Thyroid hormone (TH) plays a critical role in development, growth, and cellular metabolism. TH production is controlled by a complex mechanism of positive and negative regulation. Hypothalamic TSH-releasing hormone (TRH) stimulates TSH secretion from the anterior pituitary. TSH then initiates TH synthesis and release from the thyroid gland. The synthesis of TRH and TSH subunit genes is inhibited at the transcriptional level by TH, which also inhibits posttranslational modification and release of TSH. Although opposing TRH and TH inputs regulate the hypothalamic-pituitary-thyroid axis, TH negative feedback at the pituitary was thought to be the primary regulator of serum TSH levels. However, study of transgenic animals showed an unexpected, dominant role for TRH in regulating the hypothalamic-pituitary-thyroid axis and an unanticipated involvement of the thyroid hormone receptor ligand-dependent activation function (AF-2) domain in TH negative regulation. These results are summarized in the review.

Serum concentrations of T₄ and its biologically active form T₃ are maintained in vivo in a narrow range by the ability of thyroid hormone (TH) to limit its own production by negative feedback at the hypothalamic TSH-releasing hormone (TRH) neuron and pituitary thyrotroph. This feedback is critically dependent upon the presence of normal TH receptors (TRs), which bind to the promoters of TRH and TSH subunit genes and regulate their expression (1–5). In the presence of its ligand, T₃, TRs mediate ligand-dependent repression of the transcription of these genes, and in the absence of T₃, the transcription rate is not simply returned to baseline, but ligand-independent activation is observed (6–8). Although it is still commonly stated that the major locus of TH regulation of the hypothalamic-pituitary-thyroid (HPT) axis is the pituitary, new findings in mouse models suggest otherwise.

TH Action on the HPT Axis

Early studies using primary cell cultures of mouse thyrotropic tumor cells demonstrated that TH treatment suppressed transcription of TSH subunit genes, and as a consequence, TSH synthesis was reduced (9). At about the same time, TH was shown to suppress prepro-TRH mRNA levels from certain neurons in the hypothalamus (10). Finally, it was shown that TRH affected the bioactivity of TSH by altering its glycosylation pattern (11). Thus, TH could act at the pituitary, hypothalamic, or both levels to regulate TSH synthesis, which in turn would control TH production by the thyroid.

Generation of mouse models where TRs were either deleted or mutated helped to define better TH feedback action on the HPT axis. These mouse lines were designed to model a human disorder referred to as resistance to thyroid hormone (RTH) where a dominantly inherited mutation in the HPT axis. To confirm that the pituitary was an important locus of negative feedback by TH, a transgenic mouse expressing a pituitary-specific mutant TR was generated (13). Transgenic mice developed profound pituitary RTH, as demonstrated by markedly elevated baseline and non-T₃-suppressible serum TSH and pituitary TSH-α mRNA levels, and as expected, hypothalamic prepro-TRH mRNA levels were suppressed. Surprisingly, however, serum T₄ levels were only marginally elevated in

**Abbreviations:** CoA, Coactivator; CoR, corepressor; CART, cocaine- and amphetamine-regulated transcript; CREB, cAMP response element-binding protein; D2, type II deiodinase; DMN, dorsomedial nucleus; GR, glucocorticoid receptor; HPT, hypothalamic-pituitary-thyroid; KO, knockout; MCT, monocarboxylate transporter; NPY, neuropeptide Y; OATP, organic anion transporting polypeptide; PVN, paraventricular nucleus; RTH, resistance to thyroid hormone; Src-1, steroid receptor CoA-1; TH, thyroid hormone; TR, TRH, TSH-releasing hormone.
these mice. After TRH administration, T₄ concentrations increased in both transgenic and wild-type animals, but transgenic animals had a sustained increase over 72 h. Hypothyroid transgenic mice also exhibited a TSH response that was only 30% of the response observed in wild-type animals. These findings indicate that pituitary expression of this mutant TR impairs both T₃-independent activation and T₃-dependent suppression of TSH subunit gene expression in vivo. The discordance between basal TSH and T₄ levels and the reversal of these findings with TRH administration demonstrates that resistance at the level of both the thyrotroph and the hypothalamic TRH neuron are required to elevate TH levels in patients with RTH (13).

Although hypothalamic TRH is the major stimulator of TSH synthesis and release from the anterior pituitary (14, 15), TH negative feedback at the pituitary was believed to be the most important physiological regulator of serum TSH levels (9). Recently, the central role for TRH in normal TH feedback of the HPT axis was demonstrated. Mice that lack either TRH (TRH knockout [KO]), the β-isomers of TH receptors (TRβ KO), or both (double KO) were studied. As previously reported, TRβ KO mice have significantly higher TH and TSH levels compared with wild-type mice. In contrast, double KO mice had reduced TH and TSH levels compared with control animals. Unexpectedly, hypothalamic double KO mice also failed to mount a significant rise in serum TSH levels, and pituitary TSH immunostaining was markedly reduced compared with all other hypothyroid mouse genotypes. This impaired TSH response, however, was not due to a reduced number of pituitary thyrotrophs because thyrotroph cell number, as assessed by TSH-immunopositive cell number, was restored after chronic TRH treatment. Thus, the TRH neuron is absolutely required for both TSH and TH synthesis and appears to be the locus of the set-point in the HPT axis (16).

**TRH and the Hypophysiotropic TRH Neuron**

TRH is a tripeptide amide (pyro-Glu-His-Pro-NH₂) derived from a large precursor protein, prepro-TRH (ppTRH), by post-translational processing (prohormone convertase enzymes PC1, -2, and -3) (17). The 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, five copies of the TRH progenitor sequence Gln-His-Pro-Gly flanked by paired basic amino acids (Lys-Arg or Arg-Arg), four non-TRH peptides lying between the TRH progenitors, an N-terminal flanking peptide, and a C-terminal flanking peptide (18, 19). Rats and mice have five Gln-His-Pro-Gly TRH progenitor sequences, whereas humans have six TRH sequences (19). Serum TH levels can affect the processing of pre-TRH by altering the prohormone convertases; low TH levels stimulate TRH and prohormone convertase expression in the paraventricular nucleus (PVN) (20, 21).

The hypothalamic PVN, a triangular shaped nucleus located at the dorsal limits of the third ventricle (22–24), consists of a periventricular parvocellular part containing neurosecretory neurons (hypophysiotropic neurons) that release their hormones into the hypophyseal portal circulation in the median eminence, and a magnocellular part that contains neurosecretory cells projecting to the posterior pituitary, which release oxytocin and vasopressin (25). Studies in rats showed an inverse relationship between serum TH levels and the expression of prepro-TRH mRNA in the PVN during experimentally induced hypo- and hyperthyroidism confirming an essential role for these neurons in this classical endocrine negative feedback loop (10). This regulation was confined to a small population of TRH neurons located within the PVN.

Three main neuronal groups mediate the effects of other physiological stimuli on hypophysiotropic TRH neurons (17) (Fig. 1). First, adrenergic input from the medulla is believed to mediate the stimulatory effects of cold exposure on the TRH neuron (26, 27). Catecholamines are believed to increase the set-point for inhibition of TRH gene expression by T₃, thereby permitting high circulating levels of TH to contribute to increased thermogenesis. Catecholamines act on TRH neurons primarily through α₁ adrenergic receptors (26) that can induce the phosphorylation of the cAMP response element-binding protein (CREB) (28). CREB activates the TRH promoter by binding to a CREB response element in the promoter, which overlaps with a TR binding site (29). It is hypothesized that cold exposure increases phosphorylated CREB, which then competes with TR for binding to the promoter region of TRH (17). Adrenergic fibers in contact with TRH neurons also contain at least two neuropeptides: cocaine- and amphetamine-regulated transcript (CART) and neuropeptide Y (NPY) (30, 31). CART exerts a stimulatory effect on the synthesis and release of TRH (22) and may
potentiate the action of epinephrine on the TRH neurons during cold exposure. In contrast, NPY exerts a potent inhibitory effect on the transcription of the TRH gene (32) through inhibition of the cAMP-CREB second messenger pathway (33). NPY may play a role in antagonizing the increased release of epinephrine in the PVN in several physiological or pathological situations (34).

A second input to TRH neurons arises from peptidergic neurons in the arcuate nucleus; these neurons are believed to mediate leptin changes in the HPT axis during fasting (35). Fasting reduces leptin secretion, which results in an increased appetite, energy conservation, and alterations in neuroendocrine axes (36, 37). The HPT axis is affected by fasting resulting in reduced prepro-TRH mRNA synthesis in the PVN, and as a consequence, lower TSH and TH serum levels (38). Two separate leptin-responsive neuronal groups in the arcuate nucleus, with opposing function, send projections to TRH neurons. These neurons signal through either the anorectic peptides CART and α-MSH or the orexigenic peptides NPY and agouti-related protein (AGRP) (35). The balance between the effects of both neuronal groups may also be important in establishing the TRH neuronal set-point for TH feedback inhibition.

Finally, the hypothalamic dorsomedial nucleus (DMN) works as a metabolic sensor for hypophysiotropic TRH neurons. The arcuate nucleus sends axon terminals containing α-MSH to the DMN and then the DMN send projections to TRH neurons (39). Direct arcuate-PVN and indirect arcuate-DMN-PVN signaling to the TRH neuron may represent alternative pathways by which leptin acts to regulate this neuron (18). In summary, TRH can be synthesized and secreted in many regions of the brain (40, 41), but the hormone synthesized in the hypophysiotropic TRH neurons is the only one regulated by TH (10). In addition to TH, stress, cold, and nutrition can affect TRH expression.

TRs
TR isoforms are members of the nuclear receptor superfamily of ligand-modulated transcriptional factors (42). Alternative splicing and transcription initiation of two genes produce all known ligand-binding TR isoforms: TRα1, TRβ1, TRβ2, and TRβ3. The expression and regulation of the TRs vary with isoform and tissue type (5, 43, 44). The immunocytochemical localization of TR isoforms in the adult rat brain was reported in several areas including the hypothalamus (45). More intense TR expression was found in the PVN, the arcuate nucleus, and median eminence of the adult rat (46), and TRβ2 was found in high abundance in the PVN (5). Indeed, the restricted expression of TRβ2 (thyrotroph, TRH neurons of PVN, developing ear, and developing retina) contrasts with the more ubiquitous expression of TRα1 and TRβ1 isoforms (42). A study using siRNA delivery to the mouse hypothalamus showed that the siRNA directed against TRβ1 blocks both T3-independent activation and T3-dependent modulation of TRH transcription. In contrast, siRNA directed against TRβ2 abrogated only T3 repression of transcription (47).

In addition to these findings, the study of TRβ2-null mice (animals lacking the TRβ2 isoform) demonstrated that basal prepro-TRH expression was increased in TRβ2-null mice to levels seen in hypothyroid wild-type mice, but expression did not change significantly in response to either hypothyroidism or T3 treatment. In contrast, the suppression of prepro-TRH mRNA expression in response to fasting was preserved in TRβ2-null mice. Thus, TRβ2 is the key TR isoform responsible for T3-mediated negative-feedback regulation by hypophysiotropic TRH neurons (48).

Deiodinase and MCT8
The intracellular concentration of T3 is an essential determinant of TRH regulation. The intracellular concentration of T3 is determined by cellular uptake as well as T3 production and degradation in the central nervous system. The two most important transporter families that are involved in the TH transport in the brain are the organic anion transporting polypeptide (OATP) and the monocarboxylate transporter (MCT). Among these, MCT8 shows particularly high activity toward T3 (49). One member of the OAT family, OATP 14, is expressed in the PVN, but this is not the higher-affinity TH transporter. On the other hand, MCT8 is expressed in many tissues, including the brain, where it is predominantly localized in neurons. MCT8 plays an important role in the transport of T3 into neurons and mutations in MCT8 interfere with the action and metabolism of T3 in these cells (49). Mice lacking MCT8 have normal TSH levels despite having high T3 levels. Furthermore, mice lacking the MCT8 have low cerebral T3 levels consistent with an inability to transport T3 into neurons (50). In humans, mutations in the MCT8 gene, located on the X chromosome, result in males with neurological abnormalities, including global developmental delay, central hypotonia, spastic quadriplegia, dystonic movements, rotary nystagmus, and impaired gaze and hearing. The endocrine findings include elevated T3 and decreased T4 levels in the presence of a normal TSH secretion (51).

T3 production and degradation occurs through T4 deiodination by two separate enzymes, type II (D2) and type III (D3) deiodinase (52). D2 activates thyroid hormone by converting T4 to T3, whereas D3 inactivates thyroid hormone by converting T3 into T2 and T4 into reverse T3. In some studies, hypothyroidism induced only a moderate increase in D2 mRNA in the hypothalamus and no increase in D2 activity (53). In the same way, when hypothalamic cells were analyzed in association with iodine deficiency, there was no increase in D2 activity in the hypothalamus contrary to what was observed in other regions in the brain (54). Taken together, these studies suggest that maintenance of a constant T3 tissue level may not be the main function of D2 in the hypothalamus.

In contrast, T3 produced by tanycytes, a unique glial cell type that lines the third ventricle, may be the primary source of T3 for feedback regulation of TRH neurons. Tanycytes express high concentration of D2 mRNA and produce T3 from peripheral circulating T4 (55). T3 can then diffuse into the substance of the brain to reach the hypothalamic PVN (55) or may be released into the median eminence and transported by axon terminals to the hypophysiotropic TRH neurons (24, 56–59). The D2 activity in the tanycytes under different circulating TH levels seems to contribute to the negative feedback regulation of the HPT axis.
perhaps because it allows the hypophysiotropic TRH neurons to sense any changes in T₄ output by the thyroid gland. D₂-KO mice demonstrated the critical importance of local T₃ production to control of the HPT axis. These animals have low brain T₃ levels associated with elevated serum T₄ and TSH levels, indicating the presence of central resistance to TH due to inadequate central production of T₃ (60).

The Thyroid Feedback Mechanism

TH regulates TRH gene expression and production through a negative feedback mechanism; TRH expression is high when TH levels are low, and TRH expression is suppressed when TH levels are increased. As outlined earlier, TRH expression is regulated by TH in the PVN (10, 61). This cell-specific action of TH suggests that the TRH neurons in the PVN have all the elements necessary to sense and respond to circulating peripheral TH levels.

As noted above, circulating T₄ is converted to T₃ by D₂ in tanycytes. The balance of evidence suggests that T₃ then gains access to the TRH neuron via the MCT8 transporter. After T₃ enters TRH neurons in the PVN, regulation occurs at two levels: expression of the prepro-TRH transcript and processing of pro-TRH into the mature TRH peptide. The regulation of TRH gene expression by T₃ occurs mostly through TRβ2, presumably via a direct mechanism. It is also possible that T₃ acts in the signaling pathway of TRH gene expression via other hypothalamic nuclei because TRβ2 is also expressed in the arcuate and ventromedial nuclei, and both nuclei can alter the set-point of TRH expression to fasting.

Clearly, the basal level of TRH gene expression is important in determining the set-point for regulation by TH through either a direct or indirect mechanism. What determines the basal level of TRH gene transcription is a matter of much debate. In in vitro studies, the unliganded TR-β2 has been shown to activate the TRH gene via its unique amino-terminal domain (62). This finding is consistent with the critical in vivo role of TR-β2 in regulating the HPT axis (48). Moreover, it does not appear to be possible to dissociate T₃-independent properties of the TR, which activate genes like TRH, from its T3-dependent activities that result in TH inhibition of gene expression.

One of the first steps toward understanding TRH regulation by TH was to map TRH response elements in the promoter. Deletional analysis of the TRH gene identified a region in the proximal promotor, named site 4, that contained two structurally different negative TH response elements. This region is highly conserved both in murine and human species (2, 63). The core sequence of site 4 (TGACCTCA) is similar to a CREB response element, suggesting a mechanism for cAMP and TH cross talk at the promoter (64). Although this site is important for cAMP and T₃ regulation of the TRH gene in vitro, the physiological importance of this region in vivo has yet to be proven.

Using transgenic knock-in mouse models, the mechanism of TH negative regulation has begun to be explored in vivo. In one model, two amino acids within the P box of the DNA binding domain of TR-β were mutated to those residues found in the glucocorticoid receptor (GR). This mutation (GS125) in vitro completely abolished TRβ DNA binding while preserving T₃ binding and cofactor interactions with TR. In functional assays, the mutant displayed defective trans-activation on both positively and negatively regulated promoters (TRH, TSHα, and TSHβ). However, the mutant GS125 TRβ bound to a composite TR/GR-response element and was fully functional on this hybrid TR/GR-response element. Mice carrying this mutation in the germline of both alleles displayed abnormal T₃ regulation of the HPT axis identical to phenotypic abnormalities previously observed in TRβ KO mice. TR-β DNA binding, therefore, is essential for negative feedback regulation of the HPT axis by TH (65, 66).

A second transgenic knock-in mouse model was constructed to determine whether TR cofactor interactions were essential for TH negative regulation. In vitro studies have demonstrated that TR activity is regulated by binding to both corepressor (CoR) and coactivator (CoA) proteins on TH positively regulated genes. TH stimulation is thought to involve dissociation of CoRs, such as nuclear receptor corepressor (NCoR) and silencing mediator for retinoic acid and TR (SMRT) (67), from the transcriptional complex and recruitment of CoAs, such as steroid receptor coactivator-1 (Src-1), to the liganded TR. The physiological role of CoAs bound to TRs, however, had yet to be defined in vivo. A TR knock-in mouse was generated using the E457A TRβ mutation; this mutation completely abolished CoA recruitment in vitro while preserving normal T₃ binding and CoR interactions. As expected, mice bearing this allelic mutation displayed abnormal TH-stimulated gene expression. Interestingly, however, these animals also displayed abnormal regulation of the HPT axis. Serum TH, TSH level, and pituitary TSH subunit mRNA levels were inappropriately elevated compared with those of wild-type animals, and T₃ treatment failed to suppress serum TSH and pituitary TSH subunit mRNA levels. These data illustrate the importance of an intact CoA-binding surface for both positive and negative regulation by TH in vivo (68).

CoAs are proteins that can remodel chromatin via enzymatic acetylation of histone tails or via regulation of transcriptional complex assembly at the promoter by interactions with RNA polymerase and general transcription factors (69). One of the best studied CoA proteins is Src-1, a member of the p160 class of transcriptional factors, first identified as steroid receptor coactivator (70) and then characterized as a coactivator of other nuclear receptors, including the TR (71, 72). Src-1 KO mice showed mild resistance to TH (73), suggesting that this CoA may be critical in TH regulation of the HPT axis in the E457A mouse. How recruitment of a CoA to the liganded TR results in inhibition of gene expression in the HPT axis remains a puzzle. Perhaps binding of this cofactor to the TR AF-2 domain recruits other corepressor proteins to the transcriptional complex, which then repress gene expression.

In conclusion, the mechanism of TH negative feedback control of the HPT axis has been clarified by recent studies. Experimental models demonstrated the critical role of hypothalamic TRH in the control of HPT axis and in establishing the set-point of the axis. The realization that the TR-β2 isofrom primarily regulates the HPT axis defined a mechanistic difference between TH inhibition and stimulation. Further studies are directed at
understanding other unique features of TH inhibition at the transcriptional level. Finally, TRH gene expression is also regulated by temperature, food intake, and stress. Thus the TRH neuron is well positioned to integrate information about the environment as well as circulating TH levels and ultimately affect metabolism in response to these physiological changes.

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Address all correspondence and requests for reprints to: Fredric E. Wondisford, Division of Metabolism, Departments of Pediatrics, Medicine, and Physiology, Johns Hopkins University Medical School, Baltimore, Maryland 21287. E-mail: fwondisford@jhmi.edu.

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