60 YEARS OF NEUROENDOCRINOLOGY

TRH, the first hypophysiotropic releasing hormone isolated: control of the pituitary–thyroid axis

Patricia Joseph-Bravo, Lorraine Jaimes-Hoy, Rosa-Maria Uribe and Jean-Louis Charli
Departamento de Genética del Desarrollo y Fisiología Molecular, Instituto de Biotecnología, Universidad Nacional Autónoma de México (UNAM), A.P. 510-3, Cuernavaca, Morelos 62250, Mexico

Abstract

This review presents the findings that led to the discovery of TRH and the understanding of the central mechanisms that control hypothalamus–pituitary–thyroid axis (HPT) activity. The earliest studies on thyroid physiology are now dated a century ago when basal metabolic rate was associated with thyroid status. It took over 50 years to identify the key elements involved in the HPT axis. Thyroid hormones (TH: T₄ and T₃) were characterized first, followed by the semi-purification of TSH whose later characterization paralleled that of TRH. Studies on the effects of TH became possible with the availability of synthetic hormones. DNA recombinant techniques permitted the identification of all the elements involved in the HPT axis, including their mode of regulation. Hypophysiotropic TRH neurons, which control the pituitary–thyroid axis, were identified among other hypothalamic neurons which express TRH. Three different deiodinases were recognized in various tissues, as well as their involvement in cell-specific modulation of T₃ concentration. The role of tanycytes in setting TRH levels due to the activity of deiodinase type 2 and the TRH-degrading ectoenzyme was unraveled. TH-feedback effects occur at different levels, including TRH and TSH synthesis and release, deiodinase activity, pituitary TRH-receptor and TRH degradation. The activity of TRH neurons is regulated by nutritional status through neurons of the arcuate nucleus, which sense metabolic signals such as circulating leptin levels. Trh expression and the HPT axis are activated by energy demanding situations, such as cold and exercise, whereas it is inhibited by negative energy balance situations such as fasting, inflammation or chronic stress. New approaches are being used to understand the activity of TRHergic neurons within metabolic circuits.

Key Words

- HPT axis
- TRH
- TRH receptor
- TSH
- PPII
- cold
- fasting
- stress
- metabolism
- prolactin
- energy balance

A historical perspective on the hypothalamic control of the thyroid axis

The advancement of any scientific field requires the combination of creative new ideas with the development of technologies and knowledge in related areas; understanding the function of the hypothalamus–pituitary–thyroid axis (HPT) is no exception (Figs 1 and 2). Since the end of the 19th century, European physicians and surgeons associated neck swelling (thyroid enlargement, goiter), with iodine deficiency, cretinism, and myxoedema,
The discovery of inhibitors of thyroid function, such as propylthiouracil (PTU), aided in the cure of hyperthyroidism (Astwood 1943). PTU became useful in researching thyroid hormone (TH) metabolism, and in the discovery of different deiodinases (Escobar del Rey et al. 1961, Visser et al. 1983). The inhibition of T3-induced BMR activation in hypothyroid rats by cycloheximide helped to elucidate that the actions of T3 require protein synthesis (Tata et al. 1962). The identification of TH receptors (TRs) followed (Tata 2013), unraveling the multiplicity of effects of TH on energy metabolism (Mullur et al. 2014). The pituitary control of thyroid activity had been recognized since the beginning of the 20th century, although the purification and identification of thyroid-stimulating hormone (TSH) spanned several decades (Magner 2014). Semi-purified TSH preparations from bovine pituitaries demonstrated a similar structure to other pituitary hormones. It is composed of two subunits (a and b) and contains complex carbohydrate moieties that are essential for bioactivity and clearance (Pierce et al. 1971, Weintraub...
et al. 1989). TSH extracted from bovine or human post-mortem pituitaries was used in research, RIA and clinic for almost three decades. RIA determinations of plasma TSH concentration facilitated the conclusive demonstration of the negative feedback effects of TH on TSH secretion from the pituitary (Reichlin & Utiger 1967) and, together with serum TH (total and free) quantification, the evaluation of thyroid status (Biondi & Wartofsky 2014). TSH stimulation tests made it possible to distinguish between primary and secondary hypothyroidism (Querido & Stanbury 1950). By the 1980s, the sequence of TSH subunits became available with the isolation of their cDNAs (Fiddes & Goodman 1981, Wondisford et al. 1988) and the clinical use of recombinant human TSH (hTSH), which eliminated the health risks associated with the use of contaminated hTSH isolated from post-mortem tissues (Weintraub & Szkuflinski 1999).

Physiological support for the existence of the hypothalamic control of pituitary–thyroid function started with the pioneering work of Uotila on pituitary-stalk sections (Uotila 1939) and was further substantiated by complementary approaches such as electrical stimulation, electrolytic lesions of median eminence (ME) or diverse hypothalamic nuclei, administration of hypothalamic extracts, and histological observations under different physiological conditions (Greer 1952, Brown-Grant et al. 1957). Diminished basal thyroid activity in rabbits was observed after pituitary-stalk transections had been made, and a piece of wax paper had been placed between sections to eliminate vascular regeneration (Brown-Grant et al. 1954).
Discovery of TRH

From Harris’ initial proposal that the master gland, the adenohypophysis (or anterior pituitary), was under the control of factors released from the hypothalamus to the portal circulation (Harris 1950), it took almost 20 years to identify the first hypophysiotropic molecule. Various groups attempted to characterize the thyrotropin-releasing factor (TRF), but failed to purify it to homogeneity. They made some valid conclusions such as its non-reactivity to ninhydrin which implied a blocked NH2 terminus (Schreiber et al. 1963), its localization to several brain areas, or variations in the TRF-bioactivity of tissue extracts from animals of different thyroid status (Shibusawa et al. 1956, Reichlin 1989). Hard and competitive work for over 10 years, around 1–5 million pig or ovine hypothalami, cumbersome chromatographic techniques, and some fortuitous findings by the groups of Schally and of Guillemin enabled the isolation of the tripeptide (pyro)Glu-His-Pro-NH2, which was named thyrotropin-releasing hormone (TRH; Bøler et al. 1969, Burgus et al. 1969). The term ‘factor’ changed to ‘hormone’ when its structure was identified. An important breakthrough was the development of bio-assays to quantify pituitary hormones released in vitro (Guillemin & Rosenberg 1955). The peculiar N-(pyroGlu) and C-terminal (amide) residues, that delayed determination of TRH structure, proved essential for the biological activity of TRH, as chemical modifications were required to synthesize an active peptide based on the amino acid composition of the purified biologically active substance (Glu, His and Pro; Vale et al. 1973).

Once synthetic TRH became available, it was quantified by RIA in several tissue extracts, and detected not only in the hypothalamus but also in other brain areas, blood, and urine of several species (Jackson & Reichlin 1974, Winokur & Utiger 1974). Immunocytochemical techniques localized TRH in nerve terminals of the ME, in various hypothalamic nuclei as well as in various brain areas including the septum, nucleus accumbens or brain stem, where it plays a neuromodulatory role (Hökfelt et al. 1975, 1989, Lechan & Jackson 1982, Gary et al. 2003).

Metabolism of TRH

Biosynthesis

Soon after TRH chemical characterization, attempts began to elucidate its mode of synthesis. The initial work on the biosynthesis of neuropeptides, performed during the 1970s, was based on the incorporation of radioactive aminoacids, the availability of antibodies recognizing various forms, and sequential purification steps. Neurophysin and adrenocorticotropic hormone were found to be synthesized from precursor proteins (Mains & Eipper 1976, Gainer et al. 1977) in a similar manner to secretory proteins in other systems (Steiner et al. 1967, Kemper et al. 1972). These methods proved inadequate for TRH as incorporation of radioactive proline into the peptide was too low in the hypothalamic fragments used (McKelvy et al. 1975). The high concentrations of TRH in frog skin, and the knowledge that amidated peptides arose from glycine at their C-terminal end, allowed the isolation of a cDNA containing a partial sequence of the Trh precursor from a cDNA library screened using oligonucleotide mixtures containing the triplets that coded for Gln-His-Pro-Gly (Richter et al. 1984). This approach was unsuccessful in a hypothalamic rat cDNA library, probably because of the lower level of expression of Trh mRNA (Jackson 1989). The ingenious approach of synthesizing the peptide Cys-Lys-Arg-Gln-His-Pro-Gly-Lys-Arg-Cys, with an S—S bond linking the cysteines, left the middle portion of the molecule exposed to elicit an antibody able to detect this internal sequence. This antibody was used to identify the TRH precursor in an expression library of rat hypothalamic cDNAs, which isolated rat Trh cDNA (Lechan et al. 1986, Jackson 1989) and characterized the Trh gene (Lee et al. 1989).

The Trh-gene proximal promoter contains response elements (RE) to transcription factors whose binding was revealed by chromatin immunoprecipitation assays; for example, receptors for TH, or for glucocorticoid receptors (GR:GRE), CREB (CRE), cJun/cFos (TPA response element), STAT3, krueppel/Sp1, and GC-boxes for growth factor signaling (Joseph-Bravo et al. 2015). The protein codified by the rat (r) Trh gene is a precursor (pre-proTRH; 255 amino acids) containing five Gln-His-Pro-Gly sequences flanked by a pair of basic residues and cysteine peptides in between (Lechan et al. 1986). As for other neuropeptides (Loh et al. 2002), proTRH is processed in the secretory pathway through sequential enzyme activities: convertases, carboxypeptidase, pyroglutamylation cyclase, and peptidylglycine α-hydroxylating monoxygenase (Wu & Jackson 1988, Nilnì 2010, Fekete & Lechan 2014).

Antibodies specific for proTRH, together with Trh cDNA, were used in immunocytochemical and in situ hybridization analyses that enabled the final identification of the paraventricular nucleus (PVN) as the hypothalamic nucleus with the highest expression of proTRH precursor (Lechan & Segerson 1989). Further
studies demonstrated that TRH–hypophysiotropic cells are confined to the medial and caudal PVN of the rat (Fekete et al. 2000).

Inactivation

During the initial purification procedures it became evident that TRH was rapidly degraded in tissue homogenates (Redding & Schally 1969) and in plasma (Bassiri & Utiger 1972). Two soluble enzymes initiate hydrolysis of TRH and other peptides in vitro: proline endopeptidase (EC 3.4.21.26) cleaves the proline-amide bond; pyroglutamyl peptidase I (PPI; EC. 3.4.19.3), the pyroglutamylhistidine bond. However, these soluble enzymes do not control intracellular TRH levels in vivo because TRH is stored inside secretory granules (O’Cuinn et al. 1990, Joseph-Bravo et al. 1998). A different pyroglutamyl peptidase was initially detected in serum and termed thyroliberinase because of its strict specificity for TRH (Bauer & Nowak 1979). Later, an enzyme with similar activity and biochemical characteristics was detected in the membranes of the anterior pituitary and in several brain regions, and named PPII (EC. 3.4.19.6) or TRH-degrading ectoenzyme (O’Connor & O’Cuinn 1984, Garat et al. 1985, Heuer et al. 1998). In the hypothalamus, PPI is expressed in neurons and in tanyctyes whose cytoplasmic extensions reach the external layer of the ME, in proximity to TRH terminals (Joseph-Bravo et al. 1998, Sánchez et al. 2009). Cloning PPII (Schauder et al. 1994) led to its identification as a member of the M1 family of metallopeptidases and, by homology modeling and site-directed mutagenesis, interrogation of the structural determinants of its strict omega-peptidase specificity (Chávez-Gutiérrez et al. 2006). Because PPII is an integral membrane protein with the active site exposed on the cell surface (Charli et al. 1988), it is a prime candidate for TRH hydrolysis in the extracellular compartment, in particular before TRH reaches the ME-pituitary portal capillaries (Sánchez et al. 2009).

Release

TRH secreted from the ME enters the portal system to reach the pituitary. In vivo TRH release has been measured directly in portal blood of anesthetized animals, or by a push–pull cannula in the ME. However, these techniques are difficult to use in order to detect rapid changes in TRH secretion (Rondeel et al. 1992). In vitro systems were initially developed to study the mechanisms of neuropeptide secretion; incubates of the mediobasal hypothalamus, containing the ME, demonstrated TRH release by membrane depolarization through a Ca ++- dependent mechanism consistent with exocytosis (Joseph-Bravo et al. 1979).

TRH at the anterior pituitary

TRH stimulates, in vivo and in vitro, not only the synthesis and release of TSH from thyrotrophs but also of prolactin (PRL) from lactotrophs, and in some species also of growth hormone (GH) from somatotrophs (Galas et al. 2009). The availability of radiolabeled TRH, and later of its more stable analog 3Me-His-TRH, facilitated the characterisation of the specific binding sites in the plasma membrane of the anterior pituitary (Labrie et al. 1972), in TSH secreting pituitary tumor cells (Grant et al. 1972), and in PRL secreting GH3 cells (Hinkle & Tashjian 1973). This receptor, TRHR1, has been characterized in various species. The sequence of mouse (m) TRHR1 corresponds to a seven transmembrane-spanning GTP-binding (G) protein-coupled receptor (GPCR; Straub et al. 1990); mTRHR1 cDNA has a high similarity in the protein-coding regions with orthologs in other mammals and its expression in anterior pituitary correlates with radioligand binding studies (Gershengorn & Osman 1996). A second TRHR (TRHR2) was later cloned and found to be expressed mainly in the brain (O’Dowd et al. 2000). TRHR1 –/– mice pituitaries are devoid of any TRH-binding capacity, which suggested that TRHR1 is the only pituitary receptor (Rabeler et al. 2004).

TRH binds to TRHR1 at various residues of the extracellular (low binding affinity) and of the transmembrane (high binding affinity) domains. The extracellular site is proposed to be the initial place of interaction, which accounts for the low binding affinity and slow transformation to a tightly bound conformation with movement of TRH to the transmembrane site (Engel & Gershengorn 2007). In GH pituitary tumor cells, TRH signalling via TRHR1 is conducted through the activation of a Gq/11 protein and phospholipase C β1 mechanism: the production of inositol 3 phosphate and diacyl glycerol affects cellular calcium homeostasis (mobilizing intracellular pools) and activation of protein kinase C (PKC) (Drummond 1986). The interaction of TRH with TRHR1 induces rapid desensitization of the response due to multiple events (Hinkle et al. 2012). The ligand–receptor interaction induces receptor phosphorylation, within seconds, at multiple Ser/Thr sites in the cytoplasmic C-terminal tail by a GPCR kinase. TRH receptors bind to β-arrestin, internalize in clathrin-coated vesicles and accumulate in early sorting endosomes. They may
Journal of Endocrinology

2011). The peripheral conversion of injected T4 has been
at least in part, by stimulating Trhr

(Hinkle et al. 2012). TRH also provokes long-term transcriptional and posttranscriptional effects that
diminish Trhr mRNA levels in rat pituitary GH3 cells, at
least in part, by stimulating Trhr mRNA degradation
(Narayanan et al. 1992).

Regulation of HPT axis activity by TRH and negative feedback

The HPT axis is regulated by neuronal inputs that
stimulate or inhibit PVN–TRH hypophysiotropic neurons. Of all TRH neurons expressed in the PVN, not all project to the ME. TRH–hypophysiotropic cells are enriched in the medial and caudal PVN of the rat but this differs in the PVN of the mouse and human (Guldenaar et al. 1996, Fekete et al. 2000, Fekete & Lechan 2014).

TRH–hypophysiotropic neurons receive afferents from
multiple brain regions. Neurons from the arcuate nucleus transmit the nutritional status and the suprachiasmatic nucleus convey circadian cycle information, some neurons from the brain stem send information when external temperature drops (Fekete & Lechan 2014, Fliers et al. 2014, Joseph-Bravo et al. 2015). Stimuli that induce TRH–TSH release may coordinate increase Trh transcription.

A multifactorial control is exerted at various steps of the HPT axis. TRH stimulates TSH synthesis in pituitary cells by increasing mRNA levels of Tshb and Tsha (Shupnik et al. 1986). Transduction pathways involve Ca^{2+}/calmodulin for TSHb activation or the PKC–MAPK pathway for TSha (Hashimoto et al. 2000). TRH regulates the glycosylation pattern of TSH, which increases its biological activity and half-life (Weintraub et al. 1989, Szkudlinski et al. 2002).

TSH stimulates synthesis and release of TH which are transported in the blood by T4-bound globulin, transthyretin or albumin, in different proportions depending on the species (Zoeller et al. 2007). More than 70% of TSH-stimulated TH release corresponds to T4 (Maia et al. 2011). The peripheral conversion of injected T4 has been
recognized since the 1950s (Tata 1958), and was followed
by characterization of the enzymes responsible (Silva & Larsen 1977, Visser et al. 1983, Gereben et al. 2008). The three identified deiodinases set the intracellular and peripheral levels of T3: deiodinase type I (D1), the enzyme inhibited by PTU is mainly expressed in liver, kidney, pituitary, and thyroid, and converts T4 to either T3 or, reverse T3. D2, expressed in brain, pituitary, thyroid, BAT, and heart has a higher affinity for T4 than D1, and transforms T4 to T3; D2 is enriched in tanyocytes and makes T3 available to surrounding neurons in the hypothalamus. D3 is expressed in brain, placenta, and skin and inactivates T4 and T3. D1 and D3 are localized to the plasma membrane whereas D2 is localized to the membranes of the endoplasmic reticulum, which facilitates ready access to the nucleus for T3 (Gereben et al. 2008). The activity and expression of these enzymes are modulated, in a cell-specific manner, by various effectors including TH; T3 decreases dio2 expression and increases that of dio1 and dio3, whereas T4 decreases the activity of D2 by increasing its ubiquitination and proteosomal degradation (Gereben et al. 2008, Abdalla & Bianco 2014). Peripheral conversion of T4 to T3 arises mainly from D2 in euthyroid, or via D1 in thyrotoxic animals (Maia et al. 2011).

TH feedback on HPT axis activity was conclusively demonstrated in the pituitary when TSH RIA became available (Reichlin & Utiger 1967, Reichlin et al. 1970). At the hypothalamus evidence was indirect, supported by the diminished goitrogenic effect of PTU in animals with lesions between the PVN and the ME, and the response to thyroidectomy in lesioned–PVN rats that presented with diminished TSH secretion (Greer 1952, Martin et al. 1970). It is now evident that the HPT axis is modified by the thyroid status in a concerted fashion at multiple levels. Hypothyroidism increases Trh mRNA levels in the PVN (Koller et al. 1987, Segerson et al. 1987), proTRH processing (Perello et al. 2006), TRH release from ME (Rondeel et al. 1992), TSH and TRHR1 synthesis in the pituitary (Shupnik et al. 1986, Schomburg & Bauer 1995), and TSH serum concentration (Biondi & Wartofsky 2014). In contrast, the expression of the TRH-degrading enzyme in tanyocytes is decreased in hypothyroid animals (Lazcano et al. 2015). Opposite changes occur in hyperthyroidism (Supplementary Table 1, see section on supplementary data given at the end of this article; Chiamolera & Wondisford 2009, Costa-e-Sousa & Hollenberg 2012, Fekete & Lechan 2014, Fliers et al. 2014, Lazcano et al. 2015). The T3-negative transcriptional regulation of Trh and Tsh occurs primarily through TRb2 (Abel et al. 2001, Chiamolera & Wondisford 2009, Sugrue et al. 2010), whose
expression in the pituitary is down regulated by T₃, and modestly down-regulated by TRH (Lazar 1993). TH inhibit TSH secretion even faster than TRH or TSH transcription. This response may be related to the rapid up-regulation of expression and activity of the TRH-degrading enzyme by T₄ in tanyctyes. Increased inactivation of TRH released from the ME could account for diminished TSH release, supporting the external layer of the ME as an ultimate critical control point in modulating TRH concentration on its passage to the portal system (Sánchez et al. 2009).

At a hypothalamic level, T₄ is taken up from the circulation by tanyctyes that convert it to T₃ by D2; T₃ is then released in the surrounding neuropil and taken up by neurons (Tu et al. 1997). Several TH transporters have been identified recently. Among the best characterized in the brain is the monocarboxylate transporter 8 (MCT-8), which recognizes different TH, and is expressed in various tissues and cell types including neurons, endothelial cells, oligodendrocytes, astrocytes and tanyctyes. Another is the organic anion-transporting polypeptide 1C1 (OATP1C1), which is found in tanyctyes and endothelial cells. Its expression is modulated by TH and it has preferential substrate specificity for T₄ compared to other TH. Both participate in transporting TH across the blood brain barrier in mice but OATP1C1 does not in humans. Mutations in SLC16A2, the gene that encodes MCT-8, can produce severe neurological impairments in humans (Visser et al. 2011, Wirth et al. 2014).

The specificity of TH feedback effect on TRH expression for PVN–TRH hypophysiotropic neurons vs other hypothalamic neurons expressing TRH does not relate to an exclusive expression of THR or TH transporters (Fekete & Lechan 2014, Joseph-Bravo et al. 2015). D3 is found in only 27% of TRH-immunoreactive varicosities present in the ME (Kalio et al. 2012). An hypothesis recently put forward is that T₃, transformed from T₄ by D2 in tanyctyes, is taken up by TRH nerve terminals in the ME and transported in a retrograde fashion to the PVN, where it inhibits TRH transcription (Fekete & Lechan 2014).

Knock out (KO) animals for various elements involved in HPT axis regulation have revealed the critical steps in HPT axis function (Joseph-Bravo et al. 2015). The importance of the effects of TRH on TSH glycosylation and activity (Weintraub et al. 1989) has been demonstrated by comparing the phenotypes of TRH-KO, THRb-KO, and the double mutant. Increased TSH serum levels but reduced TSH bioactivity accounts for the low circulating T₄ concentration (Nikrodnanod et al. 2006), similar to that observed in humans with hypothalamic hypothyroidism (Beck-Peccoz et al. 1985), or in TRHR1⁻/⁻ mice that have normal TSH levels but low circulating T₃ and T₄ concentrations (Rabeler et al. 2004). D1-, D2-, and D1D2-KO show compensatory mechanisms in the interplay between hypophysiotropic TRHergic neurons, pituitary TSH expression and release, which in combination maintain serum levels of T₃ stable despite altered serum concentrations of T₄ and TSH (Abdalla & Bianco 2014, Galton et al. 2014). D2-KO specifically in the pituitary produced contradictory results regarding TRH or TSH expression, albeit data coincide that these mice maintain constant T₃ serum levels (Fonseca et al. 2014, Luongo et al. 2015). MCT8-KO mice have increased Thr expression, which further confirms that TH uptake into tanyctyes is required for negative feedback on Thr expression (Horn et al. 2013).

### Energy homeostasis and the HPT axis

#### Negative energy balance

The effects of iodine deficiency or nutritional status on BMR and thyroid activity were observed a century ago (Hinz 1920) and later confirmed when it was found that TH serum concentrations were reduced during fasting or food restriction (Reichlin 1957, Palmblad et al. 1977, Harris et al. 1978). After fasting, TSH serum levels are low or normal, but ME–TRH release and Thr mRNA levels in the PVN decreased (Blake et al. 1991, Van Haasteren et al. 1995, Fekete & Lechan 2014). Pituitary dio2, Thrb2, and Tshb mRNA levels are diminished (Boelen et al. 2006), as well as hepatic D1 activity. In contrast, dio2 hypothalamic expression and serum corticosterone are increased (Diano et al. 1998). Another element involved in the response to fasting is PPII activity in tanyctyes which is up-regulated at a time (48–72 h) when the expression of Thr in the PVN tends to reinitiate (Lazcano et al. 2015). These changes differ from those observed in primary hypothyroidism. The discovery of the adipostatic hormone leptin (Zhang et al. 1994) helped unravel the mechanism of fasting-induced inhibition of the HPT axis. Leptin is released from adipose tissue proportionally to body fat and in response to caloric intake, while its serum levels decrease rapidly during fasting (Hardie et al. 1996). Leptin administration impedes fasting-induced inhibition of Thr mRNA levels in the PVN (Légrádi et al. 1997). In response to leptin, its receptor (LepRb) activates several transcription factors including the STAT3 which binds to the Thr promoter and increases Thr transcription (Guo et al. 2004). Thr mRNA...
levels in the PVN are increased by leptin, either directly through LepRb activation, or indirectly through afferents from the arcuate nucleus. In the arcuate nucleus, two neuronal groups synthesize orexigenic (neuropeptides Y (NPY)/Agouti-related peptide (AgRP)) or anorexigenic (pro-opiomelanocortin (POMC), precursor of alpha-melanocyte stimulating hormone (aMSH)/cocaine- and amphetamine-regulated transcript (CART)) neuropeptides. These NPY/AgRP and POMC/CART neurons are tightly regulated by metabolic signals such as leptin, insulin, or ghrelin. aMSH signals through the melanocortin receptor 4 (MC4R) that also recognizes AgRP but as an inverse agonist. TRH neurons receive afferents from aMSH, NPY, and AgRP neurons, the former stimulates and the latter two inhibit Trh mRNA levels (Fekete & Lechan 2014). aMSH induces CREB phosphorylation in TRH neurons in vivo and in hypothalamic neuronal culture where it increases Trh transcription (Harris et al. 2001, Sarkar et al. 2002). The analysis of mice lacking both MC4R and NPY demonstrates that fasting-induced suppression of the central arm of the HPT axis requires NPY, and that a second pathway based in the liver, that enhances the catabolism of TH during fasting, requires MC4R and NPY (Vella et al. 2011).

Non-thyroidal illness syndrome (NTIS) is a clinical condition that presents, as in fasting, with a low T₃ but normal or slightly decreased TSH serum levels, occurring during acute or chronic inflammation, and sepsis. The mechanisms involved differ to those produced by fasting. Despite low Trh mRNA levels, those of the arcuate nucleus POMC are not changed, and deiodinase activity is higher than that detected after fasting; in particular, for D2 in tanycytes and D1 and D3 activities in liver and muscle (Boelen et al. 2011, Fekete & Lechan 2014, Fliers et al. 2014). It has been proposed that while leptin is the main regulator of fasting induced changes in the HPT axis, deiodinase activity plays a major role during NTIS (Boelen et al. 2011).

**Positive energy balance**

In contrast to the relatively detailed knowledge about the central aspects of HPT axis regulation during energy deficit, less is known about regulation during energy excess. Although hypothyroid individuals tend to gain weight, obese individuals have normal or slightly enhanced total and free T₃ levels, which are postulated as an adaptation to the increased metabolic demands of increased body weight (Strata et al. 1978, Reinehr 2010). Diet-induced obesity (DIO) enhances HPT axis activity in male rats, as demonstrated by increased Trh mRNA levels in the hypothalamus/PVN and serum TSH concentration. This increase in HPT axis activity may be due to enhanced circulating leptin levels acting directly on PVN–TRH neurons, independently from POMC neurons, thus bypassing the drop of leptin sensitivity which occurs in the ARC during DIO, or through other circuits that maintain leptin sensitivity (Araujo et al. 2010, Perello et al. 2010). Likewise, mice fed a high fat diet for 7–20 weeks have an activated HPT axis, with higher hypothalamic Trh mRNA levels, and serum TSH concentration than mice on a control diet. This study also indicates that deiodinases activities adjust in tissue, time and obesity-tendency specific ways, contributing to metabolic responses to DIO (Xia et al. 2015).

**Energy demands activate the HPT axis**

Energy demanding situations such as hypothermia activate the thyroid (Dempsey & Astwood 1943, Brown-Grant et al. 1954). The cold response is blunted in pituitary-stalk operated rats (Uotila 1939) and after PVN-electrolytic lesions (Ishikawa et al. 1984). An acute cold exposure rapidly and transiently augments Trh mRNA levels in the PVN, followed by increased TSH in serum and T₄ at a later time (Zoeller et al. 1990, Uribe et al. 1993). Cold-induced TRH expression is independent of circulating TH concentration (Zoeller et al. 1990) or of nutritional status (Jaimes-Hoy et al. 2008), but is inhibited by a previous stress exposure (Uribe et al. 2011) or corticosterone injection (Sotelo-Rivera et al. 2014). Humans exposed to cold for over 60 h activate the HPT axis, which is not inhibited if food intake is reduced (Joseph-Bravo et al. 2015).

Other examples of HPT axis activation are observed in response to an acute increase in physical activity (Fortunato et al. 2008, Gutiérrez-Mariscal et al. 2012) or after 2 weeks of voluntary exercise in rats (Uribe et al. 2014). Wheel running diminishes food intake by 18% compared to sedentary animals. In the pair-fed group, body weight gain diminished to the same extent as the exercised. However, adipose tissue mass and leptin serum levels were reduced exclusively after exercise; Trh mRNA in the PVN and TSH serum levels diminished, compared to naïve rats, more in the pair-fed than in the exercised group; only pair-fed animals had low T₃ serum levels. The inhibition of the HPT axis caused by diminished food intake was thus partially compensated with exercise and the changes to all the parameters of the HPT axis correlated with distance run and loss of fat mass (Uribe et al. 2014). These results suggest that although TH and nutritional status modulate the basal...
state of the HPT axis, immediate energy demands may override leptin or TH signaling.

**Stress interferes with HPT axis activity**

Another important modulator of HPT activity long recognized is the inhibitory effect of stress. The differential effects of physical and emotional stress on HPT activity were elegantly shown by Harris’s group, who compared thyroid activity after physical or emotional stress, in intact or adrenalectomized rabbits. A corticosterone injection, or stress, inhibits thyroid activity. However, only the effects of the emotional stressor (restraint) were avoided when pituitary-stalk sections were performed supporting an effect at hypothalamic level (Brown-Grant et al. 1957). Restraint indeed decreases rat *Trh* mRNA levels in the PVN and, as in other stressors, serum TSH (Du Ruisseau et al. 1978, Gutiérrez-Mariscal et al. 2012), but the effects of chronic stress depend on the type, intensity and duration (Armario et al. 1984). Because long-term stress affects many metabolic parameters that may regulate the HPT axis, direct cause-effects are difficult to discern (Joseph-Bravo et al. 2015).

Corticosterone affects the HPT axis. Injected into adrenalectomized rats for several days it inhibits PVN *Trh* expression (Kakucska et al. 1995) whereas, an acute injection is stimulatory. However, if injected 30 min prior to cold exposure, the cold-induced stimulation of PVN *Trh* expression or TSH serum levels is blunted (Ranta 1975, Sotelo-Rivera et al. 2014). Primary hypothalamic-cell cultures have provided information regarding potential regulators of the *Trh* promoter. TRH transcription is rapidly increased by agents that cause TRH release, such as noradrenaline or cAMP analogs that induce CREB phosphorylation and binding of pCREB to the *Trh* promoter. Corticosterone, which activates GR and its binding to GRE, also increases *Trh* expression, albeit less than cAMP analogs. However, if corticosterone and cAMP analogs treatments are combined, *Trh* transcription is no longer stimulated and pCREB or GR do not bind to their RE (Díaz-Gallardo et al. 2010), CREB phosphorylation is blunted and the catalytic subunit of phosphokinase (PKAc) interacts with GR in the cytosol, which explains the observed cAMP signaling interference induced by glucocorticoids (Sotelo-Rivera I, Cote-Vélez A, Díaz-Gallardo M, Charli JL, Joseph-Bravo P, unpublished observations). These *in vitro* results may explain why stress can alter PVN *Trh* mRNA response to an acute cold stimulus (Joseph-Bravo et al. 2015). Combining *in vitro* and *in vivo* paradigms will continue to provide important insights into the mechanisms involved in regulating the activity of the HPT axis.

**Hypophysiotropic TRH neurons are involved in PRL release**

Multiple effectors control PRL release. Soon after the discovery of TRH, evidence supported the hypothesis that TRH was one of the prolactin releasing factors. TRH stimulates PRL secretion either *in vivo* or *in vitro* (Jacobs et al. 1971, Tashjian et al. 1971). Suckling stimulates TRH biosynthesis in the PVN and release from the ME (Fink et al. 1982, Uribe et al. 1993, Van Haasteren et al. 1996, Sánchez et al. 2001). TRH antisera inhibit suckling-induced PRL release (de Greef et al. 1987). While dopamine exerts a tonic inhibition on PRL release *in vivo*, its release into the portal blood is inhibited by suckling, an event which potentiates TRH-induced PRL secretion (Martínez de la Escalera & Weiner 1992). However, TSH is neither released by suckling, nor PRL by cold exposure (Uribe et al. 1993, Van Haasteren et al. 1996, Sánchez et al. 2001). This discrepancy may be explained by CART which inhibits PRL release and its expression is upregulated in hypophysiotropic TRH neurons by cold but not by suckling (Sánchez et al. 2007). TRH and TRH-R1 KO mice have shown that while TRH is necessary to sustain PRL secretion during lactation, pups from KO dams grow normally, suggesting that TRH is not essential for suckling-induced PRL release (Rabeler et al. 2004, Yamada et al. 2006).

Contrary to data showing that anterior pituitary PPII does not regulate the response of thyrotroph response to TRH, there is evidence that in lactotrophs the intensity of TRH action is under PPII control. PPII is expressed in lactotrophs and its knockdown or inhibition enhances TRH-induced PRL release (Cruz et al. 2008). PPII expression and activity are enhanced *in vivo* by TH and down-regulated by estrogens (Schomberg & Bauer 1995, 1997). In anterior-pituitary cultured-cells, PPII activity is rapidly enhanced by the removal of dopamine and addition of TRH (Bourdais et al. 2000). These results suggest that PPII is controlled by signals that shape PRL secretion in response to TRH; regulation of PPII may in turn alter PRL release.

Although many studies show that TRH acts directly on lactotrophs, evidence that in hypothalamic slices TRH provokes a transition from phasic to tonic firing of the tuberoinfundibular dopaminergic neurons that control PRL secretion (Lyons et al. 2010) indicates additional mechanisms that link TRH and PRL secretion.
Perspectives for the 21st century

DNA recombinant techniques permitted the development of strategies that helped to characterize the various regulatory steps of the HPT axis. Transfected cells, KO and transgenic animals have provided important information, although data do not always correspond to what would be expected from the known physiology of the HPT axis (Joseph-Bravo et al. 2015). These discrepancies may be due to the redundancy of effector molecules or their receptors, and compensatory effects during development. TRH neurons are considered an important participant in energy homeostasis (Lechan & Fekete 2006, Levin 2007, Hollenberg 2008). This is further substantiated by recent findings on increased Trh and brain derived neurotropic factor (Bdnf) expression in lean animals compared to their fat counterparts; BDNF is an important participant in brain plasticity and metabolism (Byerly et al. 2009, Cao et al. 2011). Defining the circuits in which TRH neurons are involved under different circumstances now seems to be feasible with the combined techniques of Cre-recombinase, opto-genetics, pharmaco-genetics, proteomic, and genomic analysis. A recent example is the demonstration with opto- and chemo-genetic tools that a TRH projection from the PVN onto AgRP-ARC neurons drives hunger in mice (Krashes et al. 2014).

New evidence supports the hypothesis that environmental threats (nutrition, toxins) or stressful situations alter the programming of adult HPT axis activity (Joseph-Bravo et al. 2015). Considering compelling new data on epigenetic changes due to stress or other factors, and the effects of endocrine disrupting chemicals, important considerations are required in the maintenance of experimental animals. Epigenetic modifications alter the gene expression of various elements that may modify HPT axis activity. For example, early life stress increases the methylation of hippocampal GR and hence its expression, diminishing the inhibitory feedback that glucocorticoids exert during a response to stress (Turecki & Meaney 2014, Joseph-Bravo et al. 2015). The opposite is observed when raising animals in enriched environments (Cao et al. 2011). Development may also be affected by endocrine disruptors which contaminate water and food: experimental animals and cell cultures are kept in plastic bottles, cages and plates that leach endocrine disruptors (Préau et al. 2015). To obtain more reproducible data and help us understand neuroendocrine physiology, some standards, additional to those recently established (Bianco et al. 2014), are urgently needed. Neuroendocrine research should also include gender and age differences as, to date, most research has been performed in young adult male rodents.

Supplementary data
This is linked to the online version of the paper at http://dx.doi.org/10.1530/JOE-15-0124.

Declaration of interest
Authors are academic staff at UNAM with nothing to declare.

Funding
Research performed over the years has been financed by CONACYT and DGAPA. Actual grants: CONACYT-180009, 154931, 128665. DGAPA IN204913, IN206712, IA201515, IN212411.

Acknowledgements
Thanks to the introduction of PubMed (1997) that ‘socialized’ scientific knowledge by making information accessible, it is now evident that the work performed more than half a century ago included creative, ingenious and patient surgical and biochemical techniques that laid down the grounds of many questions, some of which are still unanswered. Easier access to more pre-digital journal articles is highly desirable. P-J-B expresses her gratitude to Dr S Reichlin for his generous review of the manuscript, and for being an inspiration on TRH research. We thank all students, academics, and technicians that have contributed to the work performed at the group of Molecular and Cellular Neuroendocrinology. In particular for this review, Dr M Gutiérrez-Mariscal, S Ainsworth for her willingness and cooperation in finding many of the old papers, and Dr T Nishigaki for his aid in Japanese translation.

Prominent Reviews
We apologize to those authors whose excellent work is not cited here due to space constraints. However, this list of reviews have been selected as the most prominent reviews on the history of TRH and the HPT:
Abdalla & Bianco 2014
Biondi & Wartofsky 2014
Boelen et al. 2011
Chiamolera & Wondisford 2009
Costa-e-Sousa & Hollenberg 2012
Fekete & Lechan 2014
Fliers et al. 2014
Gary et al. 2003
Gereben et al. 2008
Greer 1952
Hinkle et al. 2012
Hökfelt et al. 1989
Hollenberg 2008
Jackson 1989
Joseph-Bravo et al. 1998
Joseph-Bravo et al. 2015
Lazar 1993
Lechan & Fekete 2006
Magnier 2014
Maia et al. 2011
Martinez de la Escalera & Weiner 1992
Mullur et al. 2014
Nillni 2010
O’Cuinn et al. 1990
Reichlin 1989
References


Dempsey EW & Astwood EB 1943 A determination of the rate of thyroid hormone secretation at various environmental temperatures. *Endocrinology* **32** 509–518. (doi:10.1210/endo-32-6-509)


Fink G, Koch Y & Ben Aroya N 1982 Release of thyrotropin releasing hormone into hypophyseal portal blood is high relative to other neuropeptides and may be related to prolactin secretion. *Brain Research* **243** 186–189. (doi:10.1016/0006-8993(82)91137-4)


Hardie LJ, Rayner DV, Holmes S & Traylor P 1996 Circulating leptin levels are modulated by fasting, cold exposure and insulin administration in lean but not Zucker (fa/fa) rats as measured by ELISA. *Biochemical and Biophysical Research Communications* **223** 660–665. (doi:10.1016/bbrc.1996.0951)


Magner J 2014 Historical note: many steps led to the 'discovery' of thyroid-stimulating hormone. *European Thyroid Journal* **3** 95–100. (doi:10.1159/000360534)


Sánchez E, Vargas MA, Singru PS, Pascual I, Romero F, Fekete C, Charli JL & Lechán RM 2009 Tanscyp thyroglobulin pepitide II contributes to regulation of the hypothalamic–pituitary–thyroid axis through...


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. (doi:10.1210/endo-127-6-2955)


Received in final form 27 May 2015
Accepted 22 June 2015
Accepted Preprint published online 22 June 2015