato J, Murakami M, Iwasaki T, Hashimoto K, Satoh T, Wakabayashi K, Taketo MM, Mori M** 1997 Tertiary hypothyroidism and hyperglycemia in mice with targeted disruption of the thyrotropin-releasing hormone gene. Proc Natl Acad Sci USA 94:10862–10867
175. **Jackson IMD, Reichlin S** 1974 Thyrotropin-releasing hormone (TRH): distribution in hypothalamic and extrahypothalamic brain tissues of mammalian and submammalian chordates. Endocrinology 95:854–862
176. **Winokur A, Utiger RD** 1974 Thyrotropin-releasing hormone: regional distribution in rat brain. Science 185:265–266
177. **Morley JE** 1979 Extrahypothalamic thyrotropin-releasing hormone (TRH) – its distribution and its functions. Life Sci 25:1539–1550
178. **Yarbrough GG** 1979 On the neuropharmacology of thyrotropin-releasing hormone (TRH). Prog Neurobiol 12:291–312
179. **Reichlin S** 1986 Neural functions of TRH. Acta Endocrinol (Copenh) S276:21–33
180. **Griffiths EC** 1986 Thyrotrophin-releasing hormone: new applications in the clinic. Nature 322:212–213
181. **O'Leary RM, O'Connor B** 1995 Thyrotropin-releasing hormone. J Neurochem 65:953–963
182. **Yanagisawa T, Prasad C, Peterkofsky A** 1980 The subcellular and organ distribution and natural form of histidyl-proline diketopiperazine in rat brain determined by a specific radioimmunoassay. J Biol Chem 255:10290–10294
183. **Yamada M, Shibusawa N, Hashida T, Satoh T, Monden T, Prasad C, Mori M** 1999 Abundance of cyclo (His-Pro)-like immunoreactivity in the brain of TRH-deficient mice. Endocrinology 140:538–541
184. **Prasad C** 1989 Thyrotropin-releasing hormone: biomedical significance. In: Metcalf G, Jackson I (eds). Neurobiology of Cyclo (His-Pro). New York Academy of Science, New York, vol. 553:232–251
185. **Mori M, Yamada M, Yamaguchi M, Suzuki M, Ohshima K, Kobayashi I, Kobayashi S** 1986 Cyclo (His-Pro), a metabolite of thyrotropin-releasing hormone: specific binding to rat liver membranes. Biochem Biophys Res Commun 134:443–451
186. **Horita A** 1998 An update on the CNS actions of TRH and its analogs. Life Sci 62:1443–1448
187. **Metcalf G, Dettnar PW** 1981 Is thyrotropin-releasing hormone an endogenous substance in the brain? Lancet 1:586–589
188. **Arnold R, Klingberg F, Schaker W** 1991 Systemically applied thyrotropin-releasing hormone (TRH) modifies spontaneous behavior of rats. Biomed Biochim Acta 50:1217–1224
189. **Nishino S, Arrigoni J, Shelton J, Kanbayashi T, Dement WC, Mignot E** 1997 Effects of thyrotropin-releasing hormone and its analogs on daytime sleepiness and cataplexy in canine narcolepsy. J Neurosci 17:6401–6408
190. **Koivusalo F, Paakkari I, Leppaluoto J, Karppanen H** 1979 The effect of centrally administered TRH on blood pressure, heart rate and ventilation in the rat. Acta Physiol Scand 106:83–86
191. **Breese GR, Cott JM, Cooper BR, Prange AJ, Lipton MA** 1974 Antagonism of ethanol narcosis by thyrotropin-releasing hormone. Life Sci 14:1053–1063
192. **French TA, Masserano JM, Weiner N** 1993 Influence of thyrotropin-releasing hormone and catecholaminergic interactions on CNS ethanol sensitivity. Alcohol Clin Exp Res 17:99–106
193. **Tache Y, Lis M, Collu R** 1977 Effects of thyrotropin-releasing hormone on behavioral and hormonal changes induced by β-endorphin. Life Sci 21:841–846
194. **Bhargava HN, Matwyshyn GA** 1980 Influence of thyrotropin-releasing hormone and histidyl-proline diketopiperazine on spontaneous locomotor activity and analgesia induced by delta 9-tetrahydrocannabinol in the mouse. Eur J Pharmacol 68:147–154
195. **Glue P, Bailey J, Wilson S, Hudson A, Nutt DJ** 1992 Thyrotropin-releasing hormone selectively reverses lorazepam-induced sedation but not flowing of saccadic eye movements. Life Sci 50:PL25–PL30
196. **Sharp T, Tulloch IF, Bennett GW, Marsden CA, Metcalf G, Dettmar PW** 1984 Analeptic effects of centrally-injected TRH and analogues of TRH in the pentobarbitone-anaesthetized rat. Neuropharmacology 23:339–348
197. **Hashimoto T, Wada T, Fukuda N, Nagaoka A** 1993 Effect of thyrotropin-releasing hormone on pentobarbitone-induced sleep in rats: continuous treatment with a sustained release injectable formulation. J Pharm Pharmacol 45:94–97
198. **Lighton C, Bennett GW, Marsden CA** 1986 Chronic infusion of endogenous thyrotropin-releasing hormone (TRH) in brain alters

the behavioral response to pentobarbital and a TRH analogue. Brain Res 378:385–389
199. **Prasad C, Matsui T, Peterkofsky A** 1977 Antagonism of ethanol narcosis by histidyl-proline diketopiperazine [letter]. Nature 268: 142–144
200. **Peterkofsky A, Battani F, Koch Y, Takahara Y, Dannies P** 1982 Histidyl-proline diketopiperazine: its biological role as a regulatory peptide. Mol Cell Biochem 42:45–63
201. **Kalivas PW, Horita A** 1980 Thyrotropin-releasing hormone: neurogenesis of actions in the pentobarbital narcotized rat. J Pharmacol Exp Ther 212:203–210
202. **Morzorati S, Kubek MJ** 1993 Septal TRH in alcohol-naive P and NP rats and following alcohol challenge. Brain Res Bull 31:301–304
203. **Kalivas PW, Simasko SM, Horita A** 1981 Effect of septohippocampal lesions on thyrotropin-releasing hormone antagonism of pentobarbital narcosis. Brain Res 222:253–265
204. **Horita A, Carino MA, Lai H** 1986 Pharmacology of thyrotropin-releasing hormone. Annu Rev Pharmacol Toxicol 26:311–332
205. **Giovannini MG, Casamenti F, Nistri A, Paoli F, Pepeu G** 1991 Effect of thyrotropin-releasing hormone (TRH) on acetylcholine release from different brain areas investigated by microdialysis. Br J Pharmacol 102:363–368
206. **Brunello N, Cheney DL** 1981 The septo-hippocampal cholinergic pathway: role of antagonism of pentobarbital anesthesia and regulation by various afferents. J Pharmacol Exp Ther 219:489–495
207. **Santori EM, Schmidt DE, Kalivas PW, Horita A** 1981 Failure of muscarinic blockade to antagonize analepsis induced by thyrotropin-releasing hormone and MK-771 in the rat. Psychopharmacology (Berl) 74:13–16
208. **Tanaka K, Ogawa N, Asanuma M, Kondo Y** 1997 Thyrotropin-releasing hormone prevents abnormalities of cortical acetylcholine and monoamines in mice following head injury. Regul Pept 70: 173–178
209. **Tanaka K, Ogawa N, Chou H, Mori A, Yanaihara N** 1992 Effects of thyrotropin-releasing hormone and its analogues on unconsciousness following head injury in mice. Regul Pept 38:129–133
210. **Bennett GW, Ballard TM, Watson CD, Fone KC** 1997 Effect of neuropeptides on cognitive function. Exp Gerontol 32:451–469
211. **Yamamoto M, Ozawa Y, Takeuchi H** 1993 Effects of YM-14673, a new thyrotropin-releasing hormone analogue, on impaired learning of passive avoidance in mice. Arch Int Pharmacodyn Ther 321:5–13
212. **Horita A, Carino MA, Zabawska J, Lai H** 1989 TRH-analog MK-771 reverses neurochemical and learning deficits in medial septal-lesioned rats. Peptides 10:121–124
213. **Ogasawara T, Nakagawa Y, Ukai Y, Tamura M, Kimura K** 1995 NS-3 (CG3703), a TRH analog, ameliorates scopolamine-induced memory disruption in rats. Pharmacol Biochem Behav 51:929–934
214. **Ballard TM, Hunter AJ, Bennett GW** 1996 Effect of a thyrotropin-releasing hormone analogue, RX77368, on AMPA-induced septal-hippocampal lesioned rats in an operant delayed non-matching to position test. Psychopharmacology (Berl) 127:265–275
215. **Itoh Y, Ogasawara T, Mushiroi T, Yamazaki A, Ukai Y, Kimura K** 1994 Effect of NS-3, a thyrotropin-releasing hormone analog, on *in vivo* acetylcholine release in rat brain: regional differences and its sites of action. J Pharmacol Exp Ther 271:884–890
216. **Ogasawara T, Itoh Y, Tamura M, Ukai Y, Yoshikuni Y, Kimura K** 1996 NS-3, a TRH-analog, reverses memory disruption by stimulating cholinergic and noradrenergic systems. Pharmacol Biochem Behav 53:391–399
217. **Kasparov SA, Chizh BA** 1992 The NMDA-receptor antagonist dizocilpine (MK-801) suppresses the memory facilitatory action of thyrotropin-releasing hormone. Neuropeptides 23:87–92
218. **Khan A, Mirolo MH, Claypoole K, Hughes D** 1993 Low-dose thyrotropin-releasing hormone effects in cognitively impaired alcoholics. Alcohol Clin Exp Res 17:791–796
219. **Khan A, Shim H, Mirolo MH, Horita A, Douglas R, Tucker GJ** 1992 TRH effects on arousal, locomotor activity and spontaneous alternation in ECS post-ictal state [letter]. Psychopharmacology (Berl) 106:570–571
220. **Lampe TH, Norris J, Risse SC, Owen-Williams E, Keenan T** 1991 Therapeutic potential of thyrotropin-releasing hormone and lecithin co-adminstration. In: Iqbal K, McLechlan DRC, Winbald B, Wisniewski HM (eds) Alzheimer's disease: Basic Mechanisms, Diagnosis and Therapeutic Strategies. John Wiley & Sons, Ltd., New York, pp 643–648
221. **Mellow AH, Aronson SM, Giordani B, Berent S** 1993 A peptide enhancement strategy in Alzheimer's disease: pilot study with TRH/physostigmine infusions. Biol Psychiatry 34:271–273
222. **Kalivas PW, Stanley D, Prange Jr AJ** 1987 Interaction between thyrotropin-releasing hormone and the mesolimbic dopamine system. Neuropharmacology 26:33–38
223. **McCown TJ, Breese GR** 1989 Mechanistic and functional divergence between thyrotropin-releasing hormone and RO15–4513 interactions with ethanol. Alcohol Clin Exp Res 13:660–663
224. **Miyamoto M, Nagawa Y** 1977 Mesolimbic involvement in the locomotor stimulant action of thyrotropin-releasing hormone (TRH) in rats. Eur J Pharmacol 44:143–152
225. **Sharp T, Bennett GW, Marsden CA, Tulloch IF** 1984 A comparison of the locomotor effects induced by centrally-injected TRH and TRH analogues. Regul Pept 9:305–315
226. **Shian LR, Wu MH, Lin MT, Ho LT** 1985 Hypothalamic involvement in the locomotor stimulant or satiety action of thyrotropin-releasing hormone and amphetamine. Pharmacology 30:259–265
227. **Agarwal RA, Rastogi RB, Singhal RL** 1976 Changes in brain catecholamines and spontaneous locomotor activity in response to thyrotropin-releasing hormone. Res Commun Chem Pathol Pharmacol 15:743–752
228. **Miyamoto M, Nagawa Y** 1987 Mesolimbic involvement in the locomotor stimulant action of thyrotropin-releasing hormone (TRH) in rats. Eur J Pharmacol 44:143–152
229. **Heal DJ, Sabbagh A, Youdim MB, Green AR** 1981 Behavioural effects of central and peripheral injection of various analogues and metabolites of thyrotropin-releasing hormone (TRH). Neuropharmacology 20:947–957
230. **Kerwin RW, Pycock CJ** 1979 Thyrotrophin-releasing hormone stimulates release of [^{3}H]dopamine from slices of rat nucleus accumbens *in vitro*. Br J Pharmacol 67:323–325
231. **Sharp T, Bennett GW, Marsden CA** 1982 Thyrotropin-releasing hormone analogues increase dopamine release from slices of rat brain. J Neurochem 39:1763–1766
232. **Funk D, Post RM, Pert A** 1997 Role of central dopaminergic and 5-hydroxytryptaminergic projections in the behavioral responses elicited by thyrotropin-releasing hormone in rats. Psychopharmacology (Berl) 133:356–362
233. **Ervin GN, Schmitz SA, Nemeroff CB, Prange Jr AJ** 1981 Thyrotropin-releasing hormone and amphetamine produce different patterns of behavioral excitation in rats. Eur J Pharmacol 72:35–43
234. **Lin MT, Chan HK, Chen CF, Teh GW** 1983 Involvement of both opiate and catecholaminergic receptors in the behavioral excitation provoked by thyrotropin-releasing hormone: comparisons with amphetamine. Neuropharmacology 22:463–469
235. **Nemeroff CB, Kalivas PW, Golden RN, Prange Jr AJ** 1984 Behavioral effects of hypothalamic hypophysiotropic hormones, neurotensin, substance P and other neuropeptides. Pharmacol Ther 24:1–56
236. **Drago F, Pulvirenti L, Spadaro F, Pennisi G** 1990 Effects of TRH and prolactin in the behavioral despair (swim) model of depression in rats. Psychoneuroendocrinology 15:349–356
237. **Reny-Palasse V, Constans M, Rips R** 1989 Potentiation by TRH of the effect of antidepressants in the forced-swimming test, involvement of dopaminergic and opioid systems. Br J Pharmacol 97:197–205
238. **Lighton C, Bennett GW, Marsden CA** 1985 Increase in levels and *ex vivo* release of thyrotropin-releasing hormone (TRH) in specific regions of the CNS of the rat by chronic treatment with antidepressants. Neuropharmacology 24:401–406
239. **Kastin AJ, Ehrensing RH, Schalch DS, Anderson MS** 1972 Improvement in mental depression with decreased thyrotropin response after administration of thyrotropin-releasing hormone. Lancet 2:740–742
240. **Prange Jr AJ, Lara PP, Wilson IC, Alltop LB, Breese GR** 1972 Effects of thyrotropin-releasing hormone in depression. Lancet 2:999–1002
241. **Loosen PT** 1988 TRH: behavioral and endocrine effects in man. Prog Neuropsychopharmacol Biol Psychiatry 12:S87–S117

242. **Joffe RT, Sokolov ST** 1994 Thyroid hormones, the brain, and affective disorders. Crit Rev Neurobiol 8:45–63
243. **Marangell LB, George MS, Callahan AM, Ketter TA, Pazzaglia PJ, L'Herrou TA, Leverich GS, Post RM** 1997 Effects of intrathecal thyrotropin-releasing hormone (protirelin) in refractory depressed patients. Arch Gen Psychiatry 54:214–222
244. **Callahan AM, Frye MA, Marangell LB, George MS, Ketter TA, L'Herrou T, Post RM** 1997 Comparative antidepressant effects of intravenous and intrathecal thyrotropin-releasing hormone: confounding effects of tolerance and implications for therapeutics. Biol Psychiatry 41:264–272
245. **Sattin A** 1998 A heuristic model of mental depression derived from basic and applied research on thyrotropin-releasing hormone. Thyroid 8:957–962
246. **Kubek MJ, Rea MA, Hodes ZI, Aprison MH** 1983 Quantitation and characterization of thyrotropin-releasing hormone in vagal nuclei and other regions of the medulla oblongata of the rat. J Neurochem 40:1307–1313
247. **McCann MJ, Hermann GE, Rogers RC** 1989 Thyrotropin-releasing hormone: effects on identified neurons of the dorsal vagal complex. J Auton Nerv Syst 26:107–112
248. **Palkovits M, Mezy E, Eskay RL, Brownstein MJ** 1986 Innervation of the nucleus of the solitary tract and the dorsal vagal nucleus by thyrotropin-releasing hormone-containing raphe neurons. Brain Res 373:246–251
249. **Segerson TP, Hoefler H, Childers H, Wolfe HJ, Wu P, Jackson IMD, Lechan RM** 1987 Localization of thyrotropin-releasing hormone prohormone messenger ribonucleic acid in rat brain *in situ* hybridization. Endocrinology 121:98–107
250. **Helke CJ, Sayson SC, Keeler JR, Charlton CG** 1986 Thyrotropin-releasing hormone-immunoreactive neurons project from the ventral medulla to the intermediolateral cell column: partial coexistence with serotonin. Brain Res 381:1–7
251. **Harkness DH, Brownfield MS** 1986 A thyrotropin-releasing hormone-containing system in the rat dorsal horn separate from serotonin. Brain Res 384:323–333
252. **Appel NM, Wessendorf MW, Elde RP** 1987 Thyrotropin-releasing hormone in spinal cord: coexistence with serotonin and substance P in fibers and terminals apposing identified preganglionic sympathetic neurons. Brain Res 415:137–143
253. **Sharif NA, Towle AC, Burt DR, Mueller RA, Breese GR** 1989 Cotransmitters: differential effects of serotonin (5-HT)-depleting drugs on levels of 5-HT and TRH and their receptors in rat brain and spinal cord. Brain Res 480:365–371
254. **Brownstein MJ, Palkovits M, Saavedra JM, Bassiri RM, Utiger RD** 1974 Thyrotropin-releasing hormone in specific nuclei of rat brain. Science 185:267–269
255. **Flier JS, Foster DW** 1997 Eating disorders: obesity, anorexia nervosa, and bulimia nervosa. In: Wilson JD, Foster DW, Kronenberg HM, Larsen PR (eds) Williams Textbook of Endocrinology, ed 9. W.B. Saunders Co., Philadelphia, pp 1061–1098
256. **Vogel RA, Cooper BR, Barlow TS, Prange Jr AJ, Mueller RA, Breese GR** 1979 Effects of thyrotropin-releasing hormone on locomotor activity, operant performance and ingestive behavior. J Pharmacol Exp Ther 208:161–168
257. **Woods SC, Porte Jr D** 1978 The central nervous system, pancreatic hormones, feeding, and obesity. Adv Metab Disord 9:283–312
258. **Vijayan E, McCann SM** 1977 Suppression of feeding and drinking activity in rats following intraventricular injection of thyrotropin-releasing hormone (TRH). Endocrinology 100:1727–1730
259. **Lin MT, Chu PC, Leu SY** 1983 Effects of TSH, TRH, LH, and LHRH on thermoregulation and food and water intake in the rat. Neuroendocrinology 37:206–211
260. **Morley JE, Levine AS** 1980 Thyrotropin-releasing hormone (TRH) suppresses stress-induced eating. Life Sci 27:269–274
261. **Antelman SM, Eichler AJ, Black CA, Kocan D** 1980 Interchangeability of stress and amphetamine in sensitization. Science 207: 329–331
262. **Suzuki T, Kohno H, Sakurada T, Tadano T, Kisara K** 1982 Intracranial injection of thyrotropin-releasing hormone (TRH) suppresses starvation-induced feeding and drinking in rats. Pharmacol Biochem Behav 17:249–253
263. **Ishibashi S, Oomura Y, Okajima T** 1979 Facilitatory and inhibitory effects of TRH on lateral hypothalamic and ventromedial neurons. Physiol Behav 22:785–787
264. **Kow LM, Pfaff DW** 1991 The effects of the TRH metabolite cyclo (His-Pro) and its analogs on feeding. Pharmacol Biochem Behav 38:359–364
265. **Ishihara H, Mori M, Kobayashi I, Kobayashi S** 1985 Intraventricular administration of cyclo(His-Pro), a metabolite of thyrotropin-releasing hormone (TRH), decreases water intake in the rat. Proc Soc Exp Biol Med 178:623–628
266. **Shapiro RE, Miselis RR** 1985 The central organization of the vagus nerve innervating the stomach of the rat. J Comp Neurol 238:473–488
267. **Bieger D, Hopkins DA** 1987 Viscerotopic representation of the upper alimentary tract in the medulla oblongata in the rat: the nucleus ambiguus. J Comp Neurol 262:546–562
268. **Hornby PJ, Rossiter CD, Pineo SV, Norman WP, Friedman EK, Benjamin S, Gillis RA** 1989 TRH: immunocytochemical distribution in vagal nuclei of the cat and physiological effects of microinjection. Am J Physiol 257:G454–G462
269. **Manaker S, Rainbow TC, Winokur A** 1985 Thyrotropin-releasing hormone receptors: localization in rat and human central nervous system. In: Quantitative Receptor Autoradiography. A.R. Liss, New York, pp 103–135
270. **Mantyh PW, Hunt SP** 1985 Thyrotropin-releasing hormone (TRH) receptors. Localization by light microscopic autoradiography in rat brain using (3H)(3-Me-His2)TRH as the radioligand. J Neurosci 5:551–561
271. **Barber WD, Burks TF** 1983 Brain stem response to phasic gastric distension. Am J Physiol 245:G242–G248
272. **Appia F, Ewart WR, Pittam BS, Wingate DL** 1986 Convergence of sensory information from abdominal viscera in the rat brain stem. Am J Physiol 251:G169–G175
273. **Maeda-Hagiwara M, Watanabe K** 1983 Influence of dopamine receptor agonists on gastric acid secretion induced by intraventricular administration of thyrotropin-releasing hormone in the perfused stomach of anaesthetized rats. Br J Pharmacol 79:297–303
274. **Hernandez DE, Jennes L, Emerick SG** 1987 Inhibition of gastric acid secretion by immunoneutralization of endogenous brain thyrotropin-releasing hormone. Brain Res 401:381–384
275. **Tache Y, Lesiege D, Vale W, Collu R** 1985 Gastric hypersecretion by intracisternal TRH: dissociation from hypophysiotropic activity and role of central catecholamines. Eur J Pharmacol 107:149–155
276. **Szirtes T, Kisfaludy L, Palosi E, Szporny L** 1984 Synthesis of thyrotropin-releasing hormone analogues. 1. Complete dissociation of central nervous system effects from thyrotropin-releasing activity. J Med Chem 27:741–745
277. **Stephens RL, Ishikawa T, Weiner H, Novin D, Tache Y** 1988 TRH analogue, RX77368, injected into the dorsal vagal complex stimulates gastric secretion in rats. Am J Physiol 254:G639–G643
278. **White Jr RL, Rossiter CD, Hornby PJ, Harmon JW, Kasbekar DK, Gillis RA** 1991 Excitation of neurons in the medullary raphe increases gastric acid and pepsin production in cats. Am J Physiol 260:G91–G96
279. **Okuma Y, Osumi Y, Ishigawa T, Mitsuma T** 1987 Enhancement of gastric acid output and mucosal blood flow by tripeptide thyrotropin-releasing hormone microinjected into the dorsal motor nucleus of the vagus in rats. Jpn J Pharmacol 43:173–178
280. **Yang H, Ohning G, Tache Y** 1993 TRH in dorsal vagal complex mediates acid response to excitation of raphe pallidus neurons in rat. Am J Physiol 265:G880–G886
281. **Ishikawa T, Yang H, Tache Y** 1988 Medullary sites of action of the TRH analogue, RX77368, for stimulation of gastric acid secretion in the rat. Gastroenterology 95:1470–1476
282. **Brown MR** 1981 Thyrotropin-releasing factor: a putative CNS regulator of the autonomic nervous system. Life Sci 28:1789–1795
283. **Hawkins EF, Engel WK** 1985 Analog specificity of the thyrotropin-releasing hormone receptor in the central nervous system: possible clinical implications. Life Sci 36:601–611
284. **Tache Y, Yang H, Kaneko H** 1995 Caudal raphe-dorsal vagal complex peptidergic projections: role in gastric vagal control. Peptides 16:431–435
285. **Kaneko H, Tache Y** 1995 TRH in the dorsal motor nucleus of the

vagus in involved in gastric erosion induced by excitation of raphe pallidus in rats. Brain Res 699:97–102
286. **Varanasi S, Chi J, Stephens Jr RL** 1997 5-CT or DOI augments TRH-analog-induced gastric acid secretion at the dorsal vagal complex. Am J Physiol 273:R1607–R1611
287. **Yang H, Tache Y** 1997 Substance P in the dorsal vagal complex inhibits medullary TRH-induced gastric acid secretion in rats. Am J Physiol 272:G987–G993
288. **Kiraly A, Suto G, Guth PH, Tache Y** 1998 Peripheral mediators involved in gastric hyperemia to vagal activation by central TRH analog in rats. Am J Physiol 274:G170–G177
289. **Horita A, Carino MA, Pae Y-S** 1985 Blockade by naloxone and naltrexone of the TRH-induced stimulation of colonic transit in the rabbit. Eur J Pharmacol 108:289–293
290. **Garrick T, Buack S, Veiseh A, Tache Y** 1987 Thyrotropin-releasing hormone (TRH) acts centrally to stimulate gastric contractility in rats. Life Sci 40:649–657
291. **Lahann TR, Horita A** 1982 Thyrotropin-releasing hormone: centrally-mediated effects on gastrointestinal motor activity. J Pharmacol Exp Ther 222:66–70
292. **Lynn RB, Feng HS, Han J, Brooks FP** 1991 Gastric effects of thyrotropin-releasing hormone microinjected in the dorsal vagal nucleus in cats. Life Sci 48:1247–1254
293. **Rogers RC, Hermann GE** 1987 Oxytocin, oxytocin antagonist, TRH, and hypothalamic paraventricular nucleus stimulation effects on gastric motility. Peptides 8:505–513
294. **Heitkemper MM, Bond EF** 1991 Morphine inhibits TRH-induced gastric contractile activity. Regul Pept 36:99–109
295. **Heymann-Monnikes I, Tache Y, Trauner M, Weiner H, Garrick T** 1991 CRF microinjected into the dorsal vagal complex inhibits TRH analog- and kainic acid-stimulated gastric contractility in rats. Brain Res 554:139–144
296. **Heymann-Monnikes I, Livingston EH, Tache Y, Sierra A, Weiner H, Garrick T** 1990 Bombesin microinjected into the dorsal vagal complex inhibits TRH-stimulated gastric contractility in rats. Brain Res 533:309–314
297. **Morrow NS, Quinonez G, Weiner H, Tache Y, Garrick T** 1995 Interleukin-1 beta in the dorsal vagal complex inhibits TRH analogue-induced stimulation of gastric contractility. Am J Physiol 269:G196–G202
298. **Raybould HE, Jakobsen LJ, Novin D, Tache Y** 1989 TRH stimulation and L-glutamic acid inhibition of proximal gastric motor activity in the rat dorsal vagal complex. Brain Res 495:319–328
299. **Rogers RC, McCann MJ** 1989 Effects of TRH on the activity of gastric inflation-related neurons in the solitary nucleus in the rat. Neurosci Lett 104:71–76
300. **McCann MJ, Hermann GE, Rogers RC** 1989 Nucleus raphe obscurus (nRO) influences vagal control of gastric motility in rats. Brain Res 486:181–184
301. **Hornby PJ, Rossiter CD, White RL, Norman WP, Kuhn DH, Gillis RA** 1990 Medullary raphe: a new site for vagally mediated stimulation of gastric motility in cats. Am J Physiol 258:G637–G647
302. **Garrick T, Prince M, Yang H, Ohning G, Tache Y** 1994 Raphe pallidus stimulation increases gastric contractility via TRH projections to the dorsal vagal complex in rats. Brain Res 636:343–347
303. **Garrick T, Yang H, Trauner M, Livingston E, Tache Y** 1992 Thyrotropin-releasing hormone analog injected into the raphe pallidus and obscurus increases gastric contractility in rats. Eur J Pharmacol 223:75–81
304. **Krowicki ZK** 1996 Role of selected peptides in the vagal regulation of gastric motor and endocrine pancreatic function. J Physiol Pharmacol 47:399–409
305. **Sivarao DV, Krowicki ZK, Abrahams TP, Hornby PJ** 1997 Intracisternal antisense oligonucleotides to TRH receptor abolish TRH-evoked gastric motor excitation. Am J Physiol 272:G1372–G1381
306. **Morrow NS, Novin D, Garrick T** 1994 Microinjection of thyrotropin-releasing hormone in the paraventricular nucleus of the hypothalamus stimulates gastric contractility. Brain Res 644:243–250
307. **Morrow NS, Hodgson DM, Garrick T** 1996 Microinjection of thyrotropin-releasing hormone analogue into the central nucleus of the amygdala stimulates gastric contractility in rats. Brain Res 735: 141–148
308. **Ruckebusch Y, Malbert CH** 1986 Stimulation and inhibition of food intake in sheep by centrally-administered hypothalamic releasing factors. Life Sci 38:929–934
309. **Tonoue T, Furukawa K, Nomoto T** 1981 Transition from neurogenic to myogenic receptivity for thyrotropin-releasing hormone (TRH) in the duodenum of the neonatal rat. Endocrinology 108: 723–725
310. **Carino MA, Horita A** 1987 Localization of TRH-sensitive sites in rat brain mediating intestinal transit. Life Sci 41:2663–2667
311. **Lenz HJ, Silverman TA** 1995 Effects of cerebral TRH on intestinal water transport: role of vagal, muscarinic, and VIP pathways. Am J Physiol 269:G138–G143
312. **Smith JR, La Hann TR, Chesnut RM, Carino MA, Horita A** 1977 Thyrotropin-releasing hormone: stimulation of colonic activity following intracerebroventricular administration. Science 196:660–662
313. **Dolva O, Hanssen KF, Frey HM** 1978 Actions of thyrotropin-releasing hormone on gastrointestinal function in man. I. Inhibition of glucose and xylose absorption from the gut. Scand J Gastroenterol 13:599–604
314. **Dolva LO, Hanssen KF, Flaten O, Hanssen LE, von Schenck H** 1983 The effect of thyrotropin-releasing hormone (TRH) on pancreatic hormone secretion in normal subjects. Acta Endocrinol (Copenh) 102:224–230
315. **Amir S, Butler PD** 1988 Thyrotropin-releasing hormone blocks neurally-mediated hyperglycemia through central action. Peptides 9:31–35
316. **Leduque P, Bulant M, Dubois PM, Nicolas P, Vaudry H** 1989 Processing of thyrotropin-releasing hormone prohormone (pro-TRH) in the adult rat pancreas: identification and localization of pro-TRH-related peptides in beta-cells of pancreatic islets. Endocrinology 125:1492–1497
317. **Dutour A, Giraud P, Maltese JY, Becquet D, Pesce G, Salers P, Ouafik LH, Renard M, Oliver C** 1990 Regulation of TRH release by the cultured neonate rat pancreas. Peptides 11:1081–1085
318. **Ebiou JC, Bulant M, Nicolas P, Aratan-Spire S** 1992 Pattern of thyrotropin-releasing hormone secretion from the adult and neonatal rat pancreas: comparison with insulin secretion. Endocrinology 130:1371–1379
319. **Morley JE, Levin SR, Pehlevanian M, Adachi R, Pekary AE, Hershman JM** 1979 The effects of thyrotropin-releasing hormone on the endocrine pancreas. Endocrinology 104:137–139
320. **Fragner P, Presset O, Bernad N, Mertinez J, Roze C, Aratan-Spire S** 1997 A new biological contribution of cyclo(His-Pro) to the peripheral inhibition of pancreatic secretion. Am J Physiol 273:E1127–E1132
321. **Messmer B, Zimmerman FG, Lenz HJ** 1993 Regulation of exocrine pancreatic secretion by cerebral TRH and CGRP: role of VIP, muscarinic, and adrenergic pathways. Am J Physiol 264:G237–G242
322. **Okumura T, Taylor IL, Pappas TN** 1995 Mircoinjection of TRH analogue into the dorsal vagal complex stimulates pancreatic secretion in rats. Am J Physiol 269:G328–G334
323. **Kemmer TP, Duntas L, Munz W, Malfertheiner P** 1995 Inhibitory effect of thyrotropin-releasing hormone on enzyme secretion from isolated rat pancreatic acinar cells. Horm Metab Res 27:367–371
324. **Glasbrenner B, Malfertheiner P, Duntas L, Buchler M, Bereiter T, Ditschuneit H** 1990 Effects of TRH on pancreatic growth and secretion in rats. Peptides 5:37–41
325. **Yoneda M, Tamori K, Sato Y, Yokohama S, Nakamura K, Kono T, Makino I** 1997 Central thyrotropin-releasing hormone stimulates hepatic DNA synthesis in rats. Hepatology 26:1203–1208
326. **Tamori K, Yoneda M, Nakamura K, Makino I** 1998 Effect of intracisternal thyrotropin-releasing hormone on hepatic blood flow in rats. Am J Physiol 274:G277–G282
327. **McIntosh TK, Faden AI** 1986 Thyrotropin-releasing hormone (TRH) and circulatory shock. Circ Shock 18:241–258
328. **Koskinen LO** 1989 Effects of TRH on blood flow and the microcirculation. Ann NY Acad Sci 553:353–369
329. **Koskinen LO** 1991 Naloxone and TRH affect regional blood flows in the anesthetized rabbit. Peptides 12:1273–1277
330. **Shinozuka K, Shimoura K, Hattori K** 1997 Effects of thyrotropin-releasing hormone and its analogue, NS-3, on blood pressure, heart

rate, and serum catecholamine levels in rats. Gen Pharmacol 28: 209–214
331. **Okuda C, Mizobe T, Miyazaki M** 1987 The involvement of central cholinergic mechanisms in cardiovascular responses to intracerebroventricular and intravenous administration of thyrotropin-releasing hormone. Life Sci 40:1293–1299
332. **Jedrusiak J, Brus R, Kostrzewa RM, Slowinski Z** 1995 Dopaminergic neuronal systems modulate the central cardiovascular effects of TRH in rats. Pol J Pharmacol 47:43–52
333. **Koskinen LO, Bill A** 1985 Effects of thyrotropin-releasing hormone (TRH) on microvascular control systems. Acta Physiol Scand 124: 101
334. **Sumners C, Phillips MI, Richards EM** 1982 Central pressor action of neurotensin in conscious rats. Hypertension 4:888–893
335. **Seligsohn EE, Koskinen LO** 1991 Effects of alpha 2-adrenoreceptor blockade and thyrotropin-releasing hormone (TRH) on the cardiovascular system in the rabbit. Acta Physiol Scand 143:187–194
336. **Koskinen LO** 1986 Effects of TRH on cerebral and peripheral blood flows: role of submesencephalic brain stem centres. Acta Physiol Scand 128:277–288
337. **Bao LT, Mao TL** 1982 Effects of intracerebroventricular administration of thyrotropin-releasing hormone on cardiovascular function of the rat. Neuroendocrinology 35:173–177
338. **Mattila J, Bunag RD** 1986 Sympathetic vasoconstriction and renin secretion cause pressor response to thyrotropin-releasing hormone in rats. J Pharmacol Exp Ther 238:232–236
339. **Siren AL, Lake CR, Feuerstein C** 1988 Hemodynamic and neural mechanisms of action of thyrotropin-releasing hormone in the rat. Circulation Res 62:139–154
340. **Siren A-L, Feuerstein G** 1985 Effect of thyrotropin-releasing hormone on blood pressure and peripheral blood flow in conscious rats. Fed Proc 44:721
341. **Siren A-L, Powell E, Feuerstein G** 1986 Thyrotropin-releasing hormone in hypovolemia: a hemodynamic evaluation in the rat. Am J Physiol 250:H1093–H1101
342. **Feuerstein G, Hassen AH, Faden AI** 1983 TRH: cardiovascular and sympathetic modulation in brain nuclei of the rat. Peptides 4:617–620
343. **Helke CJ, Phillips ET** 1988 Thyrotropin-releasing hormone receptor activation in the spinal cord increases blood pressure and sympathetic tone to the vasculature and the adrenals. J Pharmacol Exp Ther 245:41–46
344. **Backman SB, Sequeira-Martinho H, Henry JL** 1990 Adrenal *vs.* nonadrenal sympathetic preganglionic neurons in the lower thoracic intermediolateral nucleus of the cat: effects of serotonin, substance P, and thyrotropin-releasing hormone. Can J Physiol Pharmacol 68:1108–1118
345. **Bunag RD, Mattila J** 1988 Selective suppression of pressor and sympathetic responses to centrally infused TRH in hypothyroid rats. J Cardiovasc Pharmacol 12:51–57
346. **Dolan DH, Nichols MF, Fletcher D, Schadt JC, Zoeller RT** 1992 Cold- and ethanol-induced hypothermia reduces cellular levels of mRNA-encoding thyrotropin-0releasing hormone in neurons of the preoptic area. Mol Cell Neurosci 3:425–432
347. **Mattila J, Bunag RD** 1990 Pressor and sympathetic responses to dorsal raphe nucleus infusions of TRH in rats. Am J Physiol 258: R1464–R1471
348. **Amir S** 1982 Opiate antagonists improve survival in anaphylactic shock. Eur J Pharmacol 80:161–162
349. **Lux Jr WE, Feuerstein G, Faden AI** 1983 Thyrotropin-releasing hormone reverses experimental anaphylactic shock through non-endorphin-related mechanisms. Eur J Pharmacol 90:301–302
350. **Long JB, Lake CR, Reid AA, Holaday JW** 1986 Effects of naloxone and thyrotropin-releasing hormone on plasma catecholamines, corticosterone, and arterial pressure in normal and endotoxemic rats. Circ Shock 18:1–10
351. **Amir S** 1984 Anti-anaphylactic action in the mouse of thyrotropin-releasing hormone (TRH) is mediated through beta 1-adrenoreceptive effectors. Neurosci Lett 46:127–130
352. **Holaday JW, Long JB, Martinez-Arizala A, Chen H-S, Reynolds DG, Gurll NJ** 1989 Effects of TRH in circulatory shock and central nervous system ischemia. Ann NY Acad Sci 553:370–379
353. **Gurll NJ, Holaday JW, Reynolds DG, Ganes E** 1987 Thyrotropin-releasing hormone: effects in monkeys and dogs subjected to experimental circulatory shock. Crit Care Med 15:574–581
354. **Liu LM, Hu DY, Chen HS, Lu RQ, Yan W** 1997 The importance of delta and kappa opioid receptors in the property of thyrotropin-releasing hormone against hemorrhagic shock. Shock 7:60–64
355. **Vergoni AV, Marrama D, Guarini S, Tagliavini S, Bazzani C, Maugeri A, Bertolini A** 1991 Afferent vagal fibers and central cholinergic mechanisms are involved in the TRH-induced reversal of hemorrhagic shock. Pharmacol Res 23:271–278
356. **Ono T, Ogawa N, Mori A** 1989 The effects of hemorrhagic shock on thyrotropin-releasing hormone and its receptors in discrete regions of rat brain. Regul Pept 25:215–222
357. **Mizobe T, Okuda C** 1988 Changes in brain thryotropin-releasing hormone in reversible and irreversible hemorrhagic shock in the rat. Circ Shock 26:245–256
358. **Dulchavsky SA, Lucas CE, Ledgerwood AM, Grabow D, Brown TR, Bagchi N** 1993 Triiodothyronine (T3) improves cardiovascular function during hemorrhagic shock. Circ Shock 39:68–73
359. **Garcia SI, Dabsys SM, Martinez VN, Delorenzi A, Santajuliana D, Nahmod VE, Finkielman S, Pirola CJ** 1995 Thyrotropin-releasing hormone hyperactivity in the preoptic area of spontaneously hypertensive rats. Hypertension 26:1105–1110
360. **Bhargava HN, Das S, Bansinath M** 1987 Proliferation of thyrotropin-releasing hormone receptors in specific brain regions during the development of hypertension in spontaneously hypertensive rats. Peptides 8:231–235
361. **Shrewsbury-Gee J, Lye RH, Latham A, Slater P** 1988 The effects of TRH analogs on cerebral ischemia produced by right middle cerebral artery occlusion in the rat. Exp Brain Res 70:342–350
362. **O'Shaughnessy CT, Rothwell NJ, Shrewsbury-Gee J** 1989 Effects of an analogue of thyrotropin-releasing hormone, RX77368, on infarct size and cerebral blood flow in focal cerebral ischemia in the rat. Can J Physiol Pharmacol 67:1345–1350
363. **Yamamoto M, Shimizu M, Kawabata S, Terai M** 1989 Effects of YM-14673, a new TRH analogue, on neurological deficits in rats with experimental cerebral hematoma. Arch Int Pharmacodyn Ther 299:55–64
364. **Guarini S, Gherardi S, Calabro G, Bertolini A** 1989 A pharmacological study of the cardiovascular effects of TRH-T in hemorrhagic shock in rats. Arch Int Pharmocodyn Ther 299:65–76
365. **Holaday JW, Faden AI** 1983 Thyrotropin-releasing hormone: autonomic effects upon cardiorespiratory function in endotoxic shock. Regul Pept 7:111–125
366. **Hedner T, Hedner J, Jonason J, Lundberg D** 1982 Respiratory effects of TRH in preterm rabbits. Pediatr Res 16:543–548
367. **Hedner J, Hedner T, Wessberg P, Lundberg D, Jonason J** 1983 Effects of TRH and TRH analogues on the central regulation of breathing in the rat. Acta Physiol Scand 117:427–437
368. **Horita A, Carino MA, Chesnut RM** 1976 Influence of thyrotropin-releasing hormone (TRH) on drug-induced narcosis and hypothermia in rabbits. Psychopharmacology 49:57–62
369. **Greer JJ, al-Zubaidy Z, Carter JE** 1996 Thyrotropin-releasing hormone stimulates perinatal rat respiration *in vitro*. Am J Physiol 271:R1160–R1164
370. **Dekin MS, Richerson GB, Getting PA** 1985 Thyrotropin-releasing hormone induces rhythmic bursting in neurons of the nucleus tractus solitarius. Science 229:67–69
371. **Hedner J, McCown TJ, Mueller RA, Hedner T, Jonason J, Breese GR** 1987 Respiratory stimulant effects by TRH into the mesencephalic region in the rat. Acta Physiol Scand 130:69–75
372. **Vonhof S, Siren AL, Feuerstein GZ** 1991 Central ventilatory effects of thyrotropin-releasing hormone in the conscious rat. Neuropeptides 18:93–98
373. **Sun QJ, Llewellyn-Smith I, Minson J, Arnolda L, Chalmers J, Pilowski P** 1996 Thyrotropin-releasing hormone immunoreactive boutons form close appositions with medullary expiratory neurons in the rat. Brain Res 715:136–144
374. **Sun QJ, Pilowsky P, Llewellyn-Smith IJ** 1995 Thyrotropin-releasing hormone inputs are preferentially directed towards respiratory motoneurons in rat nucleus ambiguus. J Comp Neurol 362:320–330
375. **Bayliss DA, Viana F, Talley EM, Berger AJ** 1997 Neuromodulation of hypoglossal motoneurons: cellular and developmental mechanisms. Respir Physiol 110:139–150

376. **Rekling JC, Champagnat J, Denavit-Saubie M** 1996 Thyrotropin-releasing hormone (TRH) depolarizes a subset of inspiratory neurons in the newborn mouse brain stem *in vitro*. J Neurophysiol 75:811–819
377. **Kharkevich DA, Chizh BA, Kasparov SA** 1991 Stimulant effect of thyrotropin-releasing hormone and its analog, RGH 2202, on the diaphragm respiratory activity, and their antagonism with morphine: possible involvement of N-methyl-D-aspartate receptors. Brain Res 551:110–115
378. **Uchida H, Nemoto H, Kinoshita M** 1986 Action of thyrotropin-releasing hormone (TRH) on the occurrence of fibrillation potentials and miniature end-plate potentials (MEPPs). An experimental study. J Neurol Sci 76:125–130
379. **Cream CL, Li A, Nattie EE** 1997 RTN TRH causes prolonged respiratory stimulation. J Appl Physiol 83:792–799
380. **Ikegami M, Polk D, Tabor B, Lewis J, Yamada T, Jobe AH** 1991 Corticosteroid and thyrotropin-releasing hormone effects on preterm sheep lung function. J Appl Physiol 70:2268–2278
381. **Ballard RA, Ballard PL, Cnaan A, Pinto-Martin J, Davis DJ, Padbury JF, Phibbs RH, Parer JT, Hart MC, Mannino FL, Sawai SK** 1998 Antenatal thyrotropin-releasing hormone to prevent lung disease in preterm infants. North American Thyrotropin-Releasing Hormone Study Group. N Engl J Med 338:493–498
382. **Torres JP, Salinas R, Gonzalez LH, Moya F** 1998 Collaborative Santiago Surfactant Group: collaborative trial of prenatal thyrotropin-releasing hormone and corticosteroids for prevention of respiratory distress syndrome. Am J Obstet Gynecol 178:33–39
383. **Nemeroff CB, Prange Jr AJ, Bissette G, Breese GR, Lipton MA** 1975 Thyrotropin-releasing hormone (TRH) and its β-alanine analogue: potentiation of the anticonvulsant potency of phenobarbital in mice. Psychopharm Comm 1:305–317
384. **Ogawa N, Hirose Y, Mori A, Kajita S, Sato M** 1985 Involvement of thyrotropin-releasing hormone (TRH) neural system of the brain in pentylenetetrazol-induced seizures. Regul Pept 12:249–256
385. **Mori N, Fukatsu T** 1992 Anticonvulsant effect of DN-1417, a derivative of thyrotropin-releasing hormone, and liposome-entrapped DN-1417, on amygdaloid-kindled rats. Epilepsia 33:994–1000
386. **Momiyama T, Ishihara K, Kimura K, Todo N, Fujita Y, Serikawa T, Sasa M** 1996 Long-term antiepileptic effects of chronic intake of CNK-602A, a thyrotropin-releasing hormone analogue, on spontaneously epileptic rats. Epilepsia 37:328–331
387. **Inanaga K, Kumashiro H, Fukuyama Y, Ohtahara S, Shirouzu M** 1989 Clinical study of oral adminstration of DN-1417, a TRH analog, in patients with intractable epilepsy. Epilepsia 30:438–445
388. **Takeuchi Y, Tominaga M, Mitsufuji N, Yamazoe I, Kawase S, Nishimura A, Matsuo S, Sawada T** 1995 Thyrotropin-releasing hormone in treatment if intractable epilepsy: neurochemical analysis of CSF monoamine metabolites. Pediatr Neurol 12:139–145
389. **Fink M** 1988 Fifty years of electroconvulsive therapy. Convul Therapy 4:2–4
390. **Lighton C, Marsden CA, Bennett GW, Minchin M, Green AR** 1984 Decrease in levels of thyrotropin-releasing hormone (TRH) in the n. accumbens and lumbar spinal cord following repeated electroconvulsive shock. Neuropharmacology 23:963–966
391. **Sattin A, Pekary AE, Lloyd RL** 1994 TRH gene products are implicated in the antidepressant mechanisms of seizures. Ann NY Acad Sci 739:135–153
392. **Knoblach SM, Kubek MJ** 1997 Increases in thyrotropin-releasing hormone messenger RNA expression induced by a model of human temporal lobe epilepsy: effect of partial and complete kindling. Neuroscience 76:85–95
393. **Knoblach SM, Kubek MJ** 1994 Thyrotropin-releasing hormone release is enhanced in hippocampal slices after electroconvulsive shock. J Neurochem 62:119–125
394. **Wan RQ, Noguera EC, Weiss SR** 1998 Anticonvulsant effects of intra-hippocampal injection of TRH in amygdala kindled rats. Neuroreport 9:677–682
395. **Hutson PH, Semark JE, Middlemiss DN** 1990 The TRH analogue MK-771 increases acetylcholine release in hippocampus but not striatum of the conscious rat. Neurosci Lett 116:149–155
396. **Yarbrough GG** 1976 TRH potentiates excitatory actions of acetylcholine on cerebral cortical neurons. Nature 263:523–524
397. **Okada M** 1991 Effects of a new thyrotropin-releasing hormone analogue, YM-14683, on the *in vivo* release of acetylcholine as measured by intracerebral dialysis in rats. J Neurochem 56:1544–1547
398. **Hagan JJ, Salamone JD, Simpson J, Iverson SD, Morris RG** 1988 Place navigation in rats is impaired by lesions of medial septum and diagonal band but not nucleus basalis magnocellularis. Behav Brain Res 27:9–20
399. **Mandel RJ, Chen AD, Connor DJ, Thal LJ** 1989 Continuous physostigmine infusion in rats with excitotoxic lesions of the nucleus basalis magnocellularis: effects on performance in the water maze task and cortical cholinergic markers. J Pharmacol Exp Ther 251: 612–619
400. **McNamara JO, Bonhaus DW, Shin C, Crain BJ, Gellman RL, Giacchino JL** 1985 The kindling model of epilepsy: a critical review. CRC Crit Rev Clin Neurobiol 1:341–391
401. **Rosen JB, Cain CJ, Weiss SR, Post RM** 1992 Alterations in mRNA of enkephalin, dynorphin and thyrotropin-releasing hormone during amygdala kindling: an *in situ* hybridization study. Brain Res Mol Brain Res 15:247–255
402. **Rosen JB, Kim SY, Post RM** 1994 Differential regional and time course increases in thyrotropin-releasing hormone, neuropeptide Y and enkephalin mRNAs following amygdala kindled seizure. Brain Res Mol Brain Res 27:71–80
403. **Clark M, Post RM, Weiss SRB, Cain CJ, Nakajima T** 1991 Regional expression of c-fos mRNA in rat brain during the evaluation of amygdala kindled seizures. Brain Res 11:55–64
404. **Rosen JB, Abramowitz J, Post RM** 1993 TRH mRNA and fos-like immunoreactivity are co-localized in limbic structures following amygdala kindled seizures: an *in situ* hybridization study. Mol Brain Res 15:247–255
405. **Szafarczyk A, Caracchini M, Rondouin G, Ixart G, Malaval F, Assenmacher I** 1986 Plasma ACTH and corticosterone responses to limbic kindling in the rat. Exp Neurol 92:583–590
406. **Rosen JB, Weiss SR, Post RM** 1994 Contingent tolerance to carbamazepine: alterations in TRH mRNA and TRH receptor binding in limbic structures. Brain Res 651:252–260
407. **Knoblach SM, Kubek MJ** 1997 Changes in thyrotropin-releasing hormone levels in hippocampal subregions induced by a model of human temporal lobe epilepsy: effect of partial and complete kindling. Neuroscience 76:97–104
408. **Kajita S, Ogawa N, Sato M** 1987 Long term increase in striatal thyrotropin-releasing hormone receptor binding caused by amygdaloid kindling. Epilepsia 28:228–223
409. **Kubek MJ, Knoblach SM, Sharif NA, Burt DR, Buterbaugh GG, Fuson KS** 1993 Thyrotropin-releasing hormone gene expression and receptors are differentially modified in limbic foci by seizures. Ann Neurol 33:70–76
410. **Racine RJ, McIntyre D** 1986 Mechanisms of kindling: a current view. In: Doane BK, Livingston KE (eds) The Limbic System: Functional Organization and Clinical Disorders. Raven Press, New York, pp 109–121
411. **Sharif NA** 1987 Adaptive changes in brain and pituitary TRH receptors: effects of lesions, kindling, hormones, drugs, and other factors. Med Sci Res 15:223–227
412. **Kreider MS, Wolfinger BL, Winokur A** 1990 Systemic administration of kainic acid produces elevations in TRH in rat central nervous system. Regul Pept 28:83–93
413. **Shapiro S, Kubek M, Sanders S, Durbin S, Goodwin S, Javed T** 1992 Regional changes in central nervous system thyrotropin-releasing hormone after pentylenetetrazol-induced seizures in dogs. Neurosurgery 31:935–939
414. **Kubek MJ, Shih TM, Meyerhoff JL** 1997 Thyrotropin-releasing hormone (TRH) is markedly increased in the rat brain following soman-induced convulsions. Brain Res 747:328–331
415. **Sharif NA, Burt DR** 1985 Limbic, hypothalamic, cortical and spinal regions are enriched in receptors for thyrotropin-releasing hormone: evidence from (^{3}H)ultrofilm autoradiography and correlation with central effects of the tripeptide in rat brain. Neurosci Lett 60:337–342
416. **Manaker S, Eichen A, Winokur A, Rhodes CH, Rainbow TC** 1986 Autoradiographic localization of thyrotropin-releasing hormone receptors in human brain. Neurology 36:641–646

417. **Piredda S, Gale K** 1985 A crucial epileptogenic site in the deep prepiriform cortex. Nature 317:623–625
418. **Renaud LP, Blume H, Pittman Q, Lamour Y, Tan A** 1979 Thyrotropin-releasing hormone selectively depresses glutamate excitation of cerebral cortical neurons. Science 205:1275–1277
419. **Koenig ML, Yourick DL, Meyerhoff JL** 1996 Thyrotropin-releasing hormone (TRH) attenuates glutamate-stimulated increases in calcium in primary neuronal cultures. Brain Res 730:143–149
420. **Dolphin AC, Errington ML, Bliss TV** 1982 Long-term potentiation of the perforant path *in vivo* is associated with increased glutamate release. Nature 297:496–498
421. **Hokfelt T, Fuxe K, Johansson O, Jeffcoate S, White N** 1975 Distribution of thyrotropin-releasing hormone (TRH) in the central nervous system as revealed with immunohistochemistry. Eur J Pharmacol 34:389–392
422. **Johansson O, Hokfelt T, Pernow B, Jeffcoate SL, White N, Steinbuch HW, Verhofstad AA, Emson PC, Spindel E** 1981 Immunohistochemical support for three putative transmitters in one neuron: coexistence of 5-hydroxytryptamine, substance P, and thyrotropin-releasing hormone-like immunoreactivity in medullary neurons projecting to the spinal cord. Neuroscience 6:1857–1881
423. **White SR** 1985 A comparison of the effects of serotonin, substance P and thyrotropin-releasing hormone on excitability of rat spinal motoneurones *in vivo*. Brain Res 335:63–70
424. **Hultborn H, Kiehn O** 1992 Neuromodulation of vertebrate motor neuron membrane properties. Curr Opin Neurobiol 2:770–775
425. **Kardon FC, Winokur A, Utiger RD** 1977 Thyrotropin-releasing hormone (TRH) in rat spinal cord. Brain Res 122:578–581
426. **Fone KC, Bennett GW, Marsden CA** 1987 Regional distribution of substance P- and thyrotropin-releasing hormone-like immunoreactivity and indoleamines in the rabbit spinal cord. J Neurochem 48:1027–1032
427. **Bennett GW, Nathan PA, Wong KK, Marsden CA** 1986 Regional distribution of immunoreactive thyrotropin-releasing hormone, substance P, and indoleamines in human spinal cord. J Neurochem 46:1718–1724
428. **Gilbert RFT, Bennett GW, Marsden CA, Emson PC** 1981 The effects of 5-hydroxytryptamin-depleting drugs on peptides in the ventral spinal cord. Eur J Pharmacol 76:203–210
429. **Lighton C, Marsden CA, Bennett GW** 1984 The effects of 5,7-dihydroxytryptamine and p-chlorophenylalanine on thyrotrophin-releasing hormone in regions of the brain and spinal cord of the rat. Neuropharmacology 23:55–60
430. **Wu W, Elde R, Wessendorf MW** 1993 Organization of the serotonergic innervation of spinal neurons in rats. III. Differential serotonergic innervation of somatic and parasympathetic preganglionic motoneurons as determined by patterns of co-existing peptides. Neuroscience 55:223–233
431. **Winokur A, Manaker S, Kreider MS** 1989 TRH and TRH receptors in the spinal cord. Ann NY Acad Sci 553:314–324
432. **Manaker S, Winokur A, Rhodes CH, Rainbow TC** 1985 Autoradiographic localization of thyrotropin-releasing hormone (TRH) receptors in human spinal cord. Neurology 35:328–332
433. **Nicoll RA** 1977 Excitatory action of TRH on spinal motoneurones. Nature 265:242–243
434. **Deshpande SB, Warnick JE** 1994 Analogs of thyrotropin-releasing hormone in potentiating the spinal monosynaptic reflex *in vitro*. Eur J Pharmacol 271:439–444
435. **Bayliss DA, Viana F, Berger AJ** 1992 Mechanisms underlying excitatory effects of thyrotropin-releasing hormone in rat hypoglossal motorneurons *in vivo*. J Neurophysiol 68:1733–1745
436. **Fisher ND, Nistri A** 1993 A study of the barium-sensitive and -insensitive components of the action of thyrotropin-releasing hormone on lumbar motor neurons of the rat isolated spinal cord. Eur J Neurosci 5:1360–1369
437. **Clarke KA, Parker AJ, Stirk GC** 1984 Motoneuron excitability during antidromically evoked inhibition after administration of a thyrotropin-releasing hormone (TRH) analogue. Neuropeptides 4:403–411
438. **Ono JI, Fukuda H** 1982 Ventral root depolarization and spinal reflex augmentation by a TRH analogue in rat spinal cord. Neuropharmacology 21:739–744
439. **White SR, Crane GK, Jackson DA** 1989 Thyrotropin-releasing hormone (TRH) effects on spinal cord neuronal excitability. Ann NY Acad Sci 553:337–350
440. **Van den Bergh P, Kelly Jr J, Soule N, Munsat TL, Jackson IMD, Lechan R** 1988 Spinal cord TRH deficiency is associated with incomplete recovery of denervated muscle in the rat. Neurology 38:452–458
441. **Schmidt-Achert KM, Askanas V, Engel WK** 1984 Thyrotropin-releasing hormone enhances choline acetyltransferase and creatinine kinase in cultured spinal ventral horn neurons. J Neurochem 43:586–589
442. **Askanas V, Engel WK, Kobayashi T** 1985 Thyrotropin-releasing hormone enhances motor neuron-evoked contractions of cultured human muscle. Ann Neurol 18:716–719
443. **Takahashi T** 1985 Thyrotropin-releasing hormone mimics descending slow synaptic potentials in rat spinal motoneurons. Proc R Soc Lond B Biol Sci 225:391–398
444. **White SR, Neuman RS** 1983 Pharmacological antagonism of facilitatory but not inhibitory effects of serotonin and norepinephrine on excitability of spinal motoneurons. Neuropharmacology 22:489–494
445. **Kinoshita K, Fukushima T, Kodama Y, Sugihara J, Yamamura M, Matsuoka Y** 1997 Chronic anti-ataxic actions of the novel thyrotropin-releasing hormone (TRH) analog, TA-0910, during and after repeated administration in Rolling mouse Nagoya: behavioral and pharmacokinetic studies. Biol Pharm Bull 20:36–39
446. **Kinoshita K, Watanabe Y, Asai H, Yamamura M, Matsuoka Y** 1995 Anti-ataxic effects of TRH and its analogue, TA-0910, in Rolling mouse Nagoya by metabolic normalization of the ventral tegumental area. Br J Pharmacol 116:3274–3278
447. **Yamamoto M, Shimizu M** 1989 Effect of YM-14673, a new thyrotropin-releasing hormone analogue, on ataxic gait in cytosine arabinoside-treated mice. Eur J Pharmacol 166:545–547
448. **Mano Y, Matsui K, Toyoshima E, Ando K** 1986 The pharmacological effect of thyrotropin-releasing hormone on ataxic mutant mice. Acta Neurol Scand 73:352–358
449. **Matsui K, Wada K, Kwak S** 1994 Ataxia-ameliorating effects of YM-14673, a potent analog of thyrotropin-releasing hormone, in ataxic mutant mice. Eur J Pharmacol 254:295–297
450. **Kinoshita K, Watanabe Y, Yamamura M, Matsuoka Y** 1998 TRH receptor agonists ameliorate 3-acetylpyridine-induced ataxia through NMDA receptors in rats. Eur J Pharmacol 343:129–133
451. **Bonuccelli U, Nuti A, Cei G, Rossi G, Grasso L, Martino E, Muratorio A** 1988 Oral thyrotropin-releasing hormone treatment in inherited ataxias. Clin Neuropharmacol 11:520–528
452. **Sobue I, Takayanagi T, Nakanishi T, Tsubaki T, Uono M, Kinoshita M, Igata A, Miyazaki M, Yoshida M, Ando K, Maruyama S, Mirsuma T, Nihei N, Sakuma A, Kato K** 1983 Controlled trial of thyrotropin-releasing hormone tartrate in ataxia of spinocerebellar degenerations. J Neurol Sci 61:235–248
453. **Brooks BR** 1989 A summary of the current position of TRH in ALS therapy. Ann NY Acad Sci 553:431–461
454. **Munsat TL, Taft J, Jackson IM, Andres PL, Hollander D, Skerry L, Ordman M, Kasdon D, Finison L** 1992 Intrathecal thyrotropin-releasing hormone does not alter the progressive course of ALS: experience with an intrathecal drug delivery system. Neurology 42:1049–1053
455. **Faden AI, Hill TG, Kubek MJ** 1986 Changes in TRH immunoreactivity in spinal cord after experimental spinal injury. Neuropeptides 7:11–18
456. **Salzman SK, Knight PB, Hirofuji E, Llados-Eckman C, Beckman AL, Winokur A** 1987 Spinal cord TRH is elevated after local traumatic injury. Peptides 8:247–250
457. **Vita G, Haun CK, Hawkins EF, Engel WK** 1991 Down-regulation of thyrotropin-releasing hormone (TRH) receptors in spinal cord after transection as revealed by quantitative autoradiography. Exp Brain Res 83:381–384
458. **Faden AI, Pilotte NS, Burt DR** 1986 Experimental spinal cord injury: effects of trauma or ischemia on TRH and muscarinic receptors. Neurology 36:723–726
459. **Zimmerman EM, Coffield JA, Miletic V, Brooks BR** 1985 Changes in substance P (sub P), 5-hydroxytryptamine (5-HT), and thyrotropin-releasing hormone (TRH)-like immunoreactivity (LI) in spi-

nal cord during murine neurotropic retrovirus-induced motor neuron degeneration. Neurology 35:157–158
460. **Court JA, McDermott JR, Gibson AM, Marschall E, Bloxham CA, Perry RH, Edwardson JA** 1987 Raised thyrotropin-releasing hormone, pyroglutamylamino peptidase, and proline endopeptidase are present in the spinal cord of wobbler mice but not in human motor neuron disease. J Neurochem 49:1084–1090
461. **Faden AI, Jacobs TP, Smith MT, Holaday JW** 1983 Comparison of thyrotropin-releasing hormone (TRH), naloxone, and dexamethasone treatments in experimental spinal injury. Neurology 33:673–678
462. **Faden AI, Vink R, McIntosh TK** 1989 Thyrotropin-releasing hormone and central nervous system trauma. Ann NY Acad Sci 553: 380–384
463. **Hashimoto T, Fukuda N** 1991 Effect of thyrotropin-releasing hormone on the neurological impairment in rats with spinal cord injury: treatment starting 24 h and 7 days after injury. Eur J Pharmacol 203:25–32
464. **Askanas V, Engel WK, Eagleson K, Micaglio G** 1989 Influence of TRH and TRH analogues RGH-2202 and DN-1417 on cultured ventral spinal cord neurons. Ann NY Acad Sci 553:325–336
465. **Iwasaki Y, Shiojima T, Tagaya N, Kobayashi T, Kinoshita M** 1997 TRH-analog, TA-0910 (3-methyl-(s)-5,6-dihydroorotyl-L-histidyl-L-prolinamide) rescues motor neurons from axotomy-induced cell death. Neurol Res 19:613–616
466. **Holtz A, Nystrom B, Gerdin B** 1989 Blocking weight-induced spinal cord injury in rats: effects of TRH or naloxone on motor function recovery and spinal cord blood flow. Acta Neurol Scand 80:215–220
467. **Ceylan S, Ilbay K, Baykal S, Ceylan S, Sener U, Ozmenoglu M, Kalelioglu M, Akturk F, Komsuoglu SS, Ozoron A** 1992 Treatment of acute spinal cord injuries: comparison of thyrotropin-releasing hormone and nimodipine. Res Exp Med (Berl) 192:23–33
468. **Salzman SK, Hirofuyi E, Knight PB, Llados-Eckman C, Beckman AL, Winokur A** 1987 Treatment of experimental spinal trauma with thyrotropin-releasing hormone: central serotonergic and vascular mechanisms of action. Cent Nerv Syst Trauma 4:181–196
469. **Faden AI, Sacksen I, Noble LJ** 1988 Structure activity relationships of TRH analogs in experimental spinal injury. Brain Res 448:287–293
470. **Fukuda N, Saji Y, Nagawa Y** 1979 [Behavioral and EEG alterations with brainstem compression and effect of thyrotropin-releasing hormone (TRH) in chronic cats.] Nippon Yakurigaku Zasshi 75: 321–331
471. **Manaka S, Sano K** 1978 Thyrotropin-releasing hormone tartrate (TRH-T) shortens concussion effects following head impact in mice. Neurosci Lett 8:255–258
472. **McIntosh TK, Vink R, Faden AI** 1988 An analogue of thyrotropin-releasing hormone improves outcome after brain injury: ^{31}P-NMR studies. Am J Physiol 254:R785–R792
473. **Redfern JS, O'Dorisio TM** 1993 Therapeutic uses of gastrointestinal peptides. Endocrinol Metab Clin North Am 22:845–873
474. **Pitts LH, Ross A, Chase GA, Faden AI** 1995 Treatment with thyrotropin-releasing hormone (TRH) in patients with traumatic spinal cord injuries. J Neurotrauma 12:235–243
475. **Cuenca E, Serrano MI, Gibert-Rahola J, Carrasco MS, Esteban JM** 1978 [The analgesic activity of TRH and MSH.] Arch Farmacol Toxicol 4:71–76
476. **Boschi G, Desiles M, Reny V, Rips R, Wrigglesworth S** 1983 Antinociceptive properties of TRH in mice: comparison with morphine. Br J Pharmacol 79:85–92
477. **Reny-Palasse V, Monier C, Rips R** 1989 Opioid involvement in TRH-induced antinociception in the rat following intracerebral administration. Pain 38:193–201
478. **Butler PD, Bodnar RJ** 1984 Potentiation of foot shock analgesia by thyrotropin-releasing hormone. Peptides 5:635–639
479. **Brewster D** 1983 Species variations in TRH inactivation: advantages of stable analogues. In: Griffiths EC, Bennett GW (eds) Thyrotropin Releasing Hormone. Raven Press, New York, pp 109–118
480. **Kawamura S, Sakurada S, Sakurada T, Kisara K, Sasaki Y, Suzuki K** 1985 The antinociceptive effects of histidyl-proline diketopiperazine and thyrotropin-releasing hormone in the mouse. Eur J Pharmacol 112:287–294
481. **Reny-Palasse V, Rips R** 1987 Antinociceptive activity of TRH metabolites in the mouse. Pain 30:259–269
482. **Bandler R, Shipley MT** 1994 Columnar organization in the midbrain periaqueductal gray: modules for emotional expression. Trends Neurosci 17:379–388
483. **Eskay RL, Long RT, Palkovits M** 1983 Localization of immunoreactive thyrotropin-releasing hormone in the lower brainstem of the rat. Brain Res 277:159–162
484. **Sharif NA** 1989 Quantitative autoradiography of TRH receptors in discrete brain regions of different mammalian species. Ann NY Acad Sci 553:147–175
485. **Robertson JA, Bodnar RJ** 1993 Site-specific modulation of morphine and swin-induced antinociception following thyrotropin-releasing hormone in the rat periaqueductal gray. Pain 55:71–84
486. **Webster VA, Griffiths EC, Slater P** 1983 Antinociceptive effects of thyrotropin-releasing hormone and its analogues in the rat periaqueductal grey region. Neurosci Lett 42:67–70
487. **Reny-Palasse V, Poncet J, Rips R** 1987 TRH-induced antinociception: interaction with the opioid systems? Gen Pharmacol 18:179–183
488. **Nie ZG, Liu ZY** 1991 Effect of injection of thyrotropin-releasing hormone into nucleus accumbens on pain discharges in nuclei parafascicularis of the thalamus in rats. J Tongji Med Univ 11:198–203
489. **Osbahr III AJ, Nemeroff CB, Luttinger D, Mason GA, Prange Jr AJ** 1981 Neurotensin-induced antinociception in mice: antagonism by thyrotropin-releasing hormone. J Pharmacol Exp Ther 217:645–651
490. **Bhargava HN, Yousif DJ, Matwyshyn GA** 1983 Interactions of thyrotropin-releasing hormone, its metabolites and analogues with endogenous and exogenous opiates. Gen Pharmacol 14:565–570
491. **Rips R, Reny V, Desiles M** 1983 Suppression of naloxone hyperalgesia by TRH. IRCS Med Sci 11:110–111
492. **Watkins LR, Suberg SN, Thurston CL, Culhane ES** 1986 Role of spinal cord neuropeptides in pain sensitivity and analgesia: thyrotropin-releasing hormone and vasopressin. Brain Res 362:308–317
493. **Heinricher MM, Rosenfeld JP** 1985 Microinjection of morphine into nucleus reticularis paragigantocellularis of the rat: suppression of noxious-evoked activity of nucleus raphe magnus neurons. Brain Res 359:388–391
494. **Liu ZY, Xiao HM, Liu CJ** 1989 Effect of intracerebroventricular injection of thyrotropin-releasing hormone on the nociceptive discharges in mesencephalic reticular formation in the rat. J Tongji Med Univ 9:251–256
495. **Korte SM, Jaarsma D, Luiten PG, Bohus B** 1992 Mesencephalic cuneiform nucleus and its ascending and descending projections serve stress-related cardiovascular responses in the rat. J Auton Nerv Syst 41:157–176
496. **Behbehani MM, Zemlan FP** 1990 Bulbospinal and intraspinal thyrotropin-releasing hormone systems: modulation of spinal cord pain transmission. Neuropeptides 15:161–168
497. **Chizh BA, Headley PM** 1996 Thyrotropin-releasing hormone facilitates spinal nociceptive responses by potentiating NMDA receptor-mediated transmission. Eur J Pharmacol 300:183–189
498. **Boschi G, Rips R** 1981 Effects of thyrotropin-releasing hormone injections into different loci of rat brain on core temperature. Neurosci Lett 23:93–98
499. **Bhargava HN** 1981 Dissociation of tolerance to the analgesic and hypothermic effects of morphine by using thyrotropin-releasing hormone. Life Sci 29:1015–1020
500. **Prasad C, Jacobs J, Wilber JF** 1980 Immunological blockade of endogenous thyrotropin-releasing hormone produces hypothermia in rats. Brain Res 193:580–583
501. **Hori T, Yamasaki M, Asami T, Koga H, Kiyohara T** 1988 Responses of anterior hypothalamic-preoptic thermosensitive neurons to thyrotropin-releasing hormone and cyclo(His-Pro). Neuropharmacology 27:895–901
502. **Chi ML, Lin MT** 1983 Involvement of adrenergic receptor mechanisms within hypothalamus in fever induced by amphetamine and thyrotropin-releasing hormone in the rat. J Neural Transm 58:213–222
503. **Ishikawa K, Suzuki M** 1986 Antagonism by thyrotropin-releasing

hormone (TRH) of pentobarbital-induced hypothermia in rats with brain lesions. Experientia 42:1029–1031
504. **Zoeller RT, Kabeer N, Albers HE** 1990 Cold exposure elevates cellular levels of messenger ribonucleic acid encoding thyrotropin-releasing hormone in paraventricular nucleus despite elevated levels of thyroid hormones. Endocrinology 127:2955–2962
505. **Ishikawa K, Kakegawa T, Suzuki M** 1984 Role of the hypothalamic paraventricular nucleus in the secretion of thyrotropin under adrenergic and cold-stimulated conditions in the rat. Endocrinology 114:352–358
506. **Carvalho SD, Kimura ET, Bianco AC, Silva JE** 1991 Central role of brown adipose tissue thyroxine 5′deiodinase on thyroid hormone-dependent thermogenic response to cold. Endocrinology 128:2149–2159
507. **Bird JA, Clarke L, Symonds ME** 1998 Influence of thyrotropin-releasing hormone on thermoregulation in newborn lambs. Biol Neonate 73:52–59
508. **Arancibia S, Tapia-Arancibia S, Assenmacher I** 1987 Effect of acute exposure to cold on SRIF release in unanesthetized rats and its temporal correlation with the cold-induced TRH peak. Exp Clin Endocrinol 6:265–268
509. **Arancibia S, Rage F, Astier H, Tapia-Arancibia L** 1996 Neuroendocrine and autonomous mechanisms underlying thermoregulation in cold environment. Neuroendocrinology 64:257–267
510. **Yang H, Wu SV, Ishikawa T, Tache Y** 1994 Cold exposure elevates thyrotropin-releasing hormone gene expression in medullary raphe nuclei: relationship with vagally-mediated gastric erosions. Neuroscience 61:655–663
511. **Siaud P, Tapia-Arancibia L, Szafarczyk A, Alonso G** 1987 Increase of thyrotropin-releasing hormone immunoreactivity in the nucleus of the solitary tract following bilateral lesions of the hypothalamic paraventricular nuclei. Neurosci Lett 79:47–52
512. **Broqua P, Laude D, Bluet-Pajot MT, Schmidt B, Baudrie V, Chaouloff F** 1993 Are 5-HT1A autoreceptors involved in the inhibitory effect of ipsapirone on cold-elicited thyrotropin secretion? Neuroendocrinology 57:640–647
513. **Fyda DM, Cooper KE, Veale WL** 1991 Modulation of brown adipose tissue-mediated thermogenesis by lesions to the nucleus tractus solitarius in the rat. Brain Res 546:203–210
514. **Cechetto DF, Saper CB** 1988 Neurochemical organization of the hypothalamic projection to the spinal cord in rat. J Comp Neurol 272:579–604
515. **Prasad C, Matsui T, Williams J, Peterkofsky A** 1978 Thermoregulation in rats: opposing effects of thyrotropin-releasing hormone and its metabolite histidyl-proline diketopiperazine. Biochem Biophys Res Commun 85:1582–1587
516. **Jacobs JJ, Prasad C, Wilber JF** 1982 Cyclo (His-Pro): mapping hypothalamic sites for its hypothermic action. Brain Res 250:205–209
517. **Bhargava HN** 1980 The effects of thyrotropin-releasing hormone and histidyl-proline diketopiperazine on δ-9-tetrahydrocannabinol-induced hypothermia. Life Sci 26:845–850
518. **Wise RA, Bozarth MA** 1987 A psychomotor stimulant theory of addiction. Psychol Rev 94:462–492
519. **Koob GF, Bloom FE** 1988 Cellular and molecular mechanisms of drug dependence. Science 242:715–723
520. **Trujillo KA, Herman JP, Schaffer MK-H, Monsour A, Meador-Woodruff JH, Watson SJ, Akil H** 1993 Drug reward and brain circuitry: recent advances and future directions. In: Korenman SG, Barchas JD (eds) Biological Basis of Substance Abuse. Oxford University Press, New York, pp 119–142
521. **Crespi F, Keane PE, Morre M** 1986 *In vivo* evaluation by differential pulse voltammetry of the effect of thyrotropin-releasing hormone (TRH) on dopaminergic and serotonergic synaptic activity in the striatum and nucleus accumbens of the rat. Exp Brain Res 62:329–334
522. **Kreutz MR, Acworth IN, Lehnert H, Wurtman RJ** 1990 Systemic administration of thyrotropin-releasing hormone enhances striatal dopamine release *in vivo*. Brain Res 536:347–352
523. **Przegalinski E, Jaworska L, Budziszewska B** 1993 The role of dopamine receptors in the release of thyrotropin-releasing hormone from the rat striatum and nucleus accumbens: an *in vitro* study. Neuropeptides 25:277–282
524. **Farwell AP, Braverman LE** 1996 Thyroid and antithyroid drugs. In: Hardman JG, Limbird LE, Molinoff PB, Ruddon RW, Gilman AG (eds) Goodman and Gilman's The Pharmacological Basis of Therapeutics, ed 9. McGraw Hill, New York, pp 1383–1409
525. **Piazza PV, Marinelli M, Jodogne C, Deroche V, Rouge-Pont F, Maccari S, LeMoal M, Simon H** 1994 Inhibition of corticosterone synthesis by metapyrone decreases cocaine-induced locomotion and relapse of cocaine self-administration. Brain Res 658:259–264
526. **Ortiz J, Fitzgerald LW, Lane S, Terwilliger R, Nestler EJ** 1996 Biochemical adaptations in the mesolimbic dopamine system in response to repeated stress. Neuropsychopharmacology 14:443–452
527. **Spindel ER, Pettibone DJ, Wurtman RJ** 1981 Thyrotropin-releasing hormone (TRH) content of rat striatum: modification by drugs and lesions. Brain Res 216:323–331
528. **Jaworska-Feil L, Budziszewska B, Lason W** 1995 The effects of repeated amphetamine administration on thyrotropin-releasing hormone level, its release and receptors in rat brain. Neuropeptides 29:171–176
529. **Sevarino KA, Primus RJ** 1993 Cocaine regulation of brain preprothyrotropin-releasing hormone mRNA. J Neurochem 60:1151–1154
530. **Breese GR, Cott JM, Cooper BR, Prange Jr AJ, Lipton MA, Plotnikoff NP** 1975 Effects of thyrotropin-releasing hormone (TRH) on the actions of pentobarbital and other centrally acting drugs. J Pharmacol Exp Ther 193:11–22
531. **Bhargava HN, Thorat SN** 1993 Effect of thyrotropin-releasing hormone on U-50,488H-induced pharmacological responses in mice. Brain Res 625:120–124
532. **Holaday JW, Tseng LF, Loh HH, Li CH** 1978 Thyrotropin-releasing hormone antagonizes β-endorphin hypothermia and catalepsy. Life Sci 22:1537–1544
533. **Das S, Bhargava HN** 1987 Unidirectional interaction between thyrotropin-releasing hormone and opiates at the level of their brain receptors. Gen Pharmacol 18:99–102
534. **Morley JE, Yamada T, Walsh JH, Lamers CB, Wong H, Shulkes A, Damassa DA, Gordon J, Carlson HE, Hershman JM** 1980 Morphine addiction and withdrawal alters brain peptide concentrations. Life Sci 26:2239–2244
535. **Rivier C, Vale W, Ling N, Brown M, Guillemin R** 1977 Stimulation *in vivo* of the secretion of prolactin and growth hormone by β-endorphin. Endocrinology 100:238–241
536. **Muraki T, Nakadate T, Tokunaga Y, Kato R** 1980 Effect of morphine on the release on thyroid-stimulating hormone stimulated by exposure to cold, thyroidectomy and the administration of thyrotropin-releasing hormone in male rats. J Endocrinol 86:357–362
537. **O'Brien CP** 1996 Drug addiction and drug abuse. In: Hardman JG, Limbird LE, Molinoff PB, Ruddon RW, Gilman AG (eds) Goodman and Gilman's The Pharmacological Basis of Therapeutics, ed 9. McGraw-Hill, New York, pp 557–580
538. **Koob GF, Maldonado R, Stinus L** 1992 Neural substrates of opiate withdrawal. Trends Neurosci 15:186–191
539. **Harris HW, Nestler EJ** 1993 Opiate regulation of signal transduction pathways. In: Hammer Jr RP (ed) The Neurobiology of Opiates. CRC Press, Ann Arbor, MI, pp 301–332
540. **Rasmussen K, Aghajanian GK** 1989 Withdrawal-induced activation of locus coeruleus neurons in opiate-dependent rats: attenuation by lesions of the nucleus paragigantocellularis. Brain Res 505:346–350
541. **Aghajanian GK, Kogan JH, Moghaddam B** 1994 Opiate withdrawal increases glutamate and aspartate efflux in the locus coeruleus. Brain Res 636:126–130
542. **Watson SJ, Trujillo KA, Herman JP, Akil H** 1989 Neuroanatomical and neurochemical substrates of drug-seeking behavior: overview and future direction. In: Goldstein A (ed) Molecular and Cellular Aspects of the Drug Addictions. Springer-Verlag, New York, pp 29–91
543. **Rasmussen K, Brodsky M, Inturrisi C** 1995 NMDA antagonists and clonidine block c-Fos expression during morphine withdrawal. Synapse 20:68–74
544. **Chieng B, Keay KA, Christie MJ** 1995 Increased Fos-like immunoreactivity in the periaqueductal gray of anaesthetized rats during opiate withdrawal. Neurosci Lett 183:79–82
545. **Bhargava HN, Matwyshyn GA** 1985 Comparative effects of thy-

rotropin-releasing hormone, MK-771 and DN-1417 on morphine abstinence syndrome. Psychopharmacology 87:141–146
546. **Fone KC, Johnson JV, Marsden CA, Bennett GW** 1989 Comparative behavioral and biochemical effects of repeated intrathecal administration of thyrotropin-releasing hormone (TRH) or two analogues of TRH in adult rats. Neuropharmacology 28:867–875
547. **Rezvani AH, Garbutt JC, Shimoda K, Garges PL, Janowsky DS, Mason GA** 1992 Attenuation of alcohol preference in alcohol-preferring rats by a novel TRH analogue, TA-0919. Alcohol Clin Exp Res 16:326–330
548. **Rezvani AH, Garbutt JC, Overstreet DH, Li L, Walker CH, Yang Y, Mason GA** 1997 Thyrotropin-releasing hormone analog TA-0910 suppresses alcohol intake in alcohol drinking African green monkeys. Alcohol Clin Exp Res 21:261–266
549. **Mason GA, Rezvani AH, Overstreet DH, Hamedi M, Walker CH, Yang Y, Garbutt JC** 1997 Involvement of dopamine D2 receptors in the suppressive effect of the thyrotropin-releasing hormone analog TA-0910 on alcohol intake in alcohol-preferring rats. Alcohol Clin Exp Res 21:1623–1629
550. **Witkin JM, Sickle J, Barrett JE** 1984 Potentiation of the behavioral effects of pentobarbital, chlordiazepoxide, and ethanol by thyrotropin-releasing hormone. Peptides 5:809–813
551. **Mason GA, Garbutt JC, Prange Jr AJ** 1995 Thyrotropin-releasing hormone. In: Bloom FE, Kupfer DJ (eds) Psychopharmacology: The Fourth Generation of Progress. Raven Press, Ltd., New York, pp 493–503
552. **Erickson JD, Masserano JM, Zoeller RT, Eskay RL, Weiner N** 1991 Differential responsiveness of the pituitary-thyroid axis to thyrotropin-releasing hormone in mouse lines selected to differ in central nervous system sensitivity to ethanol. Endocrinology 128:3013–3020
553. **Zoeller RT, Fletcher DL, Simonyl A, Rudeen PK** 1996 Chronic ethanol treatment reduces the responsiveness of the hypothalamic-pituitary-thyroid axis to central stimulation. Alcohol Clin Exp Res 20:954–960
554. **Zoeller RT, Rudeen PK** 1992 Ethanol blocks the cold-induced increase in thyrotropin-releasing hormone mRNA in paraventricular nuclei but not the cold-induced increase in thyrotropin. Brain Res Mol Brain Res 13:321–330
555. **Shimoda K, Mason GA, Walker CH, Garbutt JC, Prange Jr AJ** 1991 Administration of chlordiazepoxide affects (^{3}H)[3-methyl-histidyl(2)] thyrotropin-releasing hormone binding in rat brain. Peptides 12:199–202
556. **Goeders NE, Irby BD, Shuster CC, Guerin GF** 1997 Tolerance and sensitization to the behavioral effects of cocaine in rats: relationship to benzodiazepine receptors. Pharmacol Biochem Behav 57:43–56
557. **Goeders NE** 1997 A neuroendocrine role in cocaine reinforcement. Psychoneuroendocrinology 22:237–259
558. **Sharif NA, Burt DR** 1984 Modulation of receptors for thyrotropin-releasing hormone by benzodiazepines: brain regional differences. J Neurochem 43:742–746
559. **Simasko S, Horita A** 1984 Chlordiazepoxide displaces thyrotropin-releasing hormone (TRH) binding. Eur J Pharmacol 98:419–423
560. **Reichlin S, Saperstein R, Jackson IMD, Boyd III AE, Patel Y** 1976 Hypothalamic hormones. Annu Rev Physiol 38:389–424
561. **Jackson IMD** 1982 Thyrotropin-releasing hormone. N Engl J Med 306:145–155
562. **Morley JE, Levin SR, Pehlevanian M, Adachi R, Pekary AE, Hershman JM** 1979 The effects of thyrotropin-releasing hormone on the endocrine pancreas. Endocrinology 104:137–139
563. **Mitsuma T, Hirooka Y, Kayama K, Tomari Y, Nogimori T** 1994 Effects of hypophysectomy on TRH and its related peptide concentrations in various rat organs. Endocr Regul 28:125–131
564. **Dutour A, Bulant M, Giraud P, Nicolas P, Vaudry H, Oliver C** 1989 Pro-TRH-connecting peptides in the rat pancreas during ontogenesis. Peptides 10:523–527
565. **Lamberton P, Wu P, Jackson IM** 1985 Thyrotropin-releasing hormone release from rat pancreas is stimulated by serotonin but inhibited by carbachol. Endocrinology 117:1834–1838
566. **Pekary AE, Bhasin S, Smith V, Sugawara M, Swerdloff RS, Hershman JM** 1987 Thyroid hormone modulation of thyrotropin-releasing hormone (TRH) and TRH-Gly levels in the male rat reproductive system. J Endocrinol 114:271–277
567. **Montagne JJ, Ladram A, Grouselle D, Nicolas P, Bulant M** 1996 Identification and cellular localization of thyrotropin-releasing hormone-related peptides in rat testis. Endocrinology 137:185–191
568. **Pekary AE, Lukaski HC, Mena I, Smith SM, Bhasin S, Hershman JM** 1993 Testosterone increases TRH biosynthesis in epididymis but not heart of zinc-deficient rats. Peptides 14:315–324
569. **Fukusumi S, Ogi K, Onda H, Hinuma S** 1995 Distribution of thyrotropin-releasing hormone receptor mRNA in rat peripheral tissues. Regul Pept 57:115–121
570. **Shambaugh III G, Kubek M, Wilber JF** 1979 Thyrotropin-releasing hormone activity in the human placenta. J Clin Endocrinol Metab 48:483–486
571. **Farnsworth WE** 1993 TRH: mediator of prolactin in the prostate? Med Hypotheses 41:450–454
572. **Schaeffer JM, Brownstein MJ, Axelrod J** 1977 Thyrotropin-releasing hormone-like material in the rat retina: changes due to environmental lighting. Proc Natl Acad Sci USA 74:3579–3581
573. **Martino E, Nardi M, Vaudagna G, Simonetti S, Cilotti A, Piunchera A, Venturi G, Seo H, Baschieri L** 1980 Thyrotropin-releasing hormone-like material in human retina. J Endocrinol Invest 3:267–271
574. **Krupin T, Webb GW, Barbosa AT, Gulli B, Levine J, Becker B** 1984 Central effects of thyrotropin-releasing hormone and arginine vasopressin on intra-ocular pressure in rabbits. Invest Opthalmol Vis Sci 25:932–937
575. **Raiden S, Polack E, Nahmod V, Labeur M, Holsboer F, Arzt E** 1995 TRH receptor on immune cells: *in vitro* and *in vivo* stimulation of human lymphocyte and rat splenocyte DNA synthesis by TRH. J Clin Immunol 15:242–249
576. **Hart R, Wagner F, Steffens W, Lersch C, Dancygier H, Duntas L, Classen M** 1990 Effect of thyrotropin-releasing hormone on immune functions of peripheral blood mononuclear cells. Regul Pept 27:335–342
577. **Komorowski J, Stepien H, Pawlikowski M** 1993 The evidence of thyroliberin/triiodothyronine control of TSH secretory response from human peripheral blood monocytes cultured *in vitro*. Neuropeptides 25:31–34
578. **Haddad EE, Mashaly MM** 1990 Effect of thyrotropin-releasing hormone, triiodothyronine, and chicken growth hormone on plasma concentrations of thyroxine, triiodothyronine, growth hormone, and growth of lymphoid organs and leukocyte populations in immature male chickens. Poult Sci 69:1094–1102
579. **Socci R, Kolbeck RC, Meszaros LG** 1996 Positive inotropic effect of thyrotropin-releasing hormone on isolated rat hearts. Gen Physiol Biophys 15:309–316
580. **Wilber JF, Xu AH** 1998 The thyrotropin-releasing hormone gene: cloning, characterization, and transcriptional regulation in the central nervous system, heart, and testis. Thyroid 8:897–901
581. **Seethaler G, Chaminade M, Vlasak R, Ericsson M, Griffiths G, Toffoletto O, Rossier J, Stunnenberg HG, Kreil G** 1991 Targeting of frog prodermorphin to the regulated secretory pathway by fusion to proenkephalin. J Cell Biol 114:1125–1133
582. **Jackson IM, Wu P, Lechan RM** 1985 Immunohistochemical localization in the rat brain of the precursor for thyrotropin-releasing hormone. Science 229:1097–1099
583. **Mitsuma T, Hirooka Y, Nogimori T** 1993 Effects of dexamethasone on TRH, TRH-glycine, and prepro-TRH (178–199) levels in various rat organs. Endocr Regul 27:49–55
584. **Wondisford FE, Magner JA, Weintraub BD** 1996 Chemistry and biosynthesis of thyrotropin. In: Braverman LA, Utiger RD (eds) Werner and Ingbar's The Thyroid: A Fundamental and Clinical Text, ed 7. Lippincott-Raven Publishers, Philadelphia, pp 190–207
585. **Bulant M, Ladram A, Montagne J-J, Delfour A, Nicolas P** 1992 Isolation and amino acid sequence of the TRH-potentiating peptide from bovine hypothalamus. Biochem Biophys Res Commun 189: 1110–1118
586. **Ladram A, Bulant M, Montagne JJ, Nicolas P** 1994 Distribution of TRH-potentiating peptide (PS4) and its receptors in rat brain and peripheral tissues. Biochem Biophys Res Commun 200:958–965
587. **Valentijn K, Vandenbulcke F, Piek E, Beauvillain JC, Vaudry H** 1998 Distribution, cellular localization, and ontogeny of prepro-thyrotropin-releasing hormone-(160–169)(Ps4)-binding sites in rat pituitary. Endocrinology 139:1306–1313

588. **Ladram A, Montagne J-J, Bulant M, Nicolas P** 1994 Analysis of structural requirements for TRH-potentiating peptide receptor binding by analogue design. Peptides 15:429–433
589. **Pekary AE, Sattin A, Lloyd RL** 1997 Electroconvulsive seizures increase levels of PS4, the TRH-enhancing peptide [prepro-TRH(160–169)], in rat brain. Neuroendocrinology 65:377–384
590. **Pekary AE** 1998 Is Ps4 (preproTRH160–169) more than an enhancer for thyrotropin-releasing hormone? Thyroid 8:963–968
591. **Bulant M, Beauvillian JC, Delfour A, Vaudry H, Nicolas P** 1990 Processing of thyrotropin-releasing hormone (TRH) prohormone in the rat olfactory bulb generates novel TRH-related peptides. Endocrinology 127:1978–1985
592. **Redei E, Hilderbrand H, Aird F** 1995 Corticotropin release inhibiting factor is encoded within preproTRH. Endocrinology 136:1813–1816
593. **McGivern RF, Rittenhouse P, Aird F, Van de Kar LD, Redei E** 1997 Inhibition of stress-induced neuroendocrine and behavioral responses in the rat by prepro-thyrotropin-releasing hormone 178–199. J Neurosci 17:4886–4894
594. **Shigemasa C, Kouchi T, Ueta Y, Mitani Y, Yoshida A, Mashiba H** 1992 Evaluation of thyroid function in patients with isolated adrenocorticotropin deficiency. Am J Med Sci 304:279–284
595. **Brien TG** 1976 The adrenocortical status of patients with thyroid disease. Clin Endocrinol (Oxf) 5:97–99
596. **Joseph-Bravo P, Uribe RM, Vargas MA, Perez-Martinez L, Zoeller T, Charli JL** 1998 Multifactorial modulation of TRH metabolism. Cell Mol Neurobiol 18:231–247
597. **Van den Bergh P, Wu P, Jackson IM, Lechan RM** 1988 Neurons containing a N-terminal sequence of the TRH-prohormone (preproTRH53–74) are present in a unique location of the midbrain periaqueductal gray of the rat. Brain Res 461:53–63
598. **Lovick TA** 1991 In: Depaulis A, Bandler R (eds) The Midbrain Periaqueductal Gray Matter: Functional, Anatomical and Neurochemical Organization. Plenum Press, New York, pp 101–120
599. **Fardin V, Oliveras JL, Besson JM** 1984 A reinvestigation of the analgesic effects induced by stimulation of the periaqueductal grey matter in the rat. I. The production of behavioral side effects together with analgesia. Brain Res 306:105–123
600. **Lewis VA, Gebhart GF** 1977 Evaluation of the periaqueductal central gray (PAG) as a morphine-specific locus of action and examination of morphine-induced and stimulation-produced analgesia at coincident PAG loci. Brain Res 124:283–303
601. **Tortorici V, Vasquez E, Vanegas H** 1996 Naloxone partial reversal of the antinociception produced by dipyrone microinjected in the periaqueductal gray of rats. Possible involvement of medullary off- and on-cells. Brain Res 725:106–110
602. **Guillery RW, Feig SL, Lozsadi DA** 1998 Paying attention to the thalamic reticular nucleus. Trends Neurosci 21:28–32
603. **Simard M, Pekary AE, Smith VP, Hershman JM** 1989 Thyroid hormones modulate thyrotropin-releasing hormone biosynthesis in tissues outside the hypothalamic-pituitary axis of male rats. Endocrinology 125:524–531
604. **Yamada M, Iwasaki T, Satoh T, Monden T, Konaka S, Murakami M, Iruchijima T, Mori M** 1995 Activation of the thyrotropin-releasing hormone (TRH) receptor by a direct precursor of TRH, TRH-Gly. Neurosci Lett 196:109–112
605. **Mori M, Murakami M, Satoh T, Miyashita K, Iriuchijima T, Yamada M, Inukai T, Kobayashi I** 1990 A possible direct precursor of thyrotropin-releasing hormone, pGlu-His-Pro-Gly, stimulates prolactin secretion in anorexia nervosa. J Clin Endocrinol Metab 71:252–255
606. **Mori M, Murakami M, Iriuchijima T, Miyashita K, Satoh T, Monden T, Michimata T, Kobayashi I, Kobayashi S** 1990 Stimulation by a TRH precursor, TRH-Gly, of TSH and PRL secretion in rats: effect of starvation. Neuropeptides 16:57–62
607. **Mitsuma T, Hirooka Y, Nogimori T, Senda Y** 1989 Radioimmunoassay for thyrotropin-releasing hormone precursor peptide Lys-Arg-Gln-His-Pro-Gly-Arg-Arg. Exp Clin Endocrinol 93:53–60
608. **Wu P, Jackson IMD** 1989 Identification, characterization, and localization of thyrotropin-releasing hormone precursor peptides in perinatal rat pancreas. Ann NY Acad Sci 553:489–491
609. **Wu P, Jackson IMD** 1988 Identification, characterization, and localization of thyrotropin-releasing hormone precursor peptides in perinatal rat pancreas. Regul Pept 22:347–360
610. **Gkonos PJ, Tavianini MA, Liu CC, Roos BA** 1989 Thyrotropin-releasing hormone gene expression in normal thyroid parafollicular cells. Mol Endocrinol 3:2101–2109
611. **Mori M, Yamada M, Satoh T, Murakami M, Iriuchijima M, Kobayashi I** 1992 Different posttranslational processing of human preprothyrotropin-releasing hormone in the human placenta and hypothalamus. J Clin Endocrinol Metab 75:1535–1539
612. **Straub R, French G, Joho R, Gershengorn M** 1990 Expression cloning of a cDNA encoding the mouse pituitary thyrotropin-releasing hormone receptor. Proc Natl Acad Sci USA 87:9514–9518
613. **De La Pena P, Delgado L, Del Camino D, Barros F** 1992 Cloning and expression of the thyrotropin-releasing hormone receptor from GH_3 rat anterior pituitary cells. Biochem J 284:891–899
614. **Matre V, Karlsen H, Wright M, Lundell I, Fjeldheim A, Gabrielsen O, Larhammar D, Gautvik K** 1993 Molecular cloning of a functional human thyrotropin-releasing hormone receptor. Biochem Biophys Res Commun 195:179–185
615. **De La Pena P, Delgado L, Del Camino D, Barros F** 1992 Two isoforms of the thyrotropin-releasing hormone receptor generated by alternative splicing have indistinguishable functional properties. J Biol Chem 267:25703–25708
616. **Cao J, O'Donnell D, Vu H, Payza K, Pou C, Godbout C, Jakob A, Pelletier M, Lembo P, Ahmad S, Walker P** 1998 Cloning and characterization of a cDNA encoding a novel subtype of rat thyrotropin-releasing hormone receptor. J Biol Chem 273:32281–32287
617. **Itadani H, Nakamura T, Itoh J, Iwaasa H, Kanatani A, Borkowski J, Ihara M, Ohta M** 1998 Cloning and characterization of a new subtype of thyrotropin-releasing hormone receptors. Biochem Biophys Res Commun 250:68–71
618. **Hseih K, Martin T** 1992 Thyrotropin-releasing hormone and gonadotropin-releasing hormone receptors activate phospholipase C by coupling to the guanosine triphosphate-binding proteins Gq and G11. Mol Endocrinol 6:1673–1681
619. **Nelson E, Hinkle P** 1994 Thyrotropin-releasing hormone activates Ca^{2-} efflux. Evidence suggesting that a plasma membrane Ca2-pump is an effector for a G-protein-coupled Ca^{2-}-mobilizing receptor. J Biol Chem 269:30854–30860
620. **Kiley S, Parker P, Fabbro D, Jaken S** 1991 Differential regulation of protein kinase C isozymes by thyrotropin-releasing hormone in GH_4C_1 cells. J Biol Chem 266:23761–23768
621. **Jefferson A, Travis S, Schulman H** 1991 Activation of multifunctional Ca^{2-}/calmodulin-dependent protein kinase in GH_3 cells. J Biol Chem 266:1484–1490
622. **Cui Z, Gorelick J, Dannies P** 1994 Calcium/calmodulin-dependent protein kinase-II activation in rat pituitary cells in the presence of thyrotropin-releasing hormone and dopamine. Endocrinology 134:2245–2250
623. **Kanda Y, Koike K, Ohmichi M, Sawada T, Hirota K, Miyake A** 1994 A possible involvement of tyrosine kinase in TRH-induced prolactin secretion in GH_3 cells. Biochem Biophys Res Commun 199:1447–1452
624. **Ohmichi M, Sawada T, Kanda Y, Koike K, Hirota K, Miyake A, Saltiel A** 1994 Thyrotropin-releasing hormone stimulates MAP kinase activity in GH_3 cells by divergent pathways. Evidence of a role for early tyrosine phosphorylation. J Biol Chem 269:3783–3788
625. **Gershengorn MC, Osman R** 1996 Molecular and cellular biology of thyrotropin-releasing hormone receptors. Physiol Rev 76:175–191
626. **Yu R, Hinkle P** 1997 Desensitization of thyrotropin-releasing hormone receptor-mediated responses involves multiple steps. J Biol Chem 272:28301–29307
627. **Anderson L, Alexander C, Faccenda E, Eidne K** 1995 Rapid desensitization of the thyrotropin-releasing receptor expressed in single human embryonal kidney 293 cells. Biochem J 311:385–392
628. **Hausdorff W, Caron M, Lefkowitz R** 1990 Turning off the signal: desensitization of β-adrenergic receptor function. FASEB J 4:2881–2889
629. **Yu R, Hinkle P** 1998 Signal transduction, desensitization, and recovery of responses to thyrotropin-releasing hormone after inhibition of receptor internalization. Mol Endocrinol:737–749
630. **Falck-Pedersen E, Heinflink M, Alvira M, Nussenzveig D,**

Gershengorn M 1994 Expression of thyrotropin-releasing hormone receptors by adenovirus-mediated gene transfer reveals that thyrotropin-releasing hormone desensitization is cell specific. Mol Pharmacol 45:684–689
631. **Hinkle P, Shanshala II E** 1989 Pituitary thyrotropin-releasing hormone (TRH) receptors: effects of TRH, drugs mimicking TRH action, and chlordiazepoxide. Mol Endocrinol 3:1337–1344
632. **Ashworth R, Yu R, Nelson E, Dermer S, Gershengorn M, Hinkle P** 1995 Visualization of the thyrotropin-releasing hormone receptor and its ligand during endocytosis and recycling. Proc Natl Acad Sci USA 92:512–516
633. **Petrou C, Tashjian Jr A** 1995 Evidence that the thyrotropin-releasing hormone receptor and its ligand are recycled dissociated from each other. Biochemistry 306:107–113
634. **Nussenzveig D, Heinflink M, Gershengorn M** 1993 Agonist-stimulated internalization of the thyrotropin-releasing hormone receptor is dependent on two domains in the receptor carboxyl terminus. J Biol Chem 268:2389–2392
635. **Nussenzveig D, Heinflink M, Gershengorn M** 1993 Decreased levels of internalized thyrotropin-releasing hormone receptors after uncoupling from guanine nucleotide-binding protein and phospholipase-C. Mol Endocrinol 7:1105–1111
636. **Hinkle P** 1989 Pituitary TRH receptors. Ann NY Acad Sci 553: 176–187
637. **Gershengorn M, Thaw C** 1991 Regulation of thyrotropin-releasing hormone receptors is cell type specific: comparison of endogenous pituitary receptors and receptors transfected into non-pituitary cells. Endocrinology 128:1204–1206
638. **Yang J, Tashjian Jr AH** 1993 Transcriptional regulation by dexamethasone of endogenous thyrotropin-releasing hormone receptor messenger ribonucleic acid in rat pituitary GH4C1 cells. Endocrinology 133:487–490
639. **Yang J, Tashjian Jr AH** 1993 Regulation of endogenous thyrotropin-releasing hormone (TRH) receptor messenger RNA by TRH in GH4C1 cells. Mol Endocrinol 7:753–758
640. **Fujimoto J, Straub R, Gershengorn M** 1991 Thyrotropin-releasing hormone (TRH) and phorbol myristate acetate decrease TRH receptor mRNA in rat pituitary GH_3 cells. Evidence that protein kinase C mediates the TRH effect. Mol Endocrinol 5:1527–1532
641. **Yamada M, Monden T, Satoh T, Iizuka M, Murakami M, Iriuchijima T, Mori M** 1992 Differential regulation of thyrotropin-releasing hormone receptor mRNA levels by thyroid hormone *in vivo* and *in vitro* (GH_3 cells). Biochem Biophys Res Commun 184: 367–372
642. **Kimura N, Arai N, Sahara Y, Suzuki H** 1994 Estradiol transcriptionally and posttranscriptionally up-regulates thyrotropin-releasing hormone receptor messenger ribonucleic acid in rat pituitary cells. Endocrinology 134:432–440
643. **Fujimoto J, Narayanan C, Benjamin J, Heinflink M, Gershengorn M** 1992 Mechanism of regulation of thyrotropin-releasing hormone receptor messenger ribonucleic acid in stable transfected rat pituitary cells. Endocrinology 130:1879–1884
644. **Gershengorn M, Narayanan C, Fujimoto J** 1994 Phorbol myristate acetate enhances degradation of TRH receptor mRNA in a pituitary cell type-specific manner. Mol Cell Neurosci 5:576–579
645. **Narayanan C, Fujimoto J, Geras-Raaka E, Gershengorn M** 1992 Regulation by thyrotropin-releasing hormone (TRH) of TRH receptor mRNA degradation in rat pituitary GH_3 cells. J Biol Chem 267:17296–17303
646. **Fujimoto J, Narayanan C, Benjamin J, Gershengorn M** 1992 Posttranscriptional up-regulation of thyrotropin-releasing hormone (TRH) receptor messenger ribonucleic acid by TRH in COS-1 cells transfected with mouse pituitary TRH receptor complementary deoxyribonucleic acid. Endocrinology 131:1716–1720
647. **Ladram A, Bulant A, Nicolas P** 1992 Characterization of receptors for thyrotropin-releasing hormone potentiating peptide on rat anterior pituitary membranes. J Biol Chem 267:25697–25702
648. **Ladram A, Bulant M, Montagne J-J, Nicolas P** 1994 Distribution of TRH-potentiating peptide (PS4) and its receptors in rat brain and peripheral tissues. Biochem Biophys Res Commun 200:958–965
649. **Kelly JA** 1995 Thyrotropin-releasing hormone: basis and potential for its therapeutic use. Essays Biochem 30:133–149
650. **Garat B, Miranda J, Charli J-L, Joseph-Bravo P** 1985 Presence of a membrane bound pyroglutamyl aminopeptidase degrading thyrotropin-releasing hormone in the rat brain. Neuropeptides 6:27–33
651. **Gallagher SP, O'Connor B** 1998 A study of highly specific pyroglutamyl aminopeptidase type-II from the membrane fraction of bovine brain. Int J Biochem Cell Biol 30:115–133
652. **Vargas MA, Cisneros M, Herrera J, Joseph-Bravo P, Charli JL** 1992 Regional distribution of pyroglutamyl peptidase II in rabbit brain, spinal; cord and organs. Peptides 13:255–260
653. **Bauer K** 1987 Adenohypophyseal degradation of thyrotropin-releasing hormone regulated by thyroid hormone. Nature 330:375–377
654. **Ponce G, Charli JL, Pasten JA, Aceves C, Joseph-Bravo P** 1988 Tissue-specific regulation of pyroglutamate amino-peptidase II activity by thyroid hormones. Neuroendocrinology 48:211–218
655. **Uribe RM, Joseph-Bravo P, Pasten J, Ponce G, Mendez M, Covarribias L, Charli JL** 1991 Some events of thyrotropin-releasing hormone metabolism are regulated in lactating and cycling rats. Neuroendocrinology 54:493–498
656. **De Gandarias JM, Irazusta JM, Fernandez D, Varona A, Casis L** 1994 Developmental changes of pyroglutamate-peptidase I activity in several regions of the female and male brain. Int J Neurosci 77:53–60
657. **Bauer K, Novak P, Kleinkauf H** 1981 Specificity of a serum peptidase hydrolyzing thyroliberin at pyroglutamyl-histidine base. Eur J Biochem 118:173–183
658. **O'Connor B, O'Cuinn G** 1985 Purification of and kinetic studies on a narrow specificity synaptosomal membrane pyroglutamate aminopeptidase from guinea pig brain. Eur J Biochem 150:47–56
659. **Salers P, Ouafik LH, Giraud P, Maltese JY, Dutour A, Oliver C** 1992 Ontogeny of prolyl endopeptidase, pyroglutamyl peptidase I, TRH, and its metabolites in rat pancreas. Am J Physiol 262:E845–E850