MECHANISMS IN ENDOCRINOLOGY

Beyond the fixed setpoint of the hypothalamus–pituitary–thyroid axis

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Abstract

The hypothalamus–pituitary–thyroid (HPT) axis represents a classical example of an endocrine feedback loop. This review discusses dynamic changes in HPT axis setpoint regulation, identifying their molecular and cellular determinants, and speculates about their functional role. Hypothalamic thyrotropin-releasing hormone neurons were identified as key components of thyroid hormone (TH) setpoint regulation already in the 1980s, and this was followed by the demonstration of a pivotal role for the thyroid hormone receptor beta in negative feedback of TH on the hypothalamic and pituitary level. Gradually, the concept emerged of the HPT axis setpoint as a fixed entity, aiming at a particular TH serum concentration. However, TH serum concentrations appear to be variable and highly responsive to physiological and pathophysiological environmental factors, including the availability or absence of food, inflammation and clock time. During food deprivation and inflammation, TH serum concentrations decrease without a concomitant rise in serum TSH, reflecting a deviation from negative feedback regulation in the HPT axis. Surprisingly, TH action in peripheral organs in these conditions cannot be simply predicted by decreased serum TH concentrations. Instead, diverse environmental stimuli have differential effects on local TH metabolism, e.g. in liver and muscle, occurring quite independently from decreased TH serum concentrations. The net effect of these differential local changes is probably a major determinant of TH action at the tissue level. In sum, hypothalamic HPT axis setpoint regulation as well as TH metabolism at the peripheral organ level is flexible and dynamic, and may adapt the organism in an optimal way to a range of environmental challenges.

Introduction

The tripeptide thyrotropin-releasing hormone (TRH) was the first hypothalamic hormone to be isolated and structurally characterised in the 1960s. Subsequent immunocytochemical studies in the rat hypothalamus revealed the presence of TRH neurons in a number of hypothalamic nuclei. A key role for TRH neurons in the paraventricular nucleus (PVN) of the hypothalamus in the neuroendocrine regulation of thyroid hormone (TH)
was revealed in the 1980s when an inverse relationship of serum TH levels with TRH mRNA expression in the PVN was observed during experimentally induced hypo- and hyperthyroidism (1). TRH neurons in the medial and periventricular parvocellular subdivisions of the PVN project to the median eminence (ME), in line with observations in experimental hypothyroidism showing increased TRH mRNA only in these subdivisions of the PVN (2).

Together, these observations led to the concept of hypothalamus–pituitary–thyroid (HPT) axis setpoint regulation, reflected by highly constant intra-individual TH serum concentrations under basal conditions. The standard model of thyroid homeostasis postulates an intraindividual logarithmic relationship between serum free thyroxine (FT4) levels and pituitary thyrotropin (TSH) release (for review see (3)). Twin studies showed that heritability accounts for > 60% of the variation in serum TSH and FT4 (4), while later studies identified a number of genetic loci linked to the HPT axis setpoint (5). The concept of a fixed intra-individual TH serum concentration was reinforced in the clinical setting once serum TSH concentrations could reliably be measured. Elevated serum TSH became the key laboratory finding in patients with primary hypothyroidism, while the reverse (suppressed serum TSH) was true in primary hyperthyroidism. Moreover, serum TSH became the most important biochemical monitor in the treatment of patients with levothyroxine. However, already in the 1980s, it became clear that a variety of illnesses, including myocardial infarction, sepsis and surgical procedures, cause a decrease in serum triiodothyronine (T3) levels and – in severe cases – T4, without an elevation in TSH. Since then, additional examples of exogenous factors (schematically represented in Fig. 1) inducing deviations from fixed setpoint regulation have been uncovered. Examples of

**Figure 1**
Exogenous determinants of the hypothalamus–pituitary–thyroid (HPT) axis setpoint. On the left side some examples are shown of physiological factors influencing the HPT axis setpoint, i.e. the day–night rhythm and the availability or absence of food. On the right side some pathophysiological factors are shown, i.e. acute inflammation and critical illness. Within the circle, the HPT axis setpoint is driven mainly by TRH neurons in the hypothalamic paraventricular nucleus (PVN).
physiological factors include the diurnal TSH rhythm with a clear nocturnal TSH surge (6), which is driven by the hypothalamic suprachiasmatic nucleus (SCN), and prolonged fasting, which induces a decrease both in serum T3 (by 30%) and serum TSH (by 70%) in healthy men (7). By inference, feeding status and time-of-day effects should be considered in careful interpretation of serum TSH in a clinical setting. Examples of pathophysiological factors inducing low serum TH without an increase in serum TSH include acute inflammation and prolonged critical illness (8). This review discusses dynamic changes in HPT axis setpoint regulation, identifying their molecular and cellular determinants, and speculates about their functional role.

### HPT axis regulation in the basal state

**Hypothalamus**

**TRH neurons** The hypothalamic tripeptide TRH was discovered in the 1960s and subsequently shown to regulate the synthesis, release and biological activity of TSH via the TRH receptor (TRHR). TRH-synthesising neurons are present in a number of hypothalamic nuclei, but only hypophysiotropic TRH neurons located in the PVN are involved in the central regulation of the HPT axis. In the rat hypothalamus, hypophysiotropic TRH neurons are found in the medial and periventricular subdivisions of the parvocellular PVN exclusively (for review see (2)). In the 1990s, the first studies on the distribution of TRH neurons in the human hypothalamus appeared (9, 10) showing that TRH-containing neurons and fibres are present in a number of hypothalamic nuclei, including the PVN, the SCN, which contains the circadian pacemaker of the brain acting as a biological clock, and the sexually dimorphic nucleus. The human PVN contains many spindle-shaped and spheric multipolar parvocellular TRH neurons, especially in its dorsocaudal portion, while only a small number of magnocellular neurons express TRH. Although the precise efferent projections of hypothalamic TRH-containing neurons in the human brain are unknown, dense TRH fibre networks, e.g. in the perifornical area, suggest an important role for nonhypophysiotropic TRH neurons in the human brain as demonstrated earlier in the rat. A key role for thyroid hormone receptor beta 2 (TRβ2) in TH negative feedback on TRH neurons in the PVN was demonstrated by studies carried out in TR isoform-specific knockout mice (11), although immunocytochemical studies showed that TRH neurons in the PVN may express all TR isoforms (12, 13).

Local TH metabolism A number of molecular determinants, including transporters and enzymes, are critical for local TH bioavailability. THs have to be transported into cells in order to be able to exert their effects. In the human hypothalamus, three types of TH transporters have been reported: the organic anion transporting polypeptide 1C1 (OATP1C1), which preferentially transports T4, and the monocarboxylate transporter 8 (MCT8) and MCT10, facilitating both the uptake and efflux of T3 and T4 (14, 15, 16). Once transported into the cell, only T3 binds to the TR in the nucleus, while the pro-hormone T4 needs to be converted into the active hormone T3 by the deiodinating enzymes (17). Deiodination of TH is catalysed by the selenoenzyme family of iodothyronine deiodinases, which consists of three deiodinases: type 1 (D1), type 2 (D2), and type 3 (D3). Both the inner (phenolic) ring and the outer (tyrosyl) ring of T4 can be deiodinated, ultimately leading to the formation of the inactive 3,3′-diiodothyronine (T2). D1 is mainly expressed in liver, kidney, thyroid, and pituitary, and it can deiodinate both the inner- and the outer-ring of T4. D2 and D3 are the major deiodinating enzymes in the central part of the HPT-axis. D2 is expressed in many areas of the brain, and also in the pituitary, brown adipose tissue (BAT), placenta and – although at remarkably low levels – in skeletal muscle. It represents the main T3-producing enzyme in these tissues (18). D2 in the cortex and pituitary gland is negatively regulated by T3 and T4 at the pre- and post-transcriptional level respectively (19). D3 is a TH-inactivating enzyme, as it can only catalyse the inner-ring deiodination of T4 and T3. D3 is highly expressed in brain, especially during development, and in placenta (18). The interplay between tissue D2 and D3 determines the local availability of intracellular T3 levels and, thereby, the level of T3-regulated gene expression. Both D2 and D3 are expressed in the hypothalamus. D2 activity was reported in the rat hypothalamus, especially in the arcuate nucleus (ARC), already in the 1980s (20), and both D2 and D3 enzyme activities were reported in human pituitary and hypothalamic tissue samples obtained during autopsy (21). Moreover, D2 immunoreactivity is present in cells throughout the ependymal layer of the third ventricle, in the glial cells within the infundibular nucleus/ME region and in hypothalamic blood vessel walls. D3 expression showed a very different distribution, as D3 immunoreactivity was reported only in neurons in various hypothalamic nuclei, including the PVN, suggesting that D3 is expressed in T3-responsive neurons to terminate T3 action (for review see (22)).
Neurally mediated effects of intrahypothalamic T₃ on metabolism ★ In addition to acting on hypophysiotropic TRH neurons in the PVN, thereby regulating the HPT axis, intrahypothalamic T₃ exerts metabolic effects in peripheral organs via neural routes, e.g. via sympathetic and parasympathetic outflow from the brain to BAT, liver, and heart (23). The first indication that metabolic effects of THs can be centrally mediated was obtained in mice heterozygous for a mutant Trα with low affinity for T₃. These mice were hypermetabolic and showed a high BAT activity with increased thermogenesis and energy expenditure. The metabolic phenotype was blunted after a functional denervation of sympathetic signalling to BAT by housing them at thermoneutrality, suggesting that the CNS controlled the hypermetabolism of these mice through the autonomic nervous system (24). Then, Lopez et al. (25) showed that the activation of the thermogenic programme in the BAT through the sympathetic nervous system (SNS) depends on T₃-mediated activation of de novo lipogenesis by inhibiting AMPK in the ventromedial nucleus of the hypothalamus (VMH), establishing a role for T₃ in the VMH in the regulation of BAT. In addition to the VMH, T₃ was shown to act within the PVN to regulate hepatic glucose production and insulin sensitivity via sympathetic and parasympathetic outflow to the liver (26, 27). A third example of modulation by TH of neural outflow from the hypothalamus was recently provided in yet another hypothalamic neuron population, i.e. the parvalbuminergic neurons in the anterior hypothalamic area (AHA). These neurons require TR signalling for proper development and function and depend on THs to integrate temperature information with the regulation of cardiovascular parameters via modulation of central autonomic outflow (28). Finally, intrahypothalamic T₃ has stimulating effects on eating behaviour. Specifically, the T₃-mediated hyperphagia was shown to be mediated by activation of the mTOR pathway in the hypothalamic ARC, where mTOR co-localises with the TRα (29). These novel and topographically highly differential metabolic effects of intrahypothalamic T₃ are schematically represented in Fig. 2.

Functional connections ★ Detailed studies in rodents have shed light on the numerous and complex neural inputs to hypophysiotropic TRH neurons. Together with humoral signals reaching the PVN via the circulation, TRH neurons can integrate metabolic and endocrine information obtained via neural projections, enabling them to adjust the activity of the HPT axis to the changing environmental conditions. The ARC is an important hypothalamic nucleus sending efferent projections to TRH neurons in the PVN, thereby conveying information about the metabolic state of the organism. Within the ARC, two neuronal populations are particularly involved in the relay of metabolic information, i.e. the orexigenic neurons that produce neuropeptide Y (NPY) and agouti-related protein and the anorexigenic neurons that produce αMSH and CART. Similar innervation patterns of TRH neurons, with the exception of CART, have been reported in the human hypothalamus (for review see (30, 31)). In addition to the ARC, anatomical and physiological experiments have shown a role for the hypothalamic dorsomedial nucleus in the regulation of hypophysiotropic TRH neurons, but little information is available on the mechanisms involved. Finally, hypophysiotropic TRH neurons receive a dense catecholaminergic innervation from the brain stem, the majority of which is from

Figure 2
Thyroid hormone (TH) modulates energy metabolism via neural routes originating in hypothalamic nuclei. In the paraventricular nucleus (PVN), TH has a negative feedback action on hypophysiotropic TRH neurons, which are a major determinant of the HPT axis setpoint. In addition, TH modulates pre-autonomic neurons in the PVN, thereby modulating autonomic (both sympathetic and parasympathetic) outflow to the liver, in turn modulating endogenous glucose production and hepatic insulin sensitivity. In addition, TH affects neurons in the VMH, thereby stimulating energy expenditure in brown adipose tissue (BAT). Finally, TH acts on neurons in the arcuate nucleus (ARC) that modulate eating behaviour. 3rdV, third ventricle; yellow lines, neural pathways; blue lines, endocrine pathways.
adrenergic neurons (32), and this input is probably involved in the response of TRH neurons to cold (33).

Over the past decade, tanycytes have been recognised as important regulators of the HPT axis. These cells are specialised glial cells that line the ventrolateral wall and the floor of the third ventricle. Although there are several subtypes, they all have a small cell body located in the ependymal layer and a long process that may project to the ME, or the ARC, VMH, or DMH. The role of tanycytes in HPT axis regulation is increasingly recognised. These cells express TRs, as well as MCT8 and OATP1C1, and are capable of adapting their morphology according to the changes in circulating TH levels, perhaps regulating TRH release from hypophysiotropic terminals into the portal circulation. Furthermore, they express the TRH-degrading enzyme PPII, which is upregulated in hyperthyroidism. Finally, tanycytes are assumed to contribute to HPT axis feedback regulation by their expression of D2 and – under defined circumstances – D3 (for review see (31)).

Anterior pituitary

The anterior pituitary contains various types of adenohypophysial cells that are defined by the hormones secreted. Thyrotrophs secrete TSH and are preferentially located in the anteromedial and anterolateral portions of the pituitary. These cells express the TRHR, which is a member of the seven transmembrane-spanning, GTP-binding, G protein-coupled receptor family. Activation of this receptor by TRH stimulates both synthesis and release of TSH. Increased hormone production is thought to be regulated via activation of protein kinase C, while rapid release of stored TSH is regulated via activation of inositol 1,4,5-triphosphate (IP3) and subsequent release of intracellular Ca2+. TRH also stimulates the glycosylation of TSH, which is necessary for its full biological activity (34). TSH production and secretion are also regulated by circulating TH levels, as high TH levels inhibit TSH production and secretion while low TH levels activate TSH production. This so-called negative feedback regulation of TSH involves local D2-mediated conversion of T4 into T3, which is subsequently bound by TRβ2, finally resulting in the repression of the TSHβ gene (11). A crucial role of pituitary D2 in TSH regulation is supported by impaired TH feedback on TSH in D2-knockout mice (35).

Additional inhibitors of TSH secretion are the hypothalamic neuropeptide somatostatin, as well as dopamine and glucocorticoids. The latter impair the sensitivity of the pituitary to TRH. Pituitary peptides such as neuromedin B and PIT1, both expressed in thyrotrophs, are further determinants of TSH secretion (36, 37, 38). Finally, IGSF1, a pituitary membrane glycoprotein, was recently identified as a novel player in TSH regulation. Loss-of-function mutations in the IGSF1 gene result in congenital central hypothyroidism. Animal studies using Igsf1-knockout male mice exhibit diminished pituitary TRH-R expression, decreased pituitary and serum TSH levels, and decreased serum T3 concentrations, in line with the clinical observations (39). The net result of these various peptidergic, enzymatic and neuroendocrine factors determines serum TSH concentration, which plays a critical role in the regulation of the thyroid gland by activating the TSHR on the follicular thyrocytes. The TSHR is also expressed by folliculo-stellate (FS) cells in the human anterior pituitary, suggesting that TSH secretion might be additionally regulated in a paracrine manner via FS cells (40).

Thyroid gland and peripheral organs

TH production by the thyroid gland is mainly regulated by TSH via binding to the TSHR on the follicular thyrocyte. Activation of the TSHR stimulates a variety of processes involved in TH synthesis, ultimately resulting in the release of T4 (the prohormone) and T3 (the active hormone) from thyroglobulin (41). In healthy individuals, 20% of daily T3 production is secreted by the thyroid gland, whereas 80% is generated extrathyroidally by iodothyronine deiodinases (42). Once released, T4 and T3 circulate in the bloodstream bound to serum proteins including thyroid hormone-binding globulin, transthyretin, and albumin. Over 99% of serum THs is bound, leaving ~1% of TH as freely available for uptake by target tissues. As mentioned earlier, TH are actively transported into cells in order to exert their effects, while the prohormone T4 needs to be converted into the active hormone T3 by deiodinating enzymes (17). It has been thought for many years that liver D1 is critical for release of T3 into the circulation, but more recent studies have suggested that liver D1 is more important for TH clearance in the hyperthyroid state (43). Its expression is positively regulated by T3 (44, 45).

In the last few years, polymorphisms of deiodinating enzymes have been reported (46). The consequences of these polymorphisms on the regulation of the HPT-axis are unknown at present, although subtle changes in serum TH concentrations occur in association with these polymorphisms. In the DIO1 gene, two polymorphisms have been identified that affect serum T3 and reverse T3 (rT3) concentrations in healthy subjects, i.e. D1-C78ST
and D1-A1814G. The D1-785T variant is associated with higher rT3 levels and a lower T3/rT3 ratio, suggesting that this substitution results in decreased D1 activity. By contrast, the D1-1814G substitution is associated with a higher T3/rT3 ratio, which indicates increased activity of D1. For the DIO2 gene an association was reported in young subjects between the serum T3/T4 ratio and a polymorphism in a short open reading frame (ORFa) in the 5′-UTR of D2 (D2-ORFa-Gly3Asp) (47). Another polymorphism in the DIO2 gene, D2-Thr92Ala, is not associated with serum TH or TSH levels, but with insulin resistance (48) and decreased bone turnover (49). The mechanism has remained enigmatic as cells transfected with D2-92A or D2-92Thr do not show altered D2 activity. As to the DIO3 gene, one polymorphism has been identified (D3-T1546G), located in the 3′-UTR, but this variant does not affect serum TH levels in healthy subjects.

In target tissues, T3 has to be bound by a TR to modulate gene transcription. The TR is a member of the nuclear receptor family, and the protein structure consists of different domains, i.e. the N-terminal activation function 1 (AF1) domain (A/B), the DNA-binding domain (C), the hinge region (D) and the C-terminal AF2 domain (E) (50). TRs are encoded by two genes: the TRHA and TRHB genes. Owing to alternative splicing and alternative promoter usage, the TRHA-gene may give rise to six isoforms: TRa1, TRa2, TRΔa1 and TRΔa2, and p46 and p28 (51). The TRHB gene encodes the TRb1 and TRb2 isoform via alternative promoter usage (52). Only the TRb1, TRβ2, and TRα1 are bona fide TRs, having a ligand-binding domain and a DNA-binding domain which modulate gene transcription (51). The function of the other isoforms is unknown, although TRα2 and the short isoforms TRΔα1 and TRΔα2 are able to inhibit TRα1 and TRβ1-mediated transcriptional activation (53). TRα binds T3 with slightly higher affinity than TRβ1 (54). The DNA-binding domain of the receptor modulates gene transcription by binding to specific DNA sequences, known as thyroid hormone-response elements (TREs). TRs can bind to a TRE as monomers, as homodimers or as heterodimers with the retinoid X receptor, which is another member of the nuclear receptor superfamily that binds 9-cis retinoic acid. The heterodimer has the highest affinity and represents the major functional form of the receptor. TRα1 and TRβ1 show extensive sequence homology, specifically in domain C, D, and E. However, TRα1 and TRβ1 have isomorph-specific roles in the mediation of T3 action, which is supported by the fact that TRs are differentially expressed during embryonic development, in different tissues and even within the same organ (21, 55).

Additional levels of transcriptional regulation can be achieved by the potential of both isoforms to homo- or heterodimerise, by the type of TRE present on the promoters of T3 target genes and by the interaction with various cellular proteins. These cellular proteins can also be expressed in a tissue-dependent and developmentally regulated manner (56).

Mutations in the TR give rise to a variety of clinical symptoms depending on the TR involved. Resistance to thyroid hormone (RTH) is a clinical syndrome wherein TH levels are increased without adequate suppression of TSH. The most common cause is heterozygous mutations in the TRHb gene, mostly affecting the ligand-binding domain and the hinge region. The mutant TRb displays either reduced affinity for the ligand T3 or disturbed interaction with cofactors necessary for T3 action. RTH occurs to a similar extent in both sexes and has a world-wide distribution with an incidence of ~1 in 40 000 (57). Classic symptoms of RTH are goitre, tachycardia, developmental delay and failure to thrive, hearing loss, and bone age retardation (58), although the clinical picture is highly variable. Serum TH levels are increased in association with TSH within or just above the reference range due to nonresponsiveness of the pituitary and/or hypothalamus to regulate TSH production upon stimulation of the TRβ2. RTH patients display a hyperthyroid phenotype in tissues mainly expressing TRα1, such as the heart (tachycardia), while in tissues mainly expressing the TRβ1 (liver and kidney) and TRβ2 (hypothalamus, pituitary, cochlea, and retina) a hypothyroid phenotype is observed. Recently, mutations in the THRA gene have been reported, which are associated with growth and developmental retardation, skeletal dysplasia, and severe constipation. Of note, serum TH levels are only slightly abnormal. The clinical phenotype is a characteristic for hypothyroidism with regard to the skeleton, intestine and neural development, reflecting TRα-responsive tissues (59, 60).

**HPT axis setpoint regulation: examples of physiological determinants**

**Clock time**

One of the physiological determinants known to affect the HPT-axis is clock time: serum TSH is low during daytime, starts to increase in the early evening and peaks around the beginning of the sleep period. This phenomenon is known as the nocturnal TSH surge in humans (61, 62). The diurnal TSH rhythm is generated by the hypothalamic SCN, which is the biological clock of the brain, as
demonstrated by a number of experimental studies in rats (63). First, efferent fibres from the SCN contact TRH neurons in the PVN. Second, neuroanatomical studies using a retrograde transneuronal tracer revealed multisynaptic neural connections between the hypothalamic SCN and the thyroid gland via sympathetic and parasympathetic outflow. In addition, pre-autonomic neurons in the PVN, including TRH-immunoreactive neurons, were labelled after injection of the tracer into the thyroid gland (for review see (63)). Finally, a role for the SCN as the driver of the diurnal TSH rhythm in the circulation was confirmed by the observation that a thermic ablation of the SCN completely eliminates the diurnal peak in circulating TSH in rats (64). A recent study in healthy volunteers has confirmed that the 24-h TSH secretion is stable and robust, and not influenced by sex, BMI, or age (65). In spite of the clear diurnal variation in serum TSH levels, a diurnal rhythm in serum T3 and T4 concentrations is less obvious, illustrating that the diurnal TSH rhythm is not driven by negative feedback of serum TH on the level of the hypothalamic or pituitary. Finally, it should be noted that the physiologic meaning of the TSH rhythm is still elusive.

Feeding status

Feeding status is a major determinant of HPT-axis regulation. Fasting induces profound changes in TH metabolism characterised by decreased serum TH levels while serum TSH does not change or even decreases. The absence of a rise in serum TSH, which would be expected as a consequence of decreased negative feedback regulation, suggests that the hypothalamus and/or pituitary is involved in the observed alterations, as they are reminiscent of central hypothyroidism. In line, animal experiments showed that the fasting-induced central hypothyroidism could be completely prevented by systemic leptin administration, i.e. by restoring the fasting-induced decrease in serum leptin concentrations (66). The primary target for leptin in this setting appeared to be the ARC, from which monosynaptic efferent connections to the PVN modulate the activity of hypophysiotropic TRH neurons. The observed downregulation of the central component of the HPT axis is further characterised by an increase in D2 expression in the mediobasal hypothalamus, presumably increasing local T3 concentrations, and a decrease in TRH expression in the PVN (67, 68) (see also (31)). Chan et al. showed that a period of 72-h fasting in healthy men induces a decrease in serum T3 by 30%, and a marked suppression of TSH secretion with a decrease in integrated area by over 70% as well as loss of the typical pulsatility characteristics observed in the fed state. Interestingly, administration of a replacement dose of leptin designed to maintain serum leptin at levels similar to those in the fed state largely prevented the starvation-induced changes in the HPT axis (7). In addition to these changes at the central level of the HPT axis during food deprivation, peripheral TH metabolism is also affected by fasting. For example, liver D3 activity increases in mice after 48 h of starvation, which may further decrease hepatic T3 availability. Leptin administration selectively restores this starvation-induced D3 increase, independently of altered serum TH concentrations (69). The combination of central and peripheral alterations is likely to account for the fasting-induced decrease in serum TH levels. At present, it is unknown to what extent peripheral changes are mediated centrally.

A recent study in mice, however, has shown that both the melanocortin receptors MC4R and NPY are required for the activation of hepatic pathways that metabolise T4 during the fasting response (70), showing that starvation reduces TH availability both through central and peripheral circuits. The fasting-induced decrease in serum TH levels is assumed to be an important adaptive mechanism to conserve energy during times of food shortage (71).

HPT axis setpoint regulation: examples of pathophysiological determinants

It has been known for many years already that profound changes in TH metabolism occur during illness, the so-called nonthyroidal illness syndrome (NTIS) or the low-T3 syndrome. NTIS is characterised by decreased serum T3 and – in severe illness – serum T4, as well as increased serum rT3 concentrations. The expected increase in serum TSH is absent, reflecting a major change in negative feedback regulation (72). NTIS is a heterogeneous entity, and may occur in the setting of a great variety of illnesses (72). Recent studies have shown that TH action at the tissue level during illness is not a simple reflection of serum TH concentrations. Instead, NTIS has differential effects on local TH metabolism in various organs, which appear to occur quite independently from decreased serum T3 and T4 concentrations. The net effect of these differential changes is probably a major determinant of TH availability and, therefore, of TH action at the tissue level.
Acute inflammation

Acute inflammation is known to induce profound alterations in both circulating serum TH levels and tissue TH metabolism. Although the inflammation-induced alterations in local TH metabolism have not been studied extensively in humans, major surgery – an example of acute NTIS – induces a rapid inflammatory response characterised by activation of neutrophils and the release of a variety of proinflammatory cytokines (73, 74, 75). Simultaneously, significant alterations in serum T₃, T₄ and rT₃ concentrations and in T₃/rT₃ and T₄/T₃ ratios are observed, suggesting impaired TH conversion. Experimental studies in rodents have shown that administration of bacterial endotoxin (lipopolysaccharide (LPS)), which represents a model for severe and acute inflammation, results in down regulation of TRH expression in the PVN of the hypothalamus, probably via a local activation of D₂ in tanyocytes lining the third ventricle (76, 77). This may explain the absence of a TSH response to the decreased serum TH concentrations. LPS administration elicits a strong inflammatory response, characterised by the production of a variety of cytokines including tumor necrosis factor alpha, interleukin 1 (IL1), and IL6. For the induction of cytokines, the activation of inflammatory signalling pathways such as NFκB and activator protein 1 is mandatory (78). LPS administration also results in marked local changes in liver and muscle TH metabolism. For instance, hepatic D1 and D3 expression and activity decrease after LPS (79), presumably resulting in decreased liver TH concentrations, while D2 expression and activity increase in close correlation with liver IL1β. Inflammation-induced D2 expression was confirmed in macrophages and was absent in hepatocytes (Fig. 3). Moreover, D2 knockdown in macrophages attenuated LPS-induced granulocyte-macrophage colony-stimulating factor (GM-CSF) expression and affected phagocytosis in a negative way. Macrophages express MCT10 and TRα1, while hepatocytes predominantly express the TRβ1. Thus, locally produced T₃, acting via the TRα, may be instrumental in the inflammatory response in the liver (Fig. 3). In line, LPS-treated TRα⁻/⁻ mice showed a markedly decreased LPS-induced Gm-csf (Csf2) mRNA expression (80).

Chronic inflammation, sepsis, and critical illness

Chronic inflammation, sepsis, and critical illness are all associated with profound decreases in serum TH levels. The magnitude of the decrease in serum T₃ is related to the severity of illness; serum T₃ may become very low or even undetectable in critical illness. In severe cases, serum T₄ decreases as well and is inversely correlated with mortality: when serum T₄ falls below 50 nmol/l the risk of death increases to 50%, and with serum T₄ below 25 nmol/l mortality increases even further to 80% (for reviews see (8, 72)). A systematic review of studies in patients with sepsis and/or septic shock confirmed a correlation between decreased thyroid function at baseline and worse outcome (81). Careful analysis of the secretory TSH profile in patients with critical illness showed a loss of the nocturnal TSH surge as well as a loss of the pulsatile fraction, with a dramatically suppressed pulse amplitude in the prolonged phase of illness (82). Thus, although a simple TSH measurement can be within the reference range, the lack of TSH pulse amplitude correlates positively with the low serum T₃. Together, these observations point to altered setpoint regulation at the level of hypophysiotropic TRH neurons in the PVN. Indeed, TRH mRNA expression in the PVN was reduced in the hypothalamus of patients who had died after prolonged illness, and correlated positively
(instead of negatively) with serum T₃ (83). The latter observations were confirmed in a rabbit model for critical illness (for review see (8)). As the infusion of exogenous TRH together with the growth hormone (GH) secretagogue GH-releasing peptide 2 in critically ill patients restored not only pulsatile TSH and GH secretion but also circulating T₃ and T₄ levels, the suppression of the HPT axis in critical illness seems primarily of hypothalamic origin. In addition to changes in HPT axis setpoint regulation, the net result of which is decreased serum TH concentrations, there are marked changes in peripheral tissue TH uptake, metabolism and signalling. Only few studies have addressed TH tissue concentrations in this setting (84). Other examples of studies in ICU patients at the tissue level have shown that the decrease in serum T₃ is associated with changes in deiodinase expression in liver and muscle (85). Two recent review articles have addressed this issue extensively (8, 72).

Although NTIS may represent an adaptive response during acute inflammation, NTIS might turn disadvantageous during prolonged critical illness, necessitating mechanical ventilation, dialysis and inotropic support. There are many studies to suggest that the neuroendocrine response to illness can be seen as a dynamic process, with distinct features in the acute and chronic phase of critical illness (86), but only very few studies have addressed the changes in local TH metabolism in patients with prolonged critical illness. These studies were mostly based on samples obtained from critically ill patients shortly after death. Liver T₃ and T₄ concentrations were reported to be low in samples of NTIS patients as compared with healthy controls, indicating that the liver may be deficient in THs during prolonged critical illness (87). In agreement with this are the decreased liver T₃ levels observed in a rabbit model of prolonged critical illness (88). Prolonged critically ill patients develop a neuroendocrine dysfunction with suppressed hypothalamic TRH expression. The mechanism behind the suppression at the central level of the HPT axis in these patients is unknown at present. It is important to note that prolonged critically ill patients may theoretically benefit from correction of the TH changes, but this challenging hypothesis has not been tested to date.

**Conclusion**

Under basal conditions, the HPT axis is regulated by negative TH feedback at the hypothalamic and pituitary level, resulting in stable circulating FT₄ concentrations. However, a number of environmental challenges induce complex interactions of novel players, including D₂ in hypothalamic tanyctyes, which result in a net TH setpoint change. For example, during food deprivation and inflammation, TH serum concentrations decrease without a concomitant rise in serum TSH. Surprisingly, TH action at the tissue level in these conditions is not a simple reflection of decreased TH serum concentrations. Instead, there appear to be differential effects on local TH metabolism in liver and muscle, which occur quite independently from TH serum concentrations. In sum, hypothalamic HPT axis setpoint regulation as well as TH metabolism at the peripheral organ level appear to be dynamic, and may help to adapt the organism to a range of environmental challenges.

**Declaration of interest**

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