Regulation of TRH neurons and energy homeostasis-related signals under stress

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Abstract

Energy homeostasis relies on a concerted response of the nervous and endocrine systems to signals evoked by intake, storage, and expenditure of fuels. Glucocorticoids (GCs) and thyroid hormones are involved in meeting immediate energy demands, thus placing the hypothalamo–pituitary–thyroid (HPT) and hypothalamo–pituitary–adrenal axes at a central interface. This review describes the mode of regulation of hypophysiotropic TRHergic neurons and the evidence supporting the concept that they act as metabolic integrators. Emphasis has been placed on i) the effects of GCs on the modulation of transcription of Trh in vivo and in vitro, ii) the physiological and molecular mechanisms by which acute or chronic situations of stress and energy demands affect the activity of TRHergic neurons and the HPT axis, and iii) the less explored role of non-hypophysiotropic hypothalamic TRH neurons. The partial evidence gathered so far is indicative of a contrasting involvement of distinct TRH cell types, manifested through variability in cellular phenotype and physiology, including rapid responses to energy demands for thermogenesis or physical activity and nutritional status that may be modified according to stress history.

Key Words

- HPA
- HPT
- stress
- metabolism

Introduction

The tripeptide pglu-his-proNH2 was isolated from hypothalami and named according to its endocrine function: ‘thyrotrophin-releasing hormone’ (TRH; Boler et al. 1969, Burgus et al. 1969). TRH is synthesized in the neuronal cell bodies of many brain regions from a precursor protein, prepro-TRH (ppTRH), and processed to yield the biological active peptide (Lechan et al. 1986, Lechan & Segerson 1989, Nillni 2010). Several hypothalamic nuclei synthesize TRH (Fig. 1), but the TRH neurons that control thyrotrophin (TSH) release are the hypophysiotropic neurons present in the mid-caudal paraventricular nucleus (PVN) (anterior-medial PVN in mouse), whose axons project to the median eminence from where TRH is released into the portal vessels of the hypothalamo–pituitary system (Fekete & Lechan 2014; Fig. 1). In humans, hypophysiotropic TRH neurons are present in the medial region of the dorsocaudal portion of the PVN (Fliers et al. 2014).

The amount of released TRH that reaches the pituitary is modulated in the median-eminence extracellular space by the activity of the TRH-degrading ectoenzyme (pyroglutamyl peptidase II (PPII)) present on tanyocytes, a group of ependymal cells lining the third ventricle whose end-feet are in proximity with TRH terminals and portal vessels (Sánchez et al. 2009). In the pituitary and upon binding to its receptor (TRH-R1), TRH controls the release
of TSH and its transcription and posttranslational modifications, such as glycosylation, that define the bioactivity of TSH (Chiamolera & Wondisford 2009). Under certain physiological conditions, TRH modulates prolactin or growth hormone synthesis and release (Galas et al. 2009). The concentration of pituitary TRH-R1 is regulated by TRH and several other hormones (Chiamolera et al. 2012, Hinkle et al. 2012). Released TSH controls several steps of the synthesis of thyroid hormones (TH) at the thyroid gland, increasing serum levels of thyroxine (T4) and to a minor degree, 3,5,3’-triiodothyronine (T3). Deiodinases 1 or 2 (D1, D2) convert T4 to T3 in various tissues and their activity is differentially regulated in a condition- and tissue-specific manner (McAninch & Bianco 2014). TH have crucial roles in basal metabolic rate, thermogenesis, lipid and carbohydrate metabolism, indicating that the hypothalamus–pituitary–thyroid (HPT) axis is an important player in energy homeostasis (Hollenberg 2008, Fekete & Lechan 2014, McAninch & Bianco 2014, Mullur et al. 2014).

The activity of the HPT axis is stringently regulated by neuronal stimuli impinging on TRH neurons and by the negative-feedback effects of TH on TRH and TSH synthesis and release (Hollenberg 2008, Fekete & Lechan 2014, McAninch & Bianco 2014, Mullur et al. 2014).
Fliers et al. 2014). T₄ downregulation of Trh mRNA levels occurs specifically in the hypophysiotropic neurons of the PVN and not in other hypothalamic nuclei expressing Trh in rat or mouse (Segerson et al. 1987, Sugrue et al. 2010). The neuronal concentration of T₃ in the PVN is controlled by D₂-conversion of T₄ in tanycytes, the presence of T₃ transporters, and the activity of neuronal D₃ that inactivates T₃, converting it to rT₃ (Fliers et al. 2006, Fekete & Lechan 2014). The most commonly used sensor of activation of TRH neurons has been the measurement of Trh mRNA levels. Rapid (<2 h) changes in TRH tissue concentration, mostly concentrated at nerve endings have been used as an indirect marker of TRH release because at later times TRH content represents the resultant of synthesis and release but not of degradation because the intracellular peptidases do not modulate TRH levels within secretory granules (Rondeel et al. 1991, van Haasteren et al. 1995, Charli et al. 1998, Aguilar-Valles et al. 2007).

TRH biosynthesis

The Trh gene encodes a protein that contains 5–8 (depending on the species, Wallis 2010) repetitions of the gln-his-pro-gly sequence (Lechan et al. 1986). Like other neuropeptides, the precursor ppTRH is synthesized in the neuronal soma at the endoplasmic reticulum (Fig. 2B) where it is cleaved to proTRH, compartmentalized at the transGolgi into secretory granules that travel together with processing enzymes: PCs cleave at the carboxy end of a pair of basic residues, a carboxypeptidase cleaves the basic residues leaving the immediate precursor of TRH: gln-his-progly (black squares) and cryptic peptides (green); pyroglutaminase converts gln to pyroglu and peptidyl-glycine-α-amidase (PAM) leaves the amino group of the glycine bound to the carboxyl end (forming the amide group) and cleaves the rest of the pro-carbon moieties. Processing occurs as the secretory granule is transported to the nerve terminal (Nillini 2010).

**Figure 2**

Schematic representation of TRH synthesis. (A) The primary transcript is synthesized in the nucleus as prepro-TRH heterogeneous nuclear RNA (hnRNA) which contains two introns and five exons. (B) After splicing, mature RNA is transported to the cytosol, binds to ribosomes, begins transcription of prepro-TRH mRNA, the leader sequence is cleaved, synthesis of pro-TRH continues with ribosomes linked to rough endoplasmic reticulum (RER) and precursor is transported inside the ER. (C) At the transGolgi pro-TRH may suffer a first cleavage by protein convertase 1 (PC1), proTRH is compartmentalized in secretory granules with the rest of the processing enzymes: PCs cleave at the carboxy end of a pair of basic residues, a carboxypeptidase cleaves the basic residues leaving the immediate precursor of TRH: gln-his-progly (black squares) and cryptic peptides (green); pyroglutaminase converts gln to pyroglu and peptidyl-glycine-α-amidase (PAM) leaves the amino group of the glycine bound to the carboxyl end (forming the amide group) and cleaves the rest of the pro-carbon moieties. Processing occurs as the secretory granule is transported to the nerve terminal (Nillini 2010).
provide binding sites for specific transcription factors (TFs) whose activity is modulated by various signals (Lee et al. 1988, Fig. 3A). Chromatin remodeling constitutes the first step in transcriptional regulation; recruitment of specific TF, coregulators, and histone acetylases loosens the DNA from histones allowing initiation of transcription by RNA polymerase II (Pol II; Fig. 3B); certain TFs recruit histone deacetylases that compact chromatin repressing transcription (Gadaleta & Magnani 2014, Fig. 3C). Transcriptional repression of Trh transcription may occur by T3 binding to TH on site 4, recruiting corepressors and deacetylases that compact chromatin avoiding binding of polymerase and basal transcriptional machinery (Hollenberg 2008, Diaz-Gallardo et al. 2010a, b). Mice with knock-outs of the different THRs were used to demonstrate THR2 to be the most important receptor involved in downregulation of Trh transcription by T3 (Abel et al. 2001, Chiamolera & Wondisford 2009); however, transfection of Thrβ-KO mice with either THRβ1 or THRβ2 rescues T3-induced TRH expression, but only THRβ1 is able to activate in a ligand-independent manner (Dupré et al. 2004). Chromatin immunoprecipitation (ChiP) from stably transfected GH4C1 cells with Trh promoter (site 4, Fig. 3A) binds THRβs as homo- or hetero-dimers with retinoid X receptors (RXRs) in vitro; in transiently transfected cells, unliganded receptors activate Trh transcription whereas repression requires T3 to be bound to the receptor (Guissouma et al. 2000, Hollenberg 2008, Chiamolera & Wondisford 2009; Fig. 4). Mice with knock-outs of the different TRHs were used to demonstrate THRβ2 to be the most important receptor involved in downregulation of Trh transcription by T3 (Abel et al. 2001, Chiamolera & Wondisford 2009); however, transfection of Thrβ-KO mice with either THRβ1 or THRβ2 rescues T3-induced TRH expression, but only THRβ1 is able to activate in a ligand-independent manner (Dupré et al. 2004). Chromatin immunoprecipitation (ChiP) from stably transfected GH4C1 cells with Trh promoter detects acetylated histones 3 and 4, THRβ, and bound Pol II; T3 treatment transiently recruits deacetylases (HDAC2, 3), diminishes Pol II binding and, after 24 h, also THRβ.
phosphorylated-CREB (pCREB) binding site (CRE2: positions −101/−92 in rat) that is protected, along with adjacent 5′ GC box and 3′ CACC sequences, by nuclear extracts of cAMP-stimulated hypothalamic cells (Fig. 3A; Díaz-Gallardo et al. 2010a,b); these adjacent sites are recognized by SP1/Krüppel-like factors (Kpl; Ren et al. 1998, Díaz-Gallardo et al. 2010b, Pérez-Monter et al. 2011). Deleted or mutated CRE2 or CACC sites resulted in a decrease of 50–80% in basal and 50% in forskolin-induced transcription (the latter also with mutated GC box; Cote-Vélez et al. 2011). Multiple-intracellular signals phosphorylate CREB in the nucleus, pCREB binds to its REs and interacts with coactivators (some with histone-acetylase activity) increasing transcription (Fig. 3B; Altarejos & Montmny 2011). SP1 binding may increase the stability of pCREB on CRE2, as has been proposed for a nonconical sequence (Lundblad et al. 1998) or, depending on the TF of the SP/KPl family, cause interference. ChIP assays revealed other REs, such as the STAT-binding site, responsive to leptin stimulation (Guo et al. 2004). A composite glucocorticoid RE (cGRE), formed from a half-site GRE next to an API RE, binds the glucocorticoid receptor (GR) as a heterodimer with cJun after dexamethasone stimulation (Cote-Vélez et al. 2005, Díaz-Gallardo et al. 2010b; Fig. 3A).

Caveats regarding biosynthesis studies

Before continuing with this review we would like to stress some problems worthy of consideration when studying the regulation of TRH biosynthesis, given the diversity of experimental paradigms used. Cell lines are homogenous but with a particular set up that differs from the physiological situation, they are usually incubated with drugs for long periods and under steady conditions in contrast to in vivo situations where events such as clearance and diffusion play important roles. Transient transfections omit the regulatory steps related to chromatin remodeling (Gadala & Magnani 2014). Primary culture of embryonic hypothalamic cells (Uribe et al. 1995a, Harris et al. 2001), or transfected hypothalami of newborn pups (Guissouma et al. 2000), involve a mixture of multiple hypothalamic nuclei whose developmental windows, afferents and receptors, might differ to those exclusive to the PVN; furthermore, they miss the developmental effects of gonadal hormones (McCutcheon & Marinelli 2009). Knockout animals provide important information (Supplementary Table 1, see section on supplementary data given at the end of this article), but early-compensatory responses may lead to altered homeostatic states and/or the animals may have phenotypes that result from the simultaneous

![Figure 4](http://joe.endocrinology-journals.org)
manipulation of many organs that contribute, for example, to altered TH levels (Astapova & Hollenberg 2013); however, they revealed the redundancy of important regulatory molecules (Chiappini et al. 2013). Notwithstanding the problems mentioned, these distinct approaches have provided important information for identifying potential modes of Trh transcriptional regulation.

The HPT axis in energy balance

Circuits involved in energy balance overlap with those involved in food intake. Afferents from neurons of the brain stem or of the arcuate nucleus (ARC) convey nutritional information to the PVN and other hypothalamic nuclei. ARC neurons detect circulating hormones levels modulated by the nutritional and energy status (ghrelin, leptin, insulin, glucocorticoid, and fatty acids) and activate or inhibit neurons that express orexigenic (neuropeptide Y (NPY) and agouti-related peptide (AgRP)) or anorexigenic (pro-opiomelanocortin (POMC) and anorexigenic (pro-opiomelanocortin (POMC) and cocaine amphetamine-regulated transcript (CART) peptides) (Schneeberger et al. 2014); Trh expression is inhibited by orexigenic and stimulated by anorexigenic peptides (Fekete & Lechan 2014). Table 1 summarizes data on mRNA levels of Trh and several ARC peptides, as well as on serum hormones in rodents studied under different paradigms. Caution is required to distinguish measurements in the hypophysiotropic area (approximately 50% of parvicellular PVN neurons (Simmons & Swanson 2009)) normally determined by in situ hybridization (ISH) of Trh mRNA in mid–caudal PVN, against total PVN by analysis in dissected tissue (Fig. 1; Fekete & Lechan 2014).

TRH–hypophysiotropic neurons are the central integrator of the HPT axis, considered to be metabolic sensors as they decode neuronal and hormonal signals related to the energy status (Lechan & Fekete 2006, Hollenberg 2008). THs have multiple targets responsible for energy distribution and expenditure (Klieverik et al. 2009, Mullur et al. 2014). Negative energy balance such as fasting, food restriction, or pathological situations, including infection or critical illness, decreases serum levels of TH, and TSH to some extent, as well as of Trh expression in hypophysiotropic TRH neurons. In humans, these conditions are recognized as nonthyroidal illness syndrome (NTIS). The principal mechanism of fasting-induced decreased Trh expression involves a drop in leptin serum concentrations that lowers α-MSH and CART stimulatory tone, and enhances the inhibitory effect of NPY/AgRP on TRHergic neurons (Fekete & Lechan 2014). Results from animal models of infection or inflammation support the hypothesis that the mechanism causing NTIS primarily entails increased D2 activity in tanycytes and reduced D1 and D3 in liver and muscle, changes probably produced by cytokines and not by corticosterone (Fekete & Lechan 2014, Fliers et al. 2014). The effects are more pronounced in chronic situations where multiple factors intervene, including feeding status. In humans, postmortem analyses of critically ill patients showed low T₄ and T₃ liver concentrations and reduced TRH mRNA expression, supporting inhibition of the HPT axis at central level (Fliers et al. 2014).

The effects of energy excess on TRHergic neurons are less clear. Body-weight gain (approximately 20%) induced by high-fat diet (HFD), or ovariectomy, is associated with increases in Trh expression in the PVN; both conditions augment circulating leptin levels, although only HFD increases TH serum concentrations in rat (Perello et al. 2010). Leptin rapidly activates TRHergic neurons, indirectly in mid-PVN through its effects on ARC neurons and directly in caudal-PVN with high concentration of leptin receptors (Huo et al. 2004, Perello et al. 2006). Leptin’s direct effect is further supported by the exclusive increase in Trh mRNA in caudal PVN of ovariectomized rats (Uribe et al. 2009). The response of TRHergic neurons to feeding conditions differs in obese and lean rodents (Perello et al. 2010); under basal conditions Trh mRNA levels in the PVN are similar in obese and in lean Zucker rats; however, fasting decreases Trh expression more drastically in obese rats than in lean rats, a difference overridden by adrenalectomy (Duclos et al. 2005). A different allostatic state in obesity, related to a particular state of receptors causing leptin or insulin resistance, could be the basis of these differences.

Events involved in the regulation of energy balance such as fat metabolism, thermogenesis, body weight, food intake, and fat distribution is sex-dependent. Estrogen modulates the expression of several orexigenic and anorexigenic peptides and hormones (Brown & Clegg 2010). Male rats are more sensitive to insulin and females to leptin; compared with males, females adapt more efficiently to energy deficits, are more susceptible to HPT inhibition by food restriction, have lower pituitary expression of TSHβ and serum TSH associated with higher levels of T₃, and reduced hepatic activity of D1 under basal conditions (Cizza et al. 1996, van Haasteren et al. 1996, Valle et al. 2005, Marassi et al. 2007). Estrogen stimulates thyroid function, increasing iodide uptake and thyroid peroxidase activity which would increase TH biosynthesis (Lima et al. 2006). The expression of Trh in the PVN varies during the estrous cycle with highest levels at diestrous 2.
Table 1  Regulation of the HPT axis under chronic or acute stress. The HPT axis is differentially regulated depending on the origin of stress (physical or psychogenic), the duration (acute or chronic), and the animal's energetic and hormonal status.

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<th>Rodent strain</th>
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<th>Chronic treatment</th>
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However, the effects of estrogen and the consequences of ovariectomy on serum TH and TSH concentrations are controversial and depend on the paradigm studied and age and previous gonadal status (Lima et al. 2006, Marassi et al. 2007, Uribe et al. 2009).

Consistent with role of TRH in prolactin secretion, Trh mRNA levels increase, parallel to prolactin serum concentration, during the lactation period and in response to suckling (Uribe et al. 1993). As lactation progresses, levels of Trh mRNA decrease whereas those of prolactin rise; removal of pups normalizes Trh mRNA expression proportional to weaning time and inversely proportional to serum corticosterone levels (Uribe et al. 1991, 1995b).

Stress and the HPT axis

Stress was defined by Selye as the nonspecific response of the body to any demand (Selye 1976). This definition has been revised (Koolhaas et al. 2011). The type of stress defines the nature of the response; physiological (systemic) stressors such as cold, pain, and disease involve neuronal circuits that usually decode stimuli at the level of the brain stem, whereas psychogenic stressors are motivated by previous experiences and involve limbic areas such as the amygdala, hippocampus, and frontal cortex; all directly or indirectly converge at the PVN and activate the sympathetic nervous system and the hypothalamus–pituitary–adrenal (HPA) axis (de Kloet 2014, Myers et al. 2014). The response of the HPA axis is characterized by increased synthesis and release of corticotrophin-releasing hormone (CRH) from the PVN, adrenocorticotrophin hormone from the pituitary, and glucocorticoids (GCs) from the adrenal cortex. In rodents, paradigms of psychogenic stressors include restraint, chronic defeat stress, or isolation; their responses differ according to the controllability of the stressor, whether the animal senses a previously encountered stressor (homotypic) that causes habituation or a new one (heterotypic) to which a hyperreaction may occur (Koolhaas et al. 2011, Myers et al. 2014).

Stress, whether chronic or acute, modifies eating patterns and metabolism, and is considered to be at the base of the metabolic syndrome (Charmandari et al. 2005, Maniam & Morris 2012). GC excess or deficiency leads to several metabolic problems (Rose & Herzig 2013). As these hormones act in multiple organs, it is difficult to distinguish direct effects from indirect effects in paradigms such as adrenalectomy or thyroideectomy and hormone replacement. Chronic stress may increase or decrease food intake in humans whereas in animals this usually diminishes unless they are offered a
palatable food, although abdominal adiposity increases (Dallman et al. 2007). Chronic GC administration increases Npy and Agrp expression, the activity of their synthesizing neurons, alters the melanocortin system; and increases food intake; it causes multiple metabolic changes such as insulin resistance, visceral fat accumulation, high serum leptin levels (Dallman et al. 2007, Maniam & Morris 2012) and decreased Trh expression in the PVN and TSH serum concentrations in rat and in human (Kakucska et al. 1995, Alkemade et al. 2005). The GC-induced increase in NPY expression could contribute to GC-inhibitory effect on Trh mRNA levels in the PVN. Patients with Cushing’s disease present hypothyroidism and Trh expression in the PVN is reduced in postmortem brains of patients that received GC treatment or suffered major depression (Alkemade et al. 2005).

Various forms of long-term psychogenic stress decrease TSH and TH serum levels in rats (Armario & Castellanos 1984, Servatius et al. 2000), but reports on Trh expression are scarce. Constriction injury of sciatic nerve decreases Trh mRNA levels in the whole hypothalamus as well as serum TH levels (Kilburn-Watt et al. 2010). Foot shock (14 sessions/day) decreases total and free T4 and T3 serum concentrations without affecting Trh in the PVN, whereas Agrp mRNA levels in the ARC increase (Helmreich et al. 2005); a milder form of stress, such as 60 min daily restraint for 2 weeks, does not affect Trh mRNA expression in the PVN nor TSH or TH serum levels (Uribe et al. 2014). The intensity of the stressors thus seems to determine HPT activity and Trh expression; however, despite significant changes not being observed in the latter two paradigms, levels of Trh mRNA correlate negatively with those of corticosterone and positively, with body-weight changes.

Situations of increased energy expenditure such as exercise and cold are also considered stressful. Cold exposure rapidly activates the autonomic nervous system and the HPT and the HPA axes. Thermogenesis is achieved by the activity of brown adipose tissue (BAT) and lipolysis (Mullur et al. 2014). Exercise is proposed to be a chronic stressor that inhibits HPT and activates HPA axes (Mastorakos & Pavlatou 2005). This depends on the type of exercise, i.e. extenuating or forced versus voluntary (as wheel running; Steinacker et al. 2005, Stranahan et al. 2008). Compared with naïve sedentary controls, 2-week voluntary running reduces food ingestion (18%); the decreased energy intake in the sedentary pair-fed group causes similar changes to those caused by 40% food restriction for 25 days in rats, decreased levels of serum leptin, TSH and T3, BAT-D2, and liver-D1 activities, and increased corticosterone (Araujo et al. 2009). Exercise blunt several of the changes produced by the 18% reduced food intake, i.e. Trh diminished 30% instead of the 50% seen in the pair-fed rats, and neither T3 or deiodinases activities were altered; however, white adipose tissue (WAT) mass diminished in exercised rats whose serum leptin concentration decreased much more than that of the pair-fed rats; furthermore, the amount of exercise performed correlated positively with T3 and Trh mRNA levels and negatively with WAT mass (Uribe et al. 2014). Exercise thus overrides the signals of energy deficiency, such as low serum leptin concentration; the intermittent activation of PVN–TRH neurons and of TH release (seen after an acute increase in physical activity, see below) may guarantee maintenance of TH levels for adequate fuel supply to oxidizing tissues, such as released fatty acids from WAT (Klieverik et al. 2009, Weber 2011). Hypophysiotropic TRHergic neurons may therefore be included in a central homeostatic circuit modulated by exercise, which by maintaining adequate TH-induced lipolysis or serum glucose levels through PVN-sympathetic and parasympathetic hepatic activity guarantee energy supply to metabolic tissues (Fliers et al. 2014).

TRH expression in the hypophysiotropic neuron at a given time depends thus on the concentration of T3, THR and co-regulators, nutritional status, stress history, and the extent of neuronal and hormonal influences that sense the
‘basal’ state of the organism and regulate TRH synthesis and release – a process further modified by the environmental influences it must contend with. The HPT axis is inhibited in response to energy deficits or chronic stress. However, animals submitted to energy-demanding situations, such as chronic cold or exercise, have altered HPT axis activity and may present high or normal circulating levels of TH, although Trh expression in the PVN and serum TSH levels may be differentially affected (Table 1).

**HPT responses to acute stimuli**

Several neuromodulators regulate the excitability of TRHergic neurons of the PVN; NPY inhibits neuronal activity whereas α-MSH and leptin stimulates it, effects consistent with the known responses on TSH or T4 release (Ghamari-Langroudi et al. 2010). GCs rapidly suppress glutamatergic excitatory inputs onto various parvicellular neurons of the PVN, including those expressing TRH; the effect is mediated through membrane receptors, whose activation causes the release of endocannabinoids that act at the presynaptic side to inhibit glutamate release (Di et al. 2003). TRH neurons in the PVN receive afferents from the suprachiasmatic nucleus that modulate TRH expression in a circadian manner (Kalsbeek et al. 2000, Zoeller et al. 1990).

Acute stress inhibits the HPT axis at different levels; 1 h restraint or 2 h immobilization decrease Trh mRNA expression in the rat PVN together with TRH and TSH release during the following hour; the response to immobilization is higher in female rats (Cizza et al. 1996, Gutiérrez-Mariscal et al. 2012). However, a stressful condition, such as the defensive burying test that involves increased physical activity (burying), augments the levels of Trh mRNA in the PVN but not TSH release (Gutiérrez-Mariscal et al. 2008). Situations of energy demand, such as physical activity or cold exposure, cause an immediate response of the HPT and the HPA axes. TH and GCs are rapidly released; the latter mobilize glucose for fast responses and the former activate fuel distribution to oxidizing tissues (Klieverik et al. 2009, Rose & Herzg 2013). This is coupled with changes in TRH biosynthesis. For example, physical activity in the open field increases the expression of Trh in the PVN accompanied by enhanced TRH and TSH release, but only if animals are tested during the dark period. The stress response is stronger in animals tested during the light hours when the HPT response is blunted (Gutiérrez-Mariscal et al. 2012). Another type of exercise, such as swimming in a water maze, activates the HPT, increasing the expression of Trh mRNA in the PVN and the release of TSH; the response of the HPT is opposite to the degree of stress induced by the test (Aguilar-Valles et al. 2005, 2007).

In response to a cold stress, catecholaminergic afferents from the brain stem onto the PVN rapidly contribute to increase Crh and Trh mRNA levels in the PVN, concomitant with the release of TRH, TSH, and corticosterone (Zoeller et al. 1990, 1995, Rondeel et al. 1991, Uribe et al. 1993). The changes in Trh mRNA are transient, with increases at 1 h and normalizing by 2 h, even when animals are kept in the cold for long periods; Trh mRNA levels rise again at 6 h but only if animals are exposed during the light period (Uribe et al. 1993, Zoeller et al. 1995). Acute administration of some drugs alters Trh expression and these changes do not always coincide with changes in TSH or TH serum concentrations (Table 1). Although ethanol increases Trh mRNA levels in the PVN, it blocks increases induced by cold-exposure (Zoeller et al. 1995). Cold increases Trh mRNA expression independent of high-TH serum levels (Zoeller et al. 1990), but a mild stress 2 h before being submitted to cold suppresses the expected increase in Trh mRNA levels in the PVN but not in serum TSH in male rats (Uribe et al. 2011).

Cold exposure activates the HPT axis more in non-lactating female rats than in males or lactating females (Sánchez et al. 2001). Ovariectomized rats with increased body weight and high leptin serum concentration respond to cold stimulation augmenting Trh mRNA levels in the PVN, but the effect is suppressed by high doses of 17β-estradiol, while serum TSH and corticosterone levels are similar to those found in the ovariectomized animals (Uribe et al. 2009). In contrast, food-restricted female rats display greater increases in Trh mRNA levels in the PVN than controls after cold exposure, but TSH response is blunted (Jaimes-Hoy et al. 2008). These results exemplify situations in which the response to a life-threatening situation such as cold exposure is altered by stress, corticosterone, sex, and other as yet unidentified effectors.

As mentioned, TRH is involved in prolactin release during lactation (Galas et al. 2009), and the levels of TRH mRNA increase in mid-PVN 30 min after initiation of suckling (Sánchez et al. 2001). TRH hypophysiotropic neurons co-express CART, which is also induced after 1 h of cold exposure (Sánchez et al. 2007) but not after suckling. Since CART inhibits prolactin release in vitro and cold exposure does not induce the release of prolactin, CART may serve as a co-modulator of TRH in this physiological circumstance, stimulating TRH and TSH release while blocking prolactin release (Sánchez et al. 2001, 2007, Raptis et al. 2004).
Because GCs affect not only the electrical activity of PVN–TRH neurons, but potentially the expression of Trh in rodents and humans, it is relevant to understand how they interact with the stimuli that control the HPT axis. The timing of GR activation is of utmost importance; GR is released from a cytosolic multi-protein complex upon ligand binding, phosphorylated, and translocated to the nucleus within minutes, where it acts as a transcription factor. The dynamics of GR transport and its half life depend on the presence of ligand, DNA binding, and activity of several kinases (Ratman et al. 2013, de Kloet 2014). The response of PVN Crh expression to restraint is blunted if animals receive corticosterone 1 h but not 3 h previously, supporting the relevance of timing of GR activation; in contrast, the increase in c-FOS due to restraint is not diminished by corticosterone pretreatment (Osterlund & Spencer 2011). Trh mRNA levels in the PVN correlate negatively with corticosterone serum concentrations in animals under several circumstances; therefore, we studied the effect of a peripheral corticosterone injection on cold-induced Trh expression in the PVN. In male rats kept at room temperature, Trh mRNA expression is enhanced (greater than threefold) 2 h after corticosterone injection in the mid- and caudal-PVN, where TRHergic neurons co-express GR (Cintra et al. 1990). Rats that received injections of vehicle responded to cold as expected, increasing Trh mRNA levels in the three zones of the PVN and TSH serum concentration; however, in corticosterone pre-treated rats, Trh expression does not increase further after cold exposure and the response of TSH is suppressed (Sotelo-Rivera et al. 2014). GCs may inhibit TRH or TSH release directly at the median eminence or the pituitary respectively (van Haasteren et al. 2015). TRH synthesis and release might be uncoupled in those circumstances when TSH is not increased within an hour of exposure.

The interference of corticosterone with the cold-induced activation of Trh synthesis may be due to a cross talk between activated GR and elements of the PKA pathway, as demonstrated in vitro. Hypothalamic cells co-stimulated with dexamethasone and forskolin do not display increased Trh mRNA levels as are detected when they are incubated separately with each drug (Pérez-Martínez et al. 1998, Cote-Vélez et al. 2005), nor the recruitment of pCREB, GR, or Pol II to the Trh promoter (Díaz-Gallardo et al. 2010b). The effect is probably due to GR–PKA protein–protein interaction with PKA impeding CREB phosphorylation and GR binding to DNA (Sotelo-Rivera et al. 2012). Similar cross talk between the PKA and GR intracellular pathways is detected also in cell lines, supporting the hypothesis that the effect is an intracellular event, and not due to the average of differential responses of TRHergic cells from different hypothalamic nuclei present in primary cultures (Cote-Vélez et al. 2005). The interference of GCs with the cAMP-stimulatory effect on Trh expression is avoided with inhibitors of the ERK/MAPK pathways (Cote-Vélez et al. 2008). These results, although obtained in vitro, support the possibility of fast cross talk between signaling pathways that may alter an immediate response (Fig. 2).

As mentioned in the previous section, long-term energy-demanding situations do not seem to keep the HPT axis activated, but the response to acute stimuli may prevail or be modified. One may envisage situations when TRH neurons are activated, TRH synthesis increases and simultaneously processed TRH is released, TSH secretion follows, causing TH release that modulates different metabolic reactions at target organs. The feedback effect includes not only TH at TRH or TSH synthesis, but molecules of the target tissues, GCs if HPA is co-stimulated and TH acting on other brain areas involved in the required circuit. TH affects metabolism acting at various hypothalamic nuclei increasing, for example, glucose production, modulating central autonomic outflow, and eating behavior or stimulating the dorso-medial hypothalamus (DMH) which activates the sympathetic-BAT response involved in thermogenesis (Fliers et al. 2014).

TRHergic neurons may thus respond transiently to energy-demanding situations and maintain energy homeostasis. However, stress could impede a fast and efficient response of the HPT axis in which, for example, in cases such as cold exposure or increased physical activity, the lack of opportune lipolysis needed for fuel supply may oblige the organism to display alternative responses, such as increased food intake and decreased activity due to fatigue, thus contributing to the metabolic syndrome.

**Hypothalamic TRH neuronal populations**

In mammals, various brain circuits contribute to maintenance of energy balance (Schneeberger et al. 2014). Central administration of TRH or TRH agonists consistently reduces food intake in normal rodents and hungry rats, and in models of stress-induced feeding increases thermogenesis and arousal (Lechan & Fekete 2006, Akieda-Asai et al. 2014). These effects may involve various hypothalamic targets because, for example, local injection of TRH into medial and lateral hypothalamus (LH) reduces feeding in rats (Suzuki et al. 1982) whereas administration into the preoptic area, dorsomedial, or ventromedial...
hypothalamus (VMH) increases BAT temperature in hamsters (Shintani et al. 2005). ISH of $pp$TRH mRNA, its receptors (TRH-R1 and TRH-R2), and inactivating enzyme (PPII) together with immunostaining of Trh and of TRH, as well as autoradiography of TRH binding sites, has been used to generate maps of brain TRHergic neurons, receptors, and inactivation sites (Vargas et al. 1987, Hökfelt et al. 1989, Lechan & Segerson 1989, Heuer et al. 2000). However, the circuits in which they are involved are currently poorly understood as few of the projection fields of the TRH neurons have been identified with anterograde and retrograde techniques (Simmons & Swanson 2009, Wittmann et al. 2009a,b, Fekete & Lechan 2014). Understanding the role of hypothalamic TRHergic neurons (Fig. 1) requires consideration of their distribution because various regions, such as the LH, PVN, preoptic, suprachiasmatic, and dorsomedial nuclei express Trh mRNA (Fig. 1). The physiological state of TRH neurons has been evaluated by few electrophysiological studies on identified neurons and by biochemical markers of activation, such as detection of immediate early genes, or of TRH biosynthesis, but the output of TRH neurons is difficult to obtain in vivo because of sampling or sensitivity issues.

Even in a single nucleus, TRH neurons are not homogeneous. In the rat PVN, TRH-expressing neurons are distributed along the rostro-caudal part of the parvocellular and in some of the magnocellular subdivisions (Fekete & Lechan 2014). Almost half of the total PVN–TRHergic cells in the parvocellular part of the PVN are non-neuroendocrine or preautonomic, concentrated in the rostral or anterior PVN (aPVN) in the rat, with only few present in the mid-PVN and even fewer in the caudal. As mentioned, the hypophysiotropic or endocrine cells are concentrated in mid- and caudal-PVN (Simmons & Swanson 2009, Fekete & Lechan 2014). In mouse PVN, 69% of TRHergic endocrine cells project to the median eminence, while 17% project to the neurohypophysis, and the rest are non-endocrine cells localized in the anterior zone (Ghamari-Langroudi et al. 2010, Kádár et al. 2010). PVN–TRHergic neurons differ not only in their afferents, projections, and receptor expression (Fekete & Lechan 2014) but also according to time of birth; rat non-neuroendocrine neurons peak at embryonic days 11–12 and neuroendocrine neurons at days 12–14 (Markakis & Swanson 1997).

The non-neuroendocrine TRHergic neurons of the rat aPVN receive a robust innervation from NPY/AgRP and α-MSH/CART neurons of the ARC (Fekete & Lechan 2014) and from adrenergic and noradrenergic fibers from the brain stem (Füzesi et al. 2009). The α-MSH input seems to be functional since central administration of α-MSH enhances the phosphorylation of CREB in Trh neurons (Sarkar et al. 2002) and mediates the effect of i.c.v. leptin injection, which rapidly increases pCREB in aPVN–TRH neurons (Perello et al. 2006). The adrenergic input may be relevant to thermogenesis because cold induces a fast increase in Trh mRNA levels in the aPVN as it does in the hypophysiotropic neurons (Sánchez et al. 2001). TRH neurons of the aPVN send projections to the ARC, dorsomedial, ventral-premammillary nuclei, and medial-preoptic region, and to several additional limbic regions such as various amygdaline nuclei, the bed nucleus of the stria terminalis, and lateral septum (Wittmann et al. 2009a). TRH neurons of the aPVN do not express GR (Cintra et al. 1990), but a corticosterone injection increases Trh mRNA levels, probably through direct stimulation of GC membrane receptors, and the cold-stimulatory effect is additive (Myers et al. 2014, Sotelo-Rivera et al. 2014). Trh expression in aPVN is not modulated after suckling or food restriction, whereas it is stimulated after dehydration-induced anorexia (DIA; Sánchez et al. 2001, Alvarez-Salas et al. 2012). As regulation of biosynthesis is often coupled with changes in peptide release, the results indicate that aPVN–TRH neurons transfer multiple modalities of metabolically relevant information to postsynaptic targets; confirmation of their role will require experimental testing, but it is interesting to note that the intestinal administration of long-chain fatty acids enhances the activity of aPVN neurons (Randich et al. 2004), and adiponectin stimulates only non-neuroendocrine PVN–TRH cells (Hoyda et al. 2009).

The ARC expresses both Trh-R1 and Trh-R2 receptor mRNAs (Heuer et al. 2000, Ebling et al. 2008), its dorsomedial part receives a dense TRHergic innervation (Lyons et al. 2010) arising, at least in part, from the aPVN and some from the perifornical area (Wittmann et al. 2009a,b). Cell-specific neuron-mapping techniques in mice demonstrate a strong excitatory drive emanating from subsets of neurons from the PVN expressing TRH and pituitary–adenylate cyclase-activating polypeptide that contact and activate AgRP neurons, inducing intense feeding (Krashes et al. 2014). The functional role of TRH in this circuit was not tested, but TRH does not regulate the electrical activity of α-MSH or NPY neurons in slices of rat ARC (Zhang & van den Pol 2012), instead TRH regulates that of tuberoinfundibular dopaminergic neurons present in the dorsal ARC which are surrounded by TRH-ir terminals; TRH causes a transition from phasic to tonic firing which probably decreases dopamine output (Lyons et al. 2010).

The DMH has an important role in energy homeostasis (Schneeberger et al. 2014). A significant population...
of TRH neurons is detected in this nucleus (Hökfelt et al. 1989, Horjales-Araujo et al. 2014), which receive afferents from the subparaventricular zone, an output region from the suprachiasmatic nucleus (SCN); the DMH sends a glutamate–TRH projection to the LH area (Chou et al. 2003) which expresses both TRH receptors, predominantly TRH-R1 (Heuer et al. 2000). TRH receptors are probably present in orexin neurons as they respond to TRH with robust increases in their action potential firing rate, an effect that persists under conditions of synaptic isolation (González et al. 2009). TRH also increases the firing activity of presynaptic GABAergic interneurons (Hara et al. 2009). The DMH–TRHergic neuronal projection onto or near orexin neurons may be part of a circuit required for the circadian activation of behavioral and endocrine functions, including the circadian control of awareness (González et al. 2009). In agreement with this possibility, the effect of TRH on locomotor activity is reduced in orexin-ablated mice (Hara et al. 2009). TRH also inhibits the activity of melanin-concentrating hormone (MCH) neurons of LH indirectly through the excitation of GABA neurons, a result consistent with the detection of TRH axons terminating on or near GABA neurons (Zhang & van den Pol 2012). Interestingly, Trh mRNA levels in the DMH are increased after 2 weeks of moderate exercise compared with the pair-fed group (Uribe et al. 2014). The LH contains another large group of TRH cells throughout the rostrocaudal axis of the hypothalamus (Hökfelt et al. 1989, Lechan & Segerson 1989, Heuer et al. 2000, Horjales-Araujo et al. 2014) and in the perifornical region (Wittmann et al. 2009a,b). These neurons do not coexpress orexin, MCH, or neuropeptide Y (Horjales-Araujo et al. 2014). In the juxtaparaventricular area, a small group of TRH-immunoreactive cells stains for enkephalin and urocortin 3, and their projections partially overlap those of the aPVN (Wittmann et al. 2009b, Horjales-Araujo et al. 2014). AgRP and α-MSH populations of terminals form close appositions onto TRH cells in the LH; the TRH-expressing cell population of the LH may link metabolic signals and the generation of arousal (Horjales-Araujo et al. 2014). Increased expression of Trh is detected in LH of male rats after 5 days of DIA and in the pair-fed group (de Gortari et al. 2009) while Crh mRNA levels increase only after DIA (Watts et al. 1999). DIA and pair-fed rats eat by the fifth day only 20% of the intake of control animals; the increase in TRH expression may be related to increased arousal as increased anxiety is produced by these conditions (Jaimes-Hoy et al. 2008).

TRH neurons heavily innervate histaminergic (HA) neurons in all subdivisions of the rat tuberomammillary nucleus (TMN) (Sárvári et al. 2012) where TRH-R2 is present (Gotoh et al. 2007). A TRH microinjection above the TMN activates HA TMN neurons and histamine turnover in PVN and VMH projections (Gotoh et al. 2007). As hypothalamic neuronal histamine is involved in the regulation of body weight and acts as an anorexigenic agent (Schneider et al. 2014), it is indeed possible that a HA projection contributes to the anorectic effect of TRH.

Are changes in the expression of neuropeptides and TRH in total hypothalamus indicative of a metabolic alteration?

Owing to the crucial involvement of TH thermogenesis and metabolism (Klieverik et al. 2009, Silva 2011, Mullur et al. 2014), a more active HPT would in principle reflect a higher metabolism. However, a cause–effect relationship between gene expression of ARC peptides and that of TRH in total PVN is difficult to identify (Table 1 and Supplementary Table 1). If, for example, there is an opposite response of Trh expression in aPVN versus mid-caudal PVN, the results of the whole PVN may overshadow the endocrine response. Even more if levels of expression are measured in total hypothalamus since TRH in several nuclei may have important roles in energy homeostasis. For example, comparison between lean and fat rats (Lou/C versus Wistar (Veyrat-Durebex et al. 2013)) or selectively bred chickens (Byerly et al. 2009) show increased Trh mRNA but also increased Npy and Agrp together with decreased Pomc in the lean animals which would seem to contradict the effects of these peptides on TRH expression in the PVN (Fekete & Lechan 2014). Although it is not possible to delineate the hypothalamic regions where these reported changes occur, coincident changes in these two species include the enhanced expression of brain-derived neurotrophic factor (BDNF) which in vitro increases Trh mRNA levels (Ubieta et al. 2007). BDNF and its receptor TRKB are expressed in several hypothalamic nuclei including TRHergic cells of the PVN (Ubieta et al. 2007). BDNF regulates neurogenesis, neuronal plasticity, and support during development and throughout the animal’s life-span (Jeanneteau & Chao 2013). In the hypothalamus, BDNF plays an important part in energy homeostasis (Levin 2007, Rothman et al. 2012). Support for this role stems from the effects of transferring the Bdnf gene into hypothalami of adult mice, which display decreased body weight and body fat and increased expression of Mc4r, Lepr, Trh, and Crh compared with wild type mice despite having strangely greater than tenfold induction of the orexigenic peptides NPY and AgRP. Under HFD, they gained less weight and little fat.
compared with normal mice and displayed increased Mc4r, Lepr, Trh, Crab, Cart (Cartpt), and Pomc expression, as well as expression of the metabolic genes of target tissues such as WAT and liver (Cao et al. 2009). These results, although not defining the particular hypothalamic nuclei involved, reveals the importance of additional participants such as BDNF in re-setting the response of several hypothalamic–peptidergic systems and their relationship with body weight and adipose tissue.

Another metabolic relevant brain area, the hindbrain

The brain stem is an important part of the circuitry involved in sensing and conveying information regarding the nutritional and energy conditions of the organism (Schneeberger et al. 2014). Trh is synthesized in the raphe pallidus (Rpa), raphe obscurus (Rob), and parapyramidal regions; these neurons project to neurons of the dorsal motor nucleus of the vagus, the nucleus tractus solitarius, and the ventrolateral medulla. They contain TRH-R1 and leptin receptors involved in coordinating vagal and sympathetic outflows (Taché et al. 2006). As in mid-PVN, Trh expression in these nuclei is downregulated in hyperthyroidism (Yuan & Yang 1999) and upregulated by cold exposure (Taché et al. 2006). Some TRH neurons in the caudal raphe receive orexin afferents and project to premotor neurons that innervate BAT increasing thermogenesis; others project to the vagal circuit involved in hepatic, pancreatic, and gastrointestinal function (Taché et al. 2006). In contrast to the inhibitory effects of fasting on HPT activity, hindbrain Trh mRNA levels are elevated in rats fasted for 24 or 48 h, and refeeding restores them to normal levels (Ao et al. 2006). Studies of the regulation of TRH expression in these neurons contribute to the understanding of the role of brain TRH in metabolism and thermogenesis.

Neuroendocrinology revisited

Recognition of the effects of environment on metabolism and epigenetic changes has led to questions with regard to what are the adequate controls in experimental research with rodents? Although laboratory rodents have been domesticated for hundreds of generations, their ‘standard’ living conditions are sedentary with no other activity but to eat ad libitum and develop obesity (Martin et al. 2010). This is evident comparing pair-fed with naïve rats; caloric restriction even as low as 18% of naïve food intake and loss of 8% body weight increased their corticosterone serum concentrations (Uribe et al. 2014). In the case of 300 g male rats, normal cages do not allow standing, reducing even further their possibility of physical activity (RSPCA 2011). Stressful conditions include differences in individual versus group housing depending on the age and sex of the animal, whether animals are transported or isolated within 1–3 h previous to the experiment, etc. (Koolhaas et al. 2011, Uribe et al. 2011).

Postnatal development, lactation, and adolescence are periods of high sensitivity to stress when epigenetic changes may program the individual to having low stress resilience, changing the expression of key molecules such as BDNF and GR in a region-specific manner; in hippocampus their inhibited expression alters the inhibitory feedback on the activity of the HPA leading to hyperactive stress responses and reduced learning (Meaney & Szyf 2005, Pervanidou & Chrousos 2012, Bath et al. 2013). The nutrition or stress experience of mothers during gestation as well as litter size has long-lasting effects on metabolism and stress response (Breton 2013). In the case of the HPT axis and TRH expression in particular, reports are scarce. Dexamethasone injections during the last week of gestation diminish Trh mRNA levels in the PVN in female offspring and TRH fibers and T4 in both sexes during the second week; only Trh expression remained low in adult females, accompanied by low body temperature during estrous and diestrous (Carbone et al. 2012a). Low levels of immunoreactive TRH are also detected in the fibers of the LH (Carbone et al. 2012b). This treatment blunts stress-induced Bdnf expression in male rats or HFD-induced increases in females (Carbone & Handa 2013).

Neonatal stress produced by maternal separation causes epigenetic changes in hippocampal Gr and Bdnf promoters, their reduced expression contributes to the altered HPA-response to stressors in adulthood (Meaney & Szyf 2005), Bdnf expression is altered in a strain-, sex-, and region-specific manner (Bath et al. 2013). Adequate programing of activity and response of the HPT axis is altered under various conditions. Maternal separation increases ppTrh mRNA in the aPVN and circulating T4 serum concentrations of adult rats, and attenuates the response to exercise or cold exposure but that to fasting is normal (Jaimes-Hoy et al. 2013). Offspring of mothers that experienced a 40% food restriction, during gestation and lactation were hypothyroid (low FT4 and high TSH) and had a blunted response to 24 h cold exposure (Ayala-Moreno et al. 2013). In contrast, post-natal overnutrition during lactation, which predisposes the offspring to being overweight, leads to leptin resistance and overexpression
of orexigenic pathways (Breton 2013), alters the HPT axis in rats in contrast to the situation opposite to what is observed after HFD because Trh expression in the PVN and T4 serum concentration are decreased and the response to fasting is suppressed (Rodrigues et al. 2009, Aréchiga-Ceballos et al. 2014) compared with HFD-induced overweight, which display increased Trh mRNA and TH serum levels, but without an altered fasting response (Perello et al. 2010).

The opposite scenarios apply to mice grown in enriched environments; their body weight is reduced, BDNF expression is increased in ARC and DMH/VMH, Trh in the PVN and in the DMH/VMH, despite decreased expression of Pomc; animals with access to wheels for running for several weeks maintain lower body weight and reduced adiposity, but Trh mRNA levels tend to decrease and Crh and Crh-receptors increase in contrast to the effects of the enriched environment (Cao et al. 2011). The contradictory results observed between the effects of neonatal stress and those of growing in an enriched environment on the relationship of GR and BDNF (Jeanne teau & Chao 2013) include the responses of the environment on the relationship of GR and BDNF (Jeannetteau & Chao 2013) of the animals’ age and body weight, as some correspond to the adolescent period (McCutcheon & Marinelli 2009). Another recently discovered but very important problem is the materials used in animal houses such as water bottles made of polycarbonate that release deleterious effects, particularly during development (Zoeller 2010), and the differential leakage that occurs depending on the state and treatment of the bottle (Guart et al. 2013).

Conclusion

This review summarizes what is known about the regulation of the TRHergic neurons involved in energy homeostasis and the interfering effects of stress. In spite of the limitations of the in vitro systems or transgenic mice models, evidence indicates demonstrate an inhibitory role of T3 and a stimulatory one of pCREB, SP1/KPL, GR-cJun, and STAT3 on Trh transcription; interaction of the transduction pathways involved may explain some of the interfering effects of stress on neuronal stimulations observed in various in vivo situations, but they have not yet been fully placed in a sub-type of TRH neuron context.

Trh expression levels in the PVN, detected during chronic ‘static’ situations, are difficult to relate to changes in other known modulators as they represent a ‘snap shot’ that might not readily correspond to the activity of the target organs involved. TRH neurons can rapidly respond to energy demands for thermogenesis or physical activity; these responses may be modified due to nutritional status, and stress history. We propose that combined approaches of chronic situations with acute responses might lead to a better understanding of TRH physiology in the PVN and in other relevant metabolic hypothalamic nuclei.

Supplementary data

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Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

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