

# Infundibular tanycytes as modulators of neuroendocrine function: hypothetical role in the regulation of the thyroid and gonadal axis

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**Abstract.** Tanycytes comprise a heterogeneous population of specialized cells of glial origin that line the floor and ventrolateral walls of the third ventricle between the rostral and caudal limits of the hypothalamic median eminence. While morphologic and ultrastructural features suggest a role as barrier cells, creating separate compartments between the cerebrospinal fluid, median eminence and hypothalamus, tanycytes likely have multiple other important functions that have yet to be fully elucidated. Possibilities to consider are a role in neuroendocrine regulation including modulation of the hypothalamic-pituitary-thyroid axis during fasting and infection, regulation of reproductive function, particularly in seasonal breeders, and in feeding. ([www.actabiomedica.it](http://www.actabiomedica.it))

**Key words:** Tanycytes, median eminence, hypothalamus, deiodinase, hypothalamic-pituitary-thyroid axis, seasonal breeders, reproduction, feeding

## Introduction

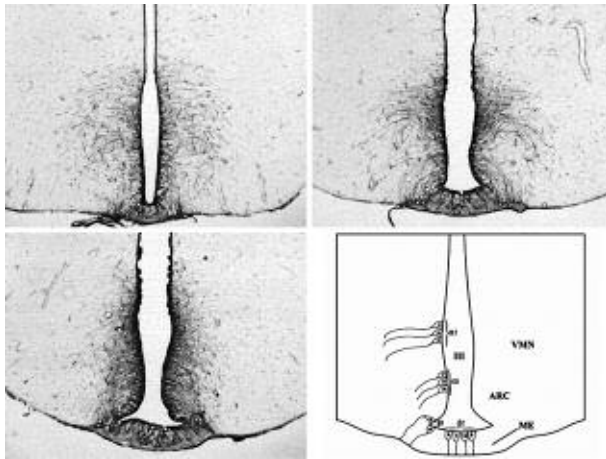
Tanycytes are specialized, elongated ependymal cells of glial origin that line the floor and ventrolateral walls of the third ventricle between the rostral and caudal limits of the hypothalamic median eminence (Fig. 1). While much is known about the anatomy of these cells, their physiologic functions remain speculative and enigmatic. In this review, we provide evidence to suggest that tanycytes may play an integral role in the regulation of the hypothalamic-pituitary-thyroid and gonadal axis and contribute to the pathogenesis of the nonthyroid illness syndrome.

## Anatomy of the tanycyte

Characteristic of the tanycyte are apical villi-like protrusions that extend into the cerebrospinal fluid (CSF), and a single, basal process that ramifies into the

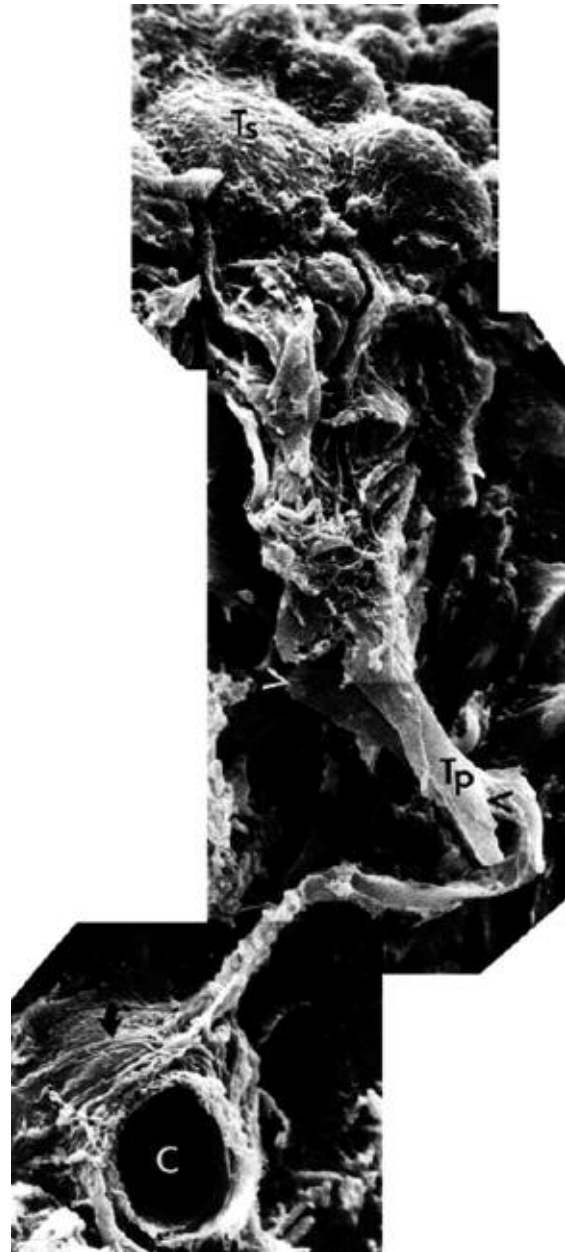
underlying neuropil. This process can encircle blood vessels in the adjacent arcuate and ventromedial nuclei, contact neurons and glia in these regions, and/or terminate in numerous endfeet processes on or near the fenestrated capillaries of the primary portal plexus in the external zone of the median eminence (1-6) (Fig. 2). Osmophilic inclusions and numerous polymorphic and round vesicles have been described within these endfeet (4), suggesting a secretory and/or transport function. An extremely high number of synaptoid or "en passant" contacts between the endfeet processes in the median eminence and nerve fibers of the hypothalamic tuberoinfundibular system have also been observed (4), indicative of glial neuronal interactions.

Tanycytes would appear to be a heterogeneous population and several subtypes have been described based on morphological and anatomical features. According to the nomenclature of Akmayev (3, 7), the subtypes include  $\beta 1$ ,  $\beta 2$ ,  $\alpha 1$  and  $\alpha 2$  tanycytes. The relative locations of the cell are indicated in Fig. 1.



**Figure 1.** Vimentin immunolabeled coronal sections (rostral to caudal) of the rat mediobasal hypothalamus showing the organization of tanycytes and their processes. The schematic diagram shows relative location of tanycyte subtypes. (Courtesy, Dr. Praful Singru)

$\beta 1$  and  $\beta 2$  tanycytes line the floor and lateral extensions of the third ventricle. Characteristic of  $\beta 1$  tanycytes are lipid inclusions confined to their cell body, an arching trajectory of their basal process that extends ventrolaterally to end at the lateral regions of the median eminence along the border between the median eminence and arcuate nucleus and synaptoid contacts by axon terminals only at their preterminal portion (5). These cells also express type I glucose transporter (GLUT I) and insulin-like growth-factor binding protein (6, 8), which can be used as markers to differentiate  $\beta 1$  from  $\beta 2$  tanycytes. Rodriguez et al (5) has further divided  $\beta 1$  tanycytes into ventral ( $\beta 1V$ ) and dorsal ( $\beta 1D$ ) subtypes on the basis of distinguishing ultrastructural characteristics, including differences in their ventricular protrusions and the observation that the basal process of  $\beta 1D$  tanycytes can completely encircle capillaries in the arcuate nucleus.  $\beta 2$  tanycytes line the floor of the third ventricle and have a fairly straight trajectory to the external zone of the median eminence where they terminate on portal capillaries (5, 9, 10). These cells receive numerous synaptoid contacts from axon terminals all along their basal process, contain lipid inclusions throughout the cell (5), and do not express GLUT I (6).



**Figure 2.** Scanning electron micrograph of a tanycyte showing its cell body (Ts) at the third ventricle surface and a single, tapering, basal process (Tp) that breaks up into multiple fine branches (endfeet, arrow) at its point of termination on a capillary (C) of the portal system. (From Bruni et al (37), with permission)

$\alpha 1$  and  $\alpha 2$  tanycytes line the walls of the third ventricle and are distinguished by the trajectory of their basal process.  $\alpha 1$  tanycytes project to the ventromedial nucleus and would appear to end on neurons,

whereas  $\alpha 2$  tanycytes project to the arcuate nucleus and either end on a single capillary vessel or a single neuron or extend to the tuberoinfundibular sulcus to join the basal processes of  $\beta 1$  tanycytes (5, 11).  $\alpha$  tanycytes can also be differentiated from the  $\beta$  tanycytes by enzyme histochemical markers such as adenosine triphosphatase and esterase (12, 13).

### Tanycytes as barrier cells

While excellent morphological descriptions of tanycytes are present in the literature (1-3, 5), the physiologic function of these cells remain poorly understood. Evidence that tanycytes are joined together by tight junctions in the floor of the third ventricle (14) has led to the belief that they function primarily as barrier cells, creating separate compartments between the CSF, median eminence and adjacent hypothalamic arcuate nucleus. This concept is supported by the observation that injection of horseradish peroxidase (HRP) into the third ventricle does not enter the median eminence, whereas injection into the bloodstream labels the median eminence (as it lies outside the blood-brain-barrier) but does not enter the arcuate nucleus (15-17). In addition, injection of HRP into the arcuate nucleus does not enter the median eminence (18). Thus, tanycytes may prevent material accumulated in the perivascular spaces in the median eminence derived from the blood stream or from hypophysiotrophic neurons of the hypothalamic tuberoinfundibular system that produce the hypothalamic releasing and inhibiting hormones, from leaking into the adjacent arcuate nucleus or the CSF. In addition, tanycytes may restrict movement of blood-borne molecules traversing the arcuate nucleus from entering the median eminence (1, 15, 16). It is also conceivable that under special circumstances, tanycytes regulate permeability of capillaries in the arcuate nucleus to peripheral hormones and metabolites, a concept supported by the apparent plasticity of tanycytes processes (19, 20) and their ability to sense metabolic parameters in the bloodstream (21). Such may be the case to explain leptin resistance in association with diet-induced obesity, when the ability of leptin to enter to the arcuate nucleus is decreased (22).

### Tanycytes and transcellular transport

It is likely that the biologic potential of tanycytes have not yet been fully realized. The fact that tanycytes are in direct contact with the CSF and have villous-like specializations on their apical surface that project into the third ventricular cavity, indicate that these cells are capable of extracting substances from the CSF by an absorptive process. Indeed, horseradish peroxidase, wheat germ agglutinin, ferritin, [ $^3$ H]dopamine or  $\beta$ -endorphin injected into the cerebrospinal fluid are avidly concentrated by tanycytes (23-26), presumably by clathrin-mediated endocytosis (25). Conversely, protrusions at the apical surface of tanycytes connected by a narrow pedicle have suggested that tanycytes can extrude substances into the cerebrospinal fluid (27). Furthermore, horseradish peroxidase injected into the bloodstream and apolipoprotein A-IV, a circulating lipoprotein, accumulate in tanycytes (28, 29), suggesting that tanycytes are also capable of extracting and concentrating substances directly from the bloodstream. The presence of caveolin-1, the main component of non-coated plasma membrane invaginations, but not clathrin in endfeet processes of  $\beta 1,2$  tanycytes (25), suggest caveolin-mediated endocytosis as the main mechanism for uptake at tanycyte terminals. Thus, tanycytes may provide a bi-directional, cytoplasmic conduit between the CSF and vascular elements in the arcuate nucleus and/or the median eminence, allowing the movement of substances from one compartment to the other. Along these lines, Lofgren (30) was the first to postulate a CSF to pituitary transport system involved in the regulation of anterior pituitary function, a concept echoed by other (31-34) but not all (35) researchers.

### Tanycytes and axonal guidance and neuronal regeneration

Neuroglial associations between tanycytes and axon terminals in the median eminence have raised the possibility that tanycytes act as a scaffolding to guide migrating axons of the tuberoinfundibular neurons during early development to reach the external zone of the median eminence (36), and/or in the reg-

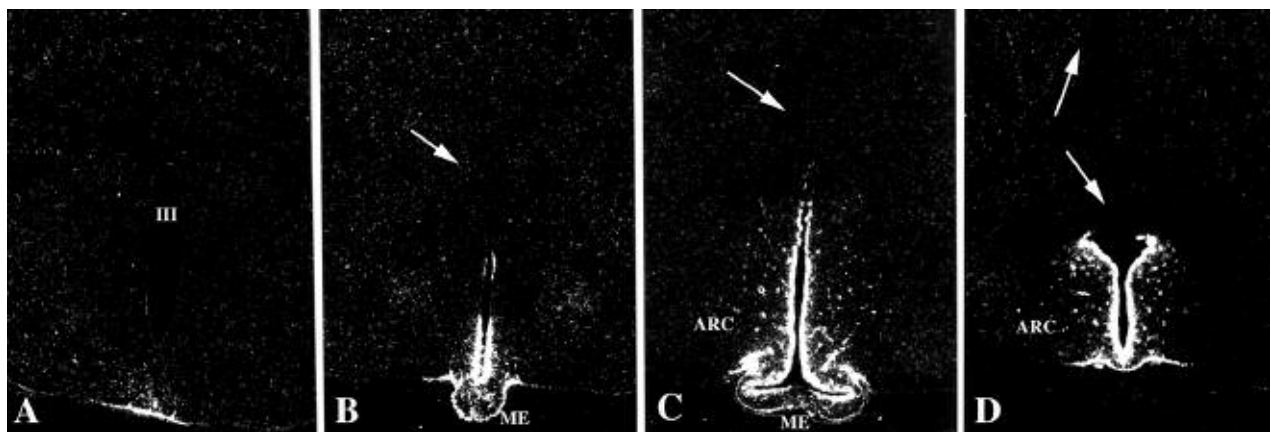
ulation of the release of hypothalamic releasing and inhibitory factors by controlling the shape of the tanycyte basal processes (37-40), allowing or disallowing substances released from these axon terminals to reach the portal circulation. Tanycytes may also release substances that promote axonal regeneration, explaining observations by Chauvet et al (41, 42) that regeneration of vasopressin and oxytocin axons always occur in close association with tanycyte processes, and that axonal sprouting of monoaminergic axons (catecholamine and serotonergic) following transection occurs only when tanycytes are present. Mechanisms to explain the regenerative capacity of tanycytes include the expression of Sonic hedgehog and associated proteins, Patched and Smoothed (43), that may participate in neuronal survival (44) and the release of growth factors such as Transforming Growth Factor (TGF)- $\beta$  (45). In addition, at least some tanycytes may serve as neural progenitor cells in the ependymal layer of the third ventricle based on studies using BrdU and nestin as markers (46).

### Tanycytes and regulation of the hypothalamic-pituitary-thyroid axis

The recent observations by Tu et al (47), Fekete et al (48) and Guadano-Ferraz et al (49) that tanycytes express one of the highest concentrations of type 2

iodothyronine deiodinase (D2) and D2 mRNA in the brain, has led to the hypothesis that tanycytes may have an integral role in the regulation of thyroid function. D2 is one of three known iodothyronine deiodinases (50-52) and the predominant 5'-iodothyronine deiodinase in the brain involved in the intracellular generation of the biologically active form of thyroid hormone, tri-iodothyronine (T3), from its less potent precursor, thyroxine (T4) (53). Since greater than 75% of nuclear T3 in the brain is derived from local conversion of T4 to T3 (53), D2 has an essential role in mediating the effects of circulating thyroid hormone on the central nervous system (CNS).

Both  $\alpha$  and  $\beta$  tanycytes would appear to express D2 mRNA as by *in situ* hybridization histochemistry, D2 mRNA is found both in the floor and infralateral walls of the third ventricle, abruptly ceasing 1/2 to 2/3 up the third ventricular wall (Fig. 3). D2 mRNA is not only present in the tanycyte cell body lining the third ventricular wall, but also extends throughout its cytoplasmic projections surrounding capillaries in the arcuate nucleus and into the basal processes in the median eminence (47-49). These findings have been confirmed by other investigators (49, 54, 55) and explain earlier observations by Riskind et al (56) that extracts of micropunches of the mediobasal hypothalamus contain high D2 activity. No hybridization is present in the roof of the third ventricle where ependymal cells reside, or in ependymal cells in other regions of

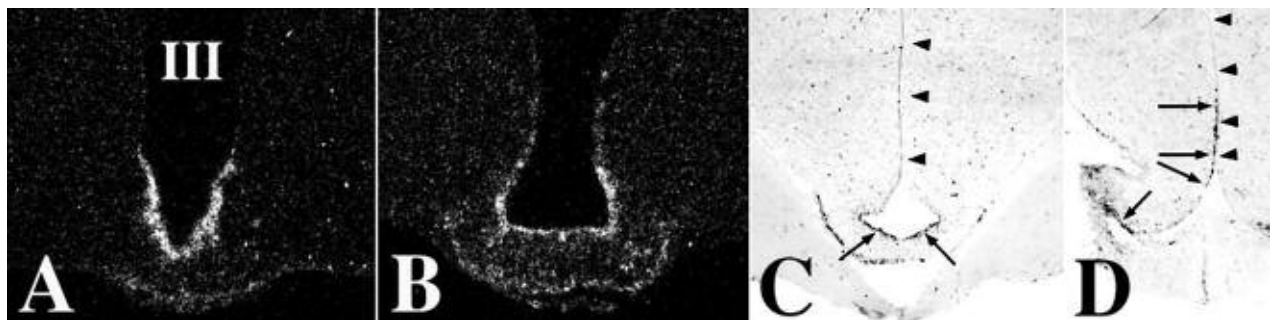


**Figure 3.** Rostral-caudal distribution of D2 mRNA in the rat mediobasal hypothalamus by *in situ* hybridization histochemistry. Note hybridization in the floor and infralateral walls of the third ventricle (III), in the arcuate nucleus (ARC) surrounding blood vessels and in the median eminence (ME). The absence of hybridization in dorsal portions of the third ventricle is denoted by the arrow. (From Tu et al (47), with permission)

the third ventricle rostral to the anterior pole of the median eminence (47). A similar distribution of D2 mRNA has been identified in the mouse (personal observations), chicken (57) and human hypothalamus (58) (Fig. 4). D2 has also been identified in tanycytes by immunocytochemistry (55).

The location of tanycytes at the interface of the blood-brain barrier and CSF-brain barrier, the endocytotic potential of these cells and their high concentration of D2 mRNA and D2 enzymatic activity, raises the possibility that tanycytes extract T4 from the bloodstream from end processes terminating on portal capillaries or capillaries in the arcuate nucleus, or from the CSF *via* apical specializations after T4 has traversed the choroid plexus (59–61). This possibility is made even stronger by the discovery that the T4 transporter, monocarboxylate transporter 8 (MCT8), is highly expressed in tanycytes in the mouse, rat and human brain (62, 63). Presumably, after the movement of T4 into the tanycyte cytoplasm, T4 is converted to T3 and then released back into the CSF, bloodstream, median eminence and/or the adjacent arcuate nucleus, thereby providing a source of T3 to the CNS. The above mechanism(s) may explain the observation that levels of free T3 in the CSF are equal to or exceed that in the systemic circulation (64–67). As the hypothalamic paraventricular nucleus (PVN) contains little, if any, D2 activity or D2 mRNA (47, 49, 56), and thereby is incapable of intracellular conversion of T4 to biologically active T3, hypophysiotropic thyrotropin-releasing hormone (TRH) neu-

rons in the PVN are dependent upon exogenous sources of T3. T3 released into the CSF, therefore, could diffuse into the substance of the brain by volume transmission (68), moving between ependymal cells lining the dorsolateral wall of the third ventricle that have poorly developed tight junctions (69), and provide a source of T3 to hypophysiotropic TRH neurons in the PVN. Indeed, in the absence of T4, nearly twice the normal circulating levels of T3 are required to restore TRH mRNA to euthyroid levels in hypothyroid animals (70), indicating that the inhibitory action of thyroid hormone on hypophysiotropic TRH is dependent upon the conversion of T4 to T3 within the CNS. Similar observations have been made for thyroid-stimulating hormone (TSH) secretion from the anterior pituitary gland (71). Were T3 released from tanycyte endings in the median eminence, it may also be taken up by axon terminals of hypophysiotropic TRH neurons of the hypothalamic tuberoinfundibular system and then transported retrogradely to their perikarya of origin in the hypothalamic paraventricular nucleus, or released into the portal vessels for transport to the pituitary to regulate TSH secretion. T3 released from tanycyte endings into the arcuate nucleus, that has known importance in the regulation of energy homeostasis (72), may also influence arcuate nucleus neurons through tanycyte-neuronal interactions. A similar paracrine mechanism has been hypothesized by Guadano-Ferraz et al (49) to take place in the cerebral cortex, whereby after conversion of T4 to T3 in astrocytes, locally released T3 can regulate



**Figure 4.** Rostral-caudal distribution of D2 mRNA in the mediobasal hypothalamus of a normal mouse (A, B) and chicken (C, D) by *in situ* hybridization histochemistry. Note hybridization in the floor and infralateral walls of the third ventricle (III), and in the median eminence (ME). At the rostrocaudal wall of the median eminence in the chicken hypothalamus, the hybridization signal (arrows) is localized to the floor of the third ventricle (C), while more caudally the D2 expressing cells cover the ventral half of the ventricular wall. Arrowheads indicate the wall of the third ventricle. (C and D from Gereben et al (57), with permission)

specific cortical functions. It is also conceivable that under certain circumstances T3 released into the median eminence might also diffuse directly into the arcuate nucleus if the blood-brain barrier created at the interface of the median eminence and arcuate nucleus by  $\beta 1$  tanycyte processes is disrupted. Morphologic evidence for the existence of a monosynaptic pathway between hypothalamic sites that can be influenced by deiodinase production and TRH neurons in the PVN has been given by Diano et al (73).

While D2 is subject to negative feedback regulation by circulating levels of thyroid hormone in the cerebral cortex and anterior pituitary (53), however, changes in circulating thyroid hormone levels have little to no effect on D2 activity or D2 mRNA in the mediobasal hypothalamus (47, 74). Accordingly, the systemic infusion of high doses of T4 into hypothyroid rats increases tissue and nuclear T3 content in the hypothalamus but maintains normal levels in the cerebral cortex (75). Presumably, therefore, the primarily role for D2 in tanycytes is regulatory rather than homeostatic.

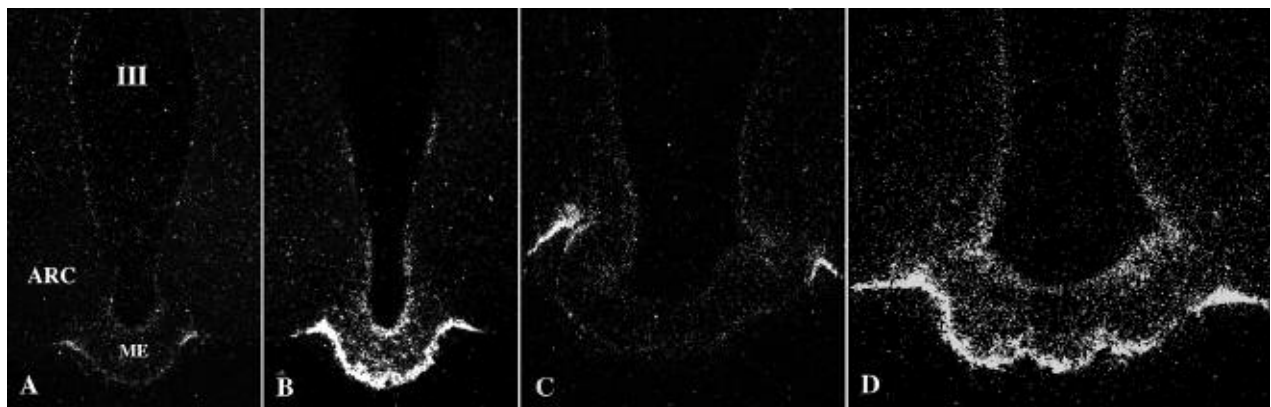
### Tanycytes and regulation of the HPT axis following immune activation and fasting

Under normal circumstances, hypophysiotropic TRH neurons in the hypothalamic paraventricular nucleus are under inverse feedback regulation by circulating levels of thyroid hormone (70, 76-78). Thus, when circulating levels of thyroid hormone fall below normal values, there is an increase in TRH mRNA in medial and periventricular parvocellular neurons in the PVN and an increase in the secretion of TRH into the portal blood for conveyance to the anterior pituitary (79-81). Conversely, increased circulating levels of T4 cause marked suppression of proTRH mRNA in the PVN and a reduction in the secretion of TRH into the portal plexus (79, 82), establishing an inverse relationship between thyroid hormone and the biosynthesis and secretion of hypophysiotropic TRH. Under certain circumstances, such as in association with infection and fasting, there is a fall in thyroid hormone levels in the peripheral blood, but a seemingly paradoxical reduction of proTRH mRNA in the

PVN, reduced secretion of TRH into the portal blood, and low or inappropriately normal plasma TSH rather than the anticipated increase in all of these parameters as seen in primary hypothyroidism (83-93). Thus, the normal feedback mechanism would appear to be overridden and a state of central hypothyroidism is induced, commonly referred to in man as the nonthyroidal illness syndrome (94). By reducing thyroid thermogenesis and preserving nitrogen stores (88, 95, 96), this mechanism is presumed to be an important adaptive response to reduce energy expenditure until the adverse stimulus has been removed.

Recent studies by Fekete et al (97) have shown that following the administration of bacterial lipopolysaccharide (LPS), D2 activity in the mediobasal hypothalamus is increased approximately 400% compared to control animals. Similar observations have been made by Boelen et al (98) in the mouse hypothalamus. The increase in D2 activity occurs primarily in tanycytes as evidenced by the marked increase in D2 mRNA in these cells following endotoxin (Fig. 5). While hypothyroidism, alone, increases D2 activity in the cerebral cortex, D2 activation in tanycytes by endotoxin would appear to be independent of the associated fall in circulating thyroid hormone levels. This is based on the observation that in thyroidectomized animals replaced with exogenously administered thyroid hormone to achieve normal circulating levels, a similar increase in D2 activity is observed in the mediobasal hypothalamus following LPS administration but not in the cerebral cortex (Fig. 6). In addition, even severe hypothyroidism is associated with only modest increase in D2 mRNA in the mediobasal hypothalamus (47).

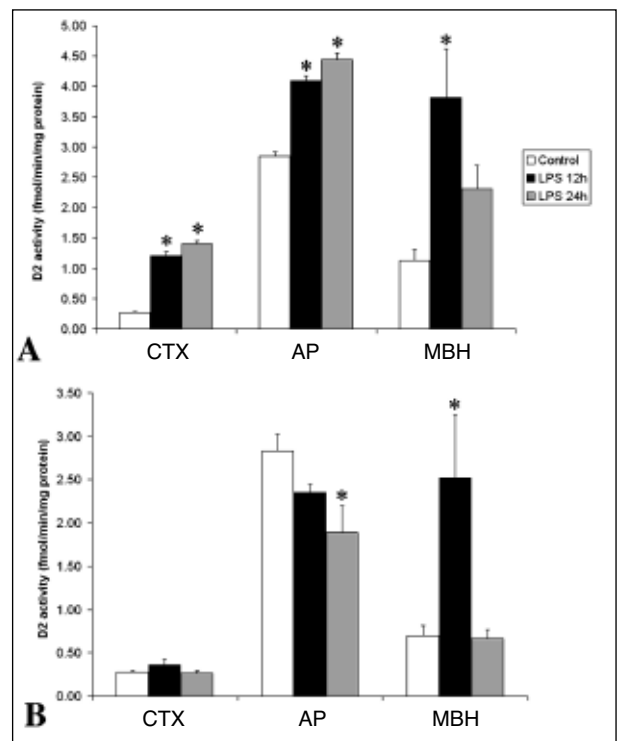
The mechanisms by which endotoxin induces D2 activation in tanycytes is not known, but observations that following endotoxin administration, tanycytes increase the expression of tumor necrosis factor (TNF) type I (p55) receptors (99) and express macrophage migration inhibitory factor (MIF) (100), indicate that tanycytes are an important target by which systemic inflammatory and infectious stimuli can affect neuroendocrine systems and the brain. One mechanism by which cytokines might induce D2 in tanycytes may be *via* NF- $\kappa$ B signaling, as the promoter of the D2 gene (*dio2*) contains multiple putative NF- $\kappa$ B binding



**Figure 5.** Low power darkfield micrographs from two different rostro-caudal levels of the median eminence (ME) showing the effect of LPS treatment on D2 gene expression in the mediobasal hypothalamus. (A,C) Controls, (B,D) LPS treated animals. The density of silver grains denoting D2 mRNA is markedly increased following LPS, particularly in the external zone of the ME. (Modified from Fekete et al (97), with permission)

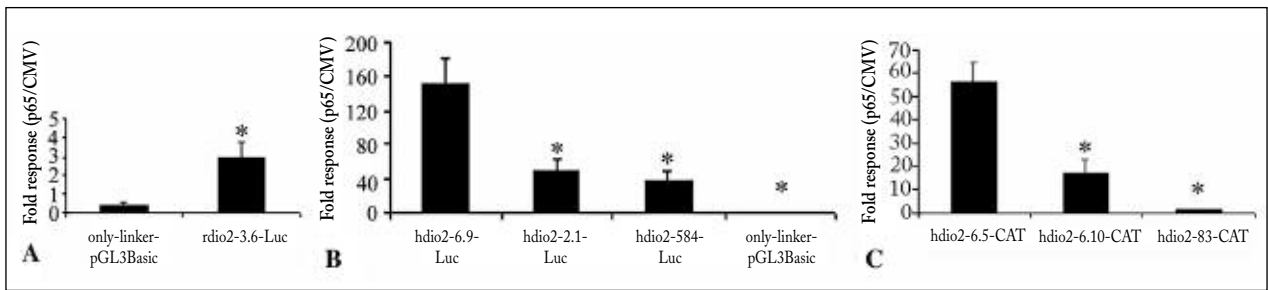
sites (97). In addition, co-expression of p65 (RelA), a required component of the activated NF- $\kappa$ B heterodimer, together with a 6.5 kb human (h) *h dio2* 5' flanking region CAT construct in a HEK-293 cell line, leads to an approximately 50-fold increase in the transcriptional activity of the *h dio2* promoter, that can be abolished by truncation of the promoter (Fig. 7). This would be in keeping with evidence that NF- $\kappa$ B is activated in the median eminence and wall of the third ventricle following LPS administration (99), although the precise cell types have not yet been identified.

We propose, therefore, that after LPS administration, TNF- $\alpha$  derived from local macrophages in the external zone of the median eminence, perhaps recruited to this region by the release of chemotactic factors from tanyocytes or local endothelial cells, or derived from the peripheral blood, may bind to TNF (p55) receptors on tanyocytes, ultimately leading to the activation of D2 through activation of NF- $\kappa$ B. Alternatively or in addition, LPS may induce CD14 receptors in tanyocytes (101) and either activate NF- $\kappa$ B directly and/or increase the expression of p55 receptors, potentiating the effect of locally released TNF- $\alpha$ . Toll-like receptor 4 (TLR4), the signal transducing molecule of the LPS receptor complex that is of critical importance in mediating the effects of LPS on NF- $\kappa$ B activation (102), may also be involved, perhaps under the influence of MIF, which has an important role in host responses to infection by modulating the expression of TLR4 (103, 104).



**Figure 6.** Effects of LPS administration on D2 activity in the cerebral cortex (CTX), anterior pituitary (AP), and mediobasal hypothalamus (MBH). (A) In intact animals, D2 activity in the cortex and anterior pituitary show a significant increase 12 and 24h after LPS administration. Peak activity in the MBH occurred 12 h after treatment. (B) In T4 replaced thyroidectomized animals, LPS has no effect on D2 activity in the CTX, an inhibitory effect in the AP, but a persistent stimulatory effect in the MBH.

\*,  $P < 0.05$  compared with saline controls.



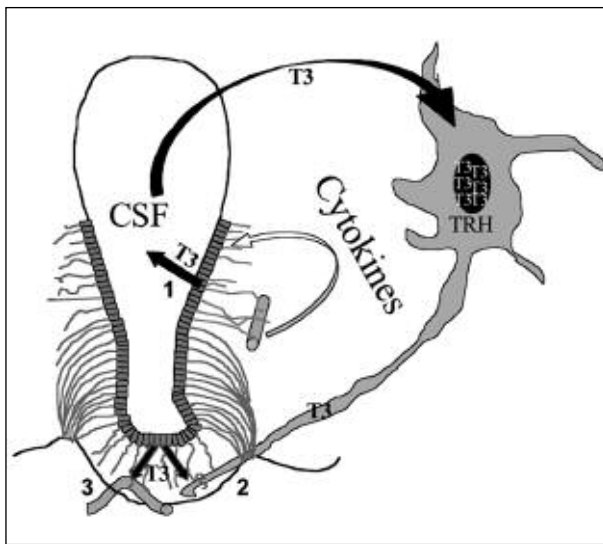
**Figure 7.** Effect of the p65 NF- $\kappa$ B subunit on the activity of the rat (r) and human (h) *dio2* promoter in HC11 and HEK cells. Results are shown as the ratio of reporter expression (corrected for transfection efficiency) in the presence and absence of p65 (p65/CMV). The ratios in experiments using the *Renilla* construct were divided by 3 to correct for the suppression of SV40-*Renilla* by p65. (A) The *rdio2*-3.6-Luc construct (containing ~3.6 kb of the rat *dio2* 5' FR) was induced 2.9-fold by P65 in HC11 cells (\*significantly different from the activation of only linker pGL3 basic vector,  $P < 0.05$ ). (B) The *hdio2*-6.9-Luc (containing ~6.9 kb of the *hdio2* 5' FR), *hdio2*-2.1-Luc (containing the 3' ~2.1 kb of the *hdio2* 5' FR), and *hdio2*-584-Luc (containing the 3' ~584 bp of the *hdio2* 5' FR), respond to p65 152-fold, 48-fold, and 38-fold, respectively. Activation of *hdio2*-584-Luc and *hdio2*-2.1-Luc constructs is significantly less than activation of *hdio2*-6.9-Luc (\* significantly different from the activation of *hdio2*-6.9-Luc,  $p < 0.01$ ). (C) The *hdio2*-6.5-CAT construct containing ~6.5 kb *hdio2* 5'FR and the *hdio2*-610-CAT (containing 610 bp of the *hdio2* 5' FR) is induced 56-fold and 17-fold, respectively, by p65 cotransfection in HEK-293 cells. The *hdio2*-83-CAT containing the 83 bp-long minimal D2 promoter is unresponsive to p65. Activation of *hdio2*-610-CAT and *hdio2*-83-CAT is significantly less than activation of *hdio2*-6.5-CAT (\* significantly different from the activation of *hdio2*-6.5-CAT vector,  $p < 0.01$ ). Data are shown as the mean of response  $\pm$  SEM of at least 4 separate experiments based on *Luc/Renilla* or *CAT/hGH* ratios. (From Fekete et al (97), with permission)

The potential role of IL-1 $\beta$ , IL-6, leptin, and other proinflammatory cytokines induced by LPS or TNF- $\alpha$  either locally or present in the circulation that signal through gp130 receptor complexes (such as CNTF, LIF, IL-11, oncostatin M, others) is not known, but may also contribute to transduction signals in tanyocytes by the activation of NF- $\kappa$ B, STATs or other second messenger systems (105). Thus, tanyocytes may be an important link between the peripheral immune system and the brain, governing neuroendocrine responses to immune activation.

It is conceivable, therefore, that endotoxin results in tissue-specific D2-mediated thyrotoxicosis in the mediobasal hypothalamus caused by increased T4 to T3 conversion by tanyocytes. Similar to that described previously, the increase in T3 may suppress the synthesis of TRH in hypophysiotropic neurons either by local feedback inhibition through the release of T3 from tanyocyte apical processes into the CSF, or uptake from hypophysiotropic TRH axonal processes in the median eminence and retrograde transport to the hypothalamic PVN. T3 may also be released into the portal capillary system for conveyance to the anterior pituitary and exert direct effects on anterior pituitary thyrotrops to inhibit the secretion of TSH. A

schematic representation of these mechanisms is shown in Fig. 8.

Fasting also has an effect to increase hypothalamic D2 activity and D2 mRNA (54, 106). Diano et al (54) reported an approximately 2-fold increase in D2 mRNA in the mediobasal hypothalamus and 1.6-fold increase in D2 activity in fasted animals compared to fed controls. These increases were unaffected by the administration of L-thyroxine, despite substantial elevations in circulating levels of T4 (54), but completely reversed by leptin administration (107). Coppola et al (107) have proposed that the suppressed circulating leptin levels and elevated glucocorticoid levels during fasting permit glucocorticoid-induced upregulation of D2. Similar to that described above, therefore, the increased D2 levels in the mediobasal hypothalamus may contribute to local tissue hyperthyroidism and suppression of hypophysiotropic TRH, contributing to the mechanism of central hypothyroidism observed during fasting. Nevertheless, the predominant central effects of the falling levels of leptin on the hypophysiotropic TRH neurons during fasting are likely to be mediated primarily by two anatomically distinct populations of neurons in the hypothalamic arcuate nucleus (108-111) that express leptin receptors and have monosy-



**Figure 8.** Proposed mechanism for D2-regulation of the hypothalamic-pituitary-thyroid axis following the administration of LPS. LPS increases D2 activity in tanycytes resulting in increased T4 to T3 conversion. [1] T3 is released from tanycyte apical processes into the CSF for conveyance to the paraventricular nucleus, or [2] taken up from hypophysiotropic TRH axonal processes in the median eminence and transported retrogradely back to its cell body in the paraventricular nucleus. [3] T3 may also be released into the portal capillary system and directly inhibit the secretion of TSH. Local tissue hyperthyroidism inhibits TRH in the paraventricular nucleus

naptic projections to TRH neurons in the PVN (108, 109, 112) or in a lesser extent by direct actions of leptin on hypophysiotropic TRH neurons (113).

### Tanycytes and regulation of reproductive function

A potential role for tanycytes in neuroendocrine regulation of reproduction has been long suspected on the basis of the close anatomical association between tanycyte endings and GnRH axon terminals (10, 114), the propensity for tanycytes to attract or induce GnRH axonal outgrowth (115, 116), and morphologic changes in tanycytes in association with the reproductive cycle or ovariectomy including size of nuclei and cell perikarya (117, 118), number of apical projections on the luminal surface (117, 119), and variations in the concentration of filaments and microtubules in processes (31). Most convincing, however, is the more recent evidence for anatomical ensheathment of

GnRH-containing axon terminals by tanycyte basal processes, suggesting the possibility that tanycytes regulate GnRH secretion to the anterior pituitary by modulating the exposure of GnRH axon terminals to the portal vessels. Indeed, subsequent studies by King and Rubin (120) and Prevot et al (121) have demonstrated dynamic changes in contacts between GnRH-containing axon terminals and tanycyte processes such that during proestrus, an increase in the number physical contacts between GnRH-containing axon terminals and the basal lamina of the portal vasculature are observed. While it is presumed that plasticity of tanycyte endings is estrogen mediated (121), other factors might also be involved such as the effect of dopamine (39, 122). Tanycytes also accumulate IGF-1 from the CSF (123, 124), and as IGF-1 can increase LH secretion *via* effects on GnRH release (125), this may be an alternative way for tanycytes to facilitate the release of GnRH from axon terminals. Furthermore, tanycytes produce and release TFG- $\alpha$  (126), which by stimulating GnRH secretion through effects on prostaglandin E2 (127), may contribute to the onset of puberty.

Tanycytes may also be involved in the regulation of reproduction in animals that are seasonal breeders. In certain temperate zone animal species, photoperiodic control of reproduction is an important adaptive mechanism to avoid giving birth during the harshest portions of the seasons. Thus, exposure to short photoperiods results in gonadal involution, reduced spermatogenesis and ovulation, and reduced mating behavior in these species, whereas exposure to long photoperiods has the opposite response (128). In the Djungarian hamster, photoperiod has profound effects on tanycytes, resulting the loss of nearly all tanycyte processes when the animals are exposed to complete darkness and its restoration or even increase in the density of processes with continuous light (19). Hence, tanycyte-neuronal interactions and thereby secretion of GnRH might be expected to be impaired during the dark period and enhanced with prolonged light. In addition, in both the Djungarian hamster and Japanese quail (129, 130), long photoperiods are associated with a marked increase in D2 mRNA in tanycytes, which as described above, is the principal enzyme involved in the conversion of T4 to T3. Of note, the peripheral or intracere-

broventricular administration of thyroid hormone in the Japanese quail can induce testicular growth and gonadotrophin secretion even though the animals are exposed to short photoperiods (130, 131). Exposure to long photoperiods is associated with a dramatic rise in the content of T3 in the mediobasal hypothalamus but not other parts of the brain, whereas administration of the D2 inhibitor, iopanoic acid, reduces testicular growth under long photoperiod conditions (130), supporting a role for D2 in transducing photoperiod regulation of reproductive function. Presumably, therefore, photoperiod changes in the concentration of D2 in tanycytes contributes to seasonal reproductive changes in susceptible animals, dependent upon upregulation of D2 in the mediobasal hypothalamus mediated by changes in the light/dark cycle.

### Tanycytes and other neuroendocrine functions

It is likely that tanycytes will be shown to be of importance in other neuroendocrine functions regulated in the mediobasal hypothalamus, and this is already suggested in the literature by several independent observations. These include reports that tanycytes are contacted by galanin-immunoreactive axon terminals in the median eminence (132), shunt potassium to CSF and vascular compartments (133), contain growth hormone-releasing hormone (134), express mu opioid receptors, IGF-1 receptors and angiotensin II receptors (8, 135, 136), and express the glucose transporter molecules, GLUT1 and GLUT2 (21). In particular, the potential importance of tanycytes to regulate feeding *via* glucose sensing mechanisms is suggested by Sanders et al (137), showing that destruction of tanycytes by the administration of alloxan into the third ventricle impairs feeding after an overnight fast, although independent effects of alloxan on glucose sensitive neurons in the arcuate nucleus neurons cannot be excluded.

### Conclusions

Despite that tanycytes were first distinguished as a specialized subtype of the ependymal wall over 50

years ago, its functions remain speculative. While considerable evidence would support a role as a barrier cell separating vascular, CSF and CNS compartments, they may also be involved in axon guidance to the median eminence, neural regeneration and the transport and regulation of secretion of substances destined for the portal vasculature or the CSF. As suggested by the unique localization of D2 primarily to tanycytes in the floor and infralateral walls of the third ventricle, however, these special glial cells may integrate hormonal and probably neuronal signals, and under specific conditions, influence neuroendocrine functions by altering local T3 tissue concentrations and perhaps the access of hypophysiotropic terminals to the portal capillaries. These functions may be of particular importance in the regulation of the hypothalamic-pituitary-thyroid axis during infection and fasting and reproduction.

### Acknowledgments

This work was supported by Grants NIH DK-37021, OTKA T046492 and Sixth EU Program (LSHM-CT-2003-503041)

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