Thyroid hormone and the response to cold exposure

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Abstract

Thyroid hormone (TH) plays a key role in regulating body temperature in mammals by stimulating energy expenditure through both obligatory and adaptive thermogenesis in a variety of tissues. Cold exposure stimulates the hypothalamus-pituitary-thyroid (HPT) axis at the hypothalamic level by activating hypophysiotropic thyrotropin-releasing hormone (TRH) producing neurons, ultimately resulting in increased plasma TH concentrations. In addition to these neuroendocrine effects, TRH neurons in the hypothalamus also have synaptic connections to brown adipose tissue (BAT) allowing sympathetic activation of thermogenesis independent of circulating TH levels. Finally, recent studies have demonstrated that intrahypothalamic TH has profound metabolic effects on BAT, liver and heart that are mediated via the autonomic nervous system. These effects originate in various hypothalamic nuclei, including the paraventricular nucleus (PVN), the ventromedial nucleus (VMN) and recently reported neurons in the anterior hypothalamic area (AHA). Although robust stimulation of the thermogenic program in BAT was shown upon TH administration in the VMH, the physiological relevance of these neurally mediated effects of TH is unclear at present. This review provides an overview of studies reporting the role of TH in the cold defense, with a focus on recent literature evidencing the centrally mediated effects of TRH and TH.

Keywords: thyroid hormone, TRH, hypothalamus, thermogenesis, cold, brown adipose tissue

Introduction

Thyroid hormone (TH) has been known to be crucially involved in thermoregulation since many decades. Hypothyroid patients or experimental animals with low TH levels have decreased metabolic rate and impaired cold tolerance. Conversely, an excess of TH induces increased metabolic rate and heat production [1]. Circulating TH levels are maintained within a narrow physiological range as a result of negative feedback action in the context of the hypothalamus-pituitary-thyroid (HPT) axis [2,3]. The thyroid gland produces thyroxine (T4), and to a lesser extent, the biologically active thyroid hormone 3,5,3'-triiodothyronine (T3). In many tissues, T4 is converted into T3 by the enzyme type 2 deiodinase (D2) , but T4 and T3 can also be inactivated by the enzyme type 3 deiodinase (D3) [4]. The relative contribution of these enzymes enables a local regulation of T3 availability which is to a certain extent independent of circulating TH concentrations [4,5]. This has been shown to be of physiological importance during specific environmental conditions such as illness [6], fasting [7] and cold exposure [8]. In addition to a direct role for T3 availability in peripheral metabolic tissues, T3 regulates energy metabolism by its effects within the hypothalamus. A number of hypothalamic nuclei including the paraventricular nucleus (PVN), ventromedial nucleus (VMN), arcuate nucleus (ARC) and anterior hypothalamic area (AHA) have been shown to mediate intrahypothalamic effects of T3 on a variety of key metabolic functions including hepatic glucose production, brown adipose tissue (BAT) thermogenesis, food intake and cardiac function [9,10]. Moreover, subsets of thyrotropin-releasing hormone (TRH) neurons within the PVN are responsible not only for dynamic HPT axis set point regulation, but also contribute to the neural control of feeding, locomotor activity and body temperature [11]. Therefore, both hypothalamic TRH and T3 are essential for energy balance and for maintaining homeostasis upon cold exposure.

Effects of TH on obligatory and adaptive thermogenesis

Homeothermic animals have developed precise thermogenic mechanisms to keep a steady body temperature in face of generally colder environmental habitats. Heat can be generated by obligatory and facultative (or adaptive) thermogenesis. Obligatory thermogenesis is the involuntary thermogenic process due to basal metabolic activity including physical activity, food digestion and other vital life processes. The term adaptive thermogenesis refers to additional heat production when obligatory thermogenesis is insufficient. During cold exposure, skeletal muscle starts shivering immediately to generate more heat. However, after some time non-shivering adaptive thermogenesis will take over for a more efficient and sustained heat production [12]. TH increases not only obligatory thermogenesis by boosting basal metabolic rate but also increases adaptive thermogenesis by stimulating different thermogenic organs including BAT and skeletal muscle.

BAT is a specialized adipose tissue with smaller cellular fat droplets and a denser population of mitochondria compared with white adipose tissue (WAT). BAT is one of the main targets for T3 induced facultative thermogenesis. Upon cold exposure, the activity of type 2 deiodinase (D2), the main T3 producing enzyme, strongly increases in BAT via enhanced sympathetic signaling, resulting in an increase of local T3 concentrations independent of circulating TH [13,14]. Therefore, D2 knockout mice display impaired BAT thermogenesis and hypothermia upon cold exposure [15]. Increased T3 further accelerates transcriptional induction of genes essential for lipogenesis and mitochondrial activation. One of the T3 targeted genes codes for uncoupling protein 1 (UCP1). This BAT specialized protein increases the proton leakage during electron transport, thereby shifting energy oxidation from ATP production to the release of heat [16]. Of note, TH also modulates some UCP1-independent effectors including creatine which has been shown to enhance mitochondrial respiration and thermogenesis in adipose tissue [17]. Additionally, T3 increases transcription

of beta-adrenergic receptors which synergistically promotes BAT thermogenesis by enhancing norepinephrine signaling via the sympathetic nervous system (SNS) [18]. In addition to direct stimulation of BAT thermogenesis, T3 also increases mitochondria biogenesis by up-regulating transcription coactivators such as peroxisome proliferator-activated receptor-γ coactivator (PGC)-1α [19]. Thyroid hormone receptor (TR) activation by a synthetic agonist was reported to induce BAT-like thermogenesis in WAT [20]. Moreover, a recent study showed that central administration of T3 induced browning markers in WAT [21]. Thus, T3 may enhance adaptive thermogenesis by browning of white or beige adipocytes [22].

Similar to the regulation of BAT thermogenesis, TH is critical for an optimal thermogenic response in skeletal muscle. Heat production is higher in euthyroid skeletal muscle compared to hypothyroid muscle. T3 induced production of UCP3, which is the isoform of uncoupling protein primarily expressed in skeletal muscle, is associated with increased energy expenditure in skeletal muscle [23]. Nevertheless, T3 treatment did increase resting metabolic rate in UCP3 knockout mice similar to wild type mice suggesting that additional pathways, including sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA1) uncoupling mechanism [24], may be involved in TH induced skeletal muscle thermogenesis [25]. Another UCP-independent thermoregulatory pathway closely controlled by TH is heat preservation. Mice with a heterozygous mutation in thyroid hormone receptor alpha (TRα) exhibited impaired tail vasoconstriction resulting in lower nocturnal body temperature despite compensatory BAT hyperactivity. This defective tail heat dissipation was restored after T3 treatment, indicating a key role of TH in heat preservation by tail vasoconstriction [26]. A schematic representation for TH regulated thermoregulation is given in Figure 1.

Effects of cold exposure on the HPT axis

Cold exposure activates the HPT axis at different levels. The TRH-containing neurons involved in HPT axis regulation are the so-called hypophysiotropic TRH neurons, groups of parvocellular neurons located in the medial and periventricular portions of the hypothalamic PVN [11]. In humans, the distribution of TRH neurons is mainly restricted to the dorsacaudal portion of the nucleus [27]. Axons from these TRH neurons project to the hypothalamic median eminence (ME), where TRH is released into the portal system thereby reaching the anterior pituitary. TRH stimulates pituitary thyrotrophs to secrete thyroid stimulating hormone (TSH) which in turn stimulates T3 and T4 production and secretion by the thyroid gland [28]. TRH neurons receive innervations from neurons of other hypothalamic nuclei including appetite-regulating neurons from the arcuate nucleus and clock neurons from the suprachiasmatic nucleus, enabling a coordinated control of temperature, feeding and the circadian rhythm [3,29,30]. Interestingly, cold exposure may activate TRH neurons independently of circulating TH concentrations or nutritional state [31,32]. Furthermore, cold activates not only the hypophysiotropic TRH neurons stimulating anterior pituitary TSH release but also the non-hypophysiotropic TRH neurons in the PVN. These non-hypophysiotropic TRH neurons project to various brain areas exerting separate and diverse actions on energy homeostasis through neuronal effects mediated within the central nervous system [11].

Effects of TRH on BAT thermogenesis

Hypothalamic TRH controls BAT thermogenesis

TRH in the PVN is best known for its regulation of the HPT axis set-point, thereby affecting thermoregulation and energy metabolism. However, the non-hypophysiotropic TRH neurons also play a key role in thermoregulation through their projections to other brain areas.

Intracerebroventricular administration of TRH to Syrian Hamsters was shown to increase BAT activity and core temperature, without affecting circulating TH levels. These effects were attenuated by sympathetic denervation of BAT [33], suggesting a central effect mediated via the autonomic nervous system (ANS). In addition, a number of studies showed that TRH injections into the preoptic area of the anterior hypothalamus (POA), a well-recognized primary site for thermoregulation, causes hyperthermia in rats [34,35]. It has been demonstrated that TRH inhibits heat-sensitive neurons and activates cold-sensitive neurons [36] in the POA, a mechanism resulting in increased heat production and conservation. Interestingly, ablation of the POA did not block the TRH antagonism of pentobarbital-induced hypothermia, suggesting that sites other than the POA may also mediate the thermogenic effect of TRH [37]. Indeed, cold exposure greatly increased TRH mRNA and peptide expression in the PVN [38,39] thereby activating both hypophysiotropic and non-hypophysiotropic TRH neurons which strongly suggests a role for TRH neurons in body temperature regulation in response to cold. Moreover, TRH injections directly into other distinct hypothalamic areas including dorsal medial hypothalamus (DMH) and VMH have also been shown to induce hyperthermia [33]. TRH receptors and TRH-immunoreactive axon terminals are present in the PVN [40].

Systemic TRH administration activates BAT in humans

The involvement of TRH in the cold response is also indicated by the fact that TRH knockout mice showed cold intolerance, which could not be corrected by TH supplementation [41][9,37]. A recent study of Heinen et al. [42] evaluated the effect of an intravenous bolus injection of TRH on BAT thermogenesis in humans in a randomize-controlled trial using ^{18}F-FDG PET. They showed that 44% of the healthy volunteers who were pre-exposed to mild cold, displayed clear increases of ^{18}F-FDG uptake in BAT after TRH compared to placebo.

This increase in ^{18}F-FDG uptake was not paralleled by any changes in plasma TH. The exact mechanism of BAT activation induced by systemic TRH is still unknown, however, evidence from animal studies suggest a central effect of TRH acting through the hypothalamus [33,34].

Systemic effects of intrahypothalamic TH

Although THs have major effects on energy metabolism and thermoregulation by directly acting on peripheral organs such as adipose tissue and muscle, an increasing number of studies demonstrated neural effects of THs acting within the hypothalamus. The hypothalamus contains a number of nuclei containing anatomically and functionally clustered neurons that sense and integrate metabolic information from the body. Some of these nuclei project to hypothalamic premotor neurons enabling a fast response via autonomic outflow to peripheral organs [43]. Thyroid hormone receptors, transporters and deiodinases are widely expressed in the hypothalamus, providing a substrate for TH to regulate energy metabolism via intrahypothalamic effects [44,45].

T3 in the PVN controls glucose metabolism

Increased energy expenditure during cold is accompanied by increased glucose and fatty acid oxidation. Thyrotoxicosis is known to increase hepatic glucose production (EGP) as well as lipolysis and proteolysis, thereby providing substrates required for the concomitant increase in energy expenditure [46,47]. Intriguingly, thyrotoxicosis has also been associated with increased sympathetic nerves activity [48]. Earlier studies from our lab demonstrated that the stimulation of EGP by thyrotoxicosis was mediated in part via the sympathetic nervous system, as selective hepatic sympathetic denervation diminished the increase in EGP induced

by thyrotoxicosis [49]. These neurally mediated effects of T3 on glucose were shown to be mediated by the hypothalamus. Local administration of T3 via microdialysis in the PVN increased plasma glucose as well as EGP, without affecting circulating T3 levels. Selective hepatic sympathectomy prevented these effects indicating that intrahypothalamic T3 regulates hepatic glucose production by a sympathetic pathway from the PVN [50,51]. It is unknown at present if this pathway is stimulated simultaneously with a cold challenge.

T3 in the VMH controls BAT thermogenesis

There is increasing interest in the relationship between intrahypothalamic T3, increased sympathetic tone and BAT activation. The first indication for a close correlation between T3 and sympathetic signaling to BAT came from mice with a mutant TRα1 which showed increased sympathetic activity and a hypermetabolic phenotype. Functional blockade of sympathetic signaling normalized BAT thermogenesis in these mice, suggesting a sympathetic regulation of BAT activation by TH [52]. This hypothesis was confirmed and elegantly expanded by subsequent studies from Lopez *et al* [53]. They showed increased SNS activity and increased thermogenic markers in BAT after administration of T3 in the VMH [53]. These effects were reversed by pharmacological blockade of the SNS. The T3 induced sympathetic activation of BAT was further shown to be mediated by *de novo* lipogenesis and inactivation of AMP-activated protein kinase in the VMH. Indeed, acute injections of T3 directly into the VMH resulted in a rapid increase in sympathetic nerve activity towards BAT. Mittag *et al*. showed in mice that intracerebroventricular T3 administration using mini-pumps for a number of days resulted in increased oxygen consumption and body temperature only during the dark phase, effects that were dependent upon UCP1 [54]. In line with these observations, mice with a deficiency of the T3 inactivating enzyme type 3 deiodinase (D3)

212 revealed elevated locomotor activity and energy expenditure leading to lower body weight.
213 Histologic examination showed reduced adipocyte size in WAT and BAT, but unaltered
214 UCP1 mRNA expression. Based on these observations it was proposed that the hyper-
215 metabolic phenotype was secondary to an increased TH action in the hypothalamus despite
216 lower circulating TH concentrations [55]. Together, these studies demonstrated an important
217 role for central T3 in regulating BAT thermogenesis.
218
219 *T3 in the AHA controls cardiovascular function*
220
221 TH status is also linked with cardiovascular function by direct effects of T3 on sino-atrial
222 node firing and by indirect actions via the autonomic nervous system. Thus, hyperthyroid
223 patients often display enhanced cardiac contractility, while hypothyroidism may induce
224 reduced cardiac output and bradycardia (for review see [56]). However, more recent studies
225 on TH control of cardiovascular function revealed an additional central mechanism
226 originating in the hypothalamus. Mittag *et al.* reported that mice expressing the mutant TRα1
227 exhibit a slight decrease in heart rate and a severely impaired cardiovascular response to
228 stress or an environmental temperature challenge. This cardiac dysfunction in TRα1 mutant
229 mice was found to be due to an unbalanced sympatho-vagal tone from the brain [57]. Further
230 studies indicated that a previously unknown population of parvalbuminergic neurons in the
231 anterior hypothalamus (AHA) may be responsible for this dysregulation of heart function.
232 Stereotaxic ablation of these neurons resulted in hypertension and temperature-dependent
233 tachycardia. Interestingly, electrophysiological recordings indicated that these
234 parvalbuminergic neurons display intrinsic temperature sensitivity while a majority is
235 sensitive to TH treatment, suggesting an interactive regulation between cardiovascular
236 function and body temperature by TH [58].

T3 in the ARC controls feeding

Thyrotoxicosis induced increased food intake accompanied by increased hypothalamic neuropeptide Y (NPY) mRNA and decreased hypothalamic pro-opiomelanocortin (POMC) mRNA expression [59]. Mice lacking the T3 inactivating enzyme D3, presumably leading to increased hypothalamic T3 levels, showed increased NPY and decreased POMC gene expression [55]. Accordingly, direct T3 administration in the ARC resulted in increased feeding, a mechanism involving up-regulation of the hypothalamic mammalian target of rapamycin (mTOR) signaling pathway [60]. Interestingly, TH induced orexigenic effects were linked with thermogenic pathways in the hypothalamus. Other studies showed that fasting increased hypothalamic D2 activity and, thereby, local T3 production. Elevated T3 in ARC appeared to accelerate UCP2-dependent mitochondrial uncoupling in NPY/AgRP neurons, leading to consequent rebound feeding following food deprivation [61]. The observation that TH regulates POMC expression in the Arc may be relevant for cold induced BAT thermogenesis as a recent study showed that cold exposure induces autophagy in hypothalamic POMC neurons, which is necessary to activate lipophagy in BAT and liver through the sympathetic network favoring cold induced thermogenesis [62].

Acute versus chronic effects of intrahypothalamic T3

The experiments involving intrahypothalamic T3 administration to modulate neural regulation of energy metabolism were mostly performed using an experimental design that only allows the investigation of acute effects, with a time range from minutes to hours. We therefore aimed to develop a model for intrahypothalamic T3 administration in a more

chronic setting to study metabolic effects of longer lasting T3 exposure. This is of clinical relevance as some of the metabolic complications of thyrotoxicosis, such as weight loss and bone loss, occur after long-term exposure to TH excess. To this end, we designed an experimental protocol using T3-containing beeswax pellets that showed stable T3 release (~5 nmol/L) for 4 weeks *in vitro*. Bilateral implantation of these T3 pellets locally in the PVN or VMH of rats resulted in selectively increased T3 concentrations either in the PVN or VMH region for 28 days. Increased local T3 concentrations were shown to increase mRNA expression of T3-responsive genes. As expected, after placement of T3-containing pellets in the PVN, plasma T3 and T4 decreased, while there was no change in plasma TH after placement of T3-containing pellets in the VMH. Thus, our model is valid to selectively and chronically deliver T3 to specific hypothalamic nuclei [63]. However, surprisingly, body weight, food intake, and body temperature were not changed after T3 administration in the PVN or VMH for 28 days despite these nuclei-specific changes. In addition, no effects on energy expenditure, locomotor activity, or respiratory exchange rate were present upon 7 days of intrahypothalamic T3 administration. In view of the substantial metabolic effects by acute T3 treatment, our results imply that the effects of intrahypothalamic T3 on metabolism largely depend on the duration of treatment [64]. In line with our results, other showed that the effects of central TH administration on sleep were not only dose- but also time-dependent. An intermediate dose, but not a high dose of T3 in the preoptic area (POA) of euthyroid rats changed EEG-defined sleep patterns. These central T3 effects on sleep occurred only after acute (hours) but not after chronic (days) T3 treatment [65,66]. An overview of the differential effects of acute and chronic intrahypothalamic T3 administration on energy metabolism is given in Figure 2.

Although the mechanism behind the differential effects of acute and chronic intrahypothalamic T3 administration is currently unknown, several theoretical possibilities

exist. First, modulation of intrahypothalamic T3 may trigger a neural response in autonomic outflow to metabolic organs that is temporary, thereby modulating the classical TR-mediated and longer lasting endocrine effects of T3 in the periphery. Second, local non-genomic actions of T3 may account for acute effects of intrahypothalamic T3 while chronic T3 exposure may be mediated by intrahypothalamic TRs which may be downregulated upon long-term T3 exposure [67,68]. Finally, the adult hypothalamus maintains neuronal plasticity. For instance, leptin may stimulate neurite growth of NPY and POMC neurons in ARC [69]. Hence, chronic T3 administration in the hypothalamus may result in structural and/or functional adaptations which mask or counteract the metabolic changes observed during acute T3 administration. At present, however, there is no evidence available to support any of these possibilities.

Summary and conclusions

For many decades, thyroid hormone has been known to be critically involved in key metabolic processes including energy expenditure and heat preservation in response to cold. TH regulates both obligatory thermogenesis by increasing basal metabolic rate and adaptive thermogenesis by targeting BAT, WAT and skeletal muscle. TH also regulates hepatic glucose production, cardiac function and feeding which are all key metabolic responses in the cold defense. Recent and compelling evidence has shown many of these effects of TH are mediated through the hypothalamus via the ANS. However, differential metabolic effects between acute and chronic intrahypothalamic T3 treatment paradigms urge us to further explore the central mechanisms involved in these neural effects of thyroid hormone. In addition to T3, TRH is also a key regulator of BAT thermogenesis and cold defense, effects that are likely mediated also via the hypothalamus.

Figure legend

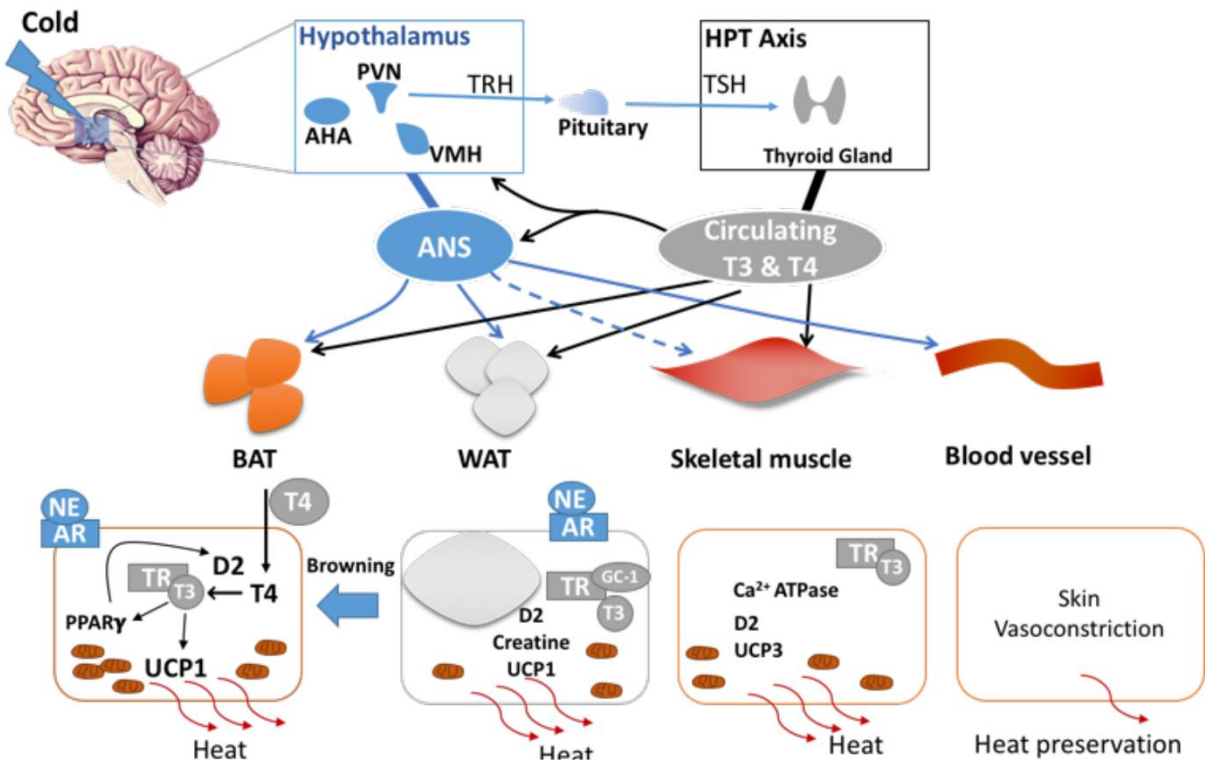
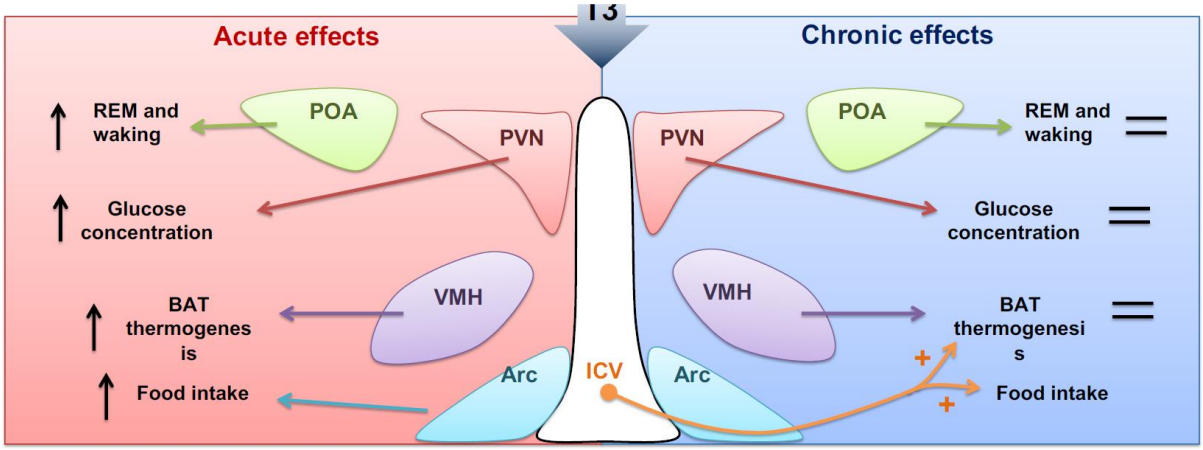


Figure 1: Diagram for combined central and systemic regulation of thermogenesis by TRH and thyroid hormone. AHA: anterior hypothalamus, VMH: ventral medial hypothalamus, PVN: paraventricular nucleus, TRH: thyrotropin-release hormone, TSH: thyroid stimulating hormone, HPT: hypothalamus-pituitary-thyroid gland, ANS: autonomic nervous system, BAT: brown adipose tissue, WAT: white adipose tissue, NE: norepinephrine, AR: adrenergic receptor, TR: thyroid hormone receptor, D2: type 2 deiodinase, UCP: uncoupling protein, GC-1:thyroid hormone receptors agonist



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322 Figure 2: Scheme summarizing reported differential effects of acute vs chronic
323 intrahypothalamic T3 administration on energy metabolism. Arc: arcuate nucleus, BAT:
324 brown adipose tissue, ICV: intracerebroventricular compartment, POA: preoptic area, PVN:
325 paraventricular nucleus, REM: rapid eye movement sleep, VMH: ventromedial nucleus. With
326 permission from Zhang et al, Mol Cell Endocrinol, Elsevier, 2017 [9].

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328

329 Table 1 Main studies involving central actions of T3 on energy metabolism
330

Sites of brain	Routes and duration	BAT	Liver	other findings
ICV	{Lopez, 2010 #114}			
	{Alvarez-Crespo, 2016 #2204}			
	{Martinez-Sanchez, 2017 #3141}			
AHA	Developmental dependent on TH {Mittag, 2013 #888}			
POA	{Moffett, 2013 #2871}			
PVN	MD {Klieverik, 2009 #112}		Increased glucose production	
	{Zhang, 2016 #2725}	No effect	No effect	Suppressed HPT axis
VMH	{Kong, 2004 #113}			
	{Lopez, 2010 #114}			
	{Zhang, 2016 #2725}	No effect	No effect	
	{Martinez-Sanchez, 2017 #3141}			
ARC	{Varela, 2012 #279}	No effect		Increased food intake

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